FATS AS INDICATORS OF PHYSIOLOGICAL CONSTRAINTS IN NEWBORN AND YOUNG REINDEER

Rangifer tarandus tarandus L.

PÄIVI SOPPELA

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and The Arctic Centre, University of Lapland

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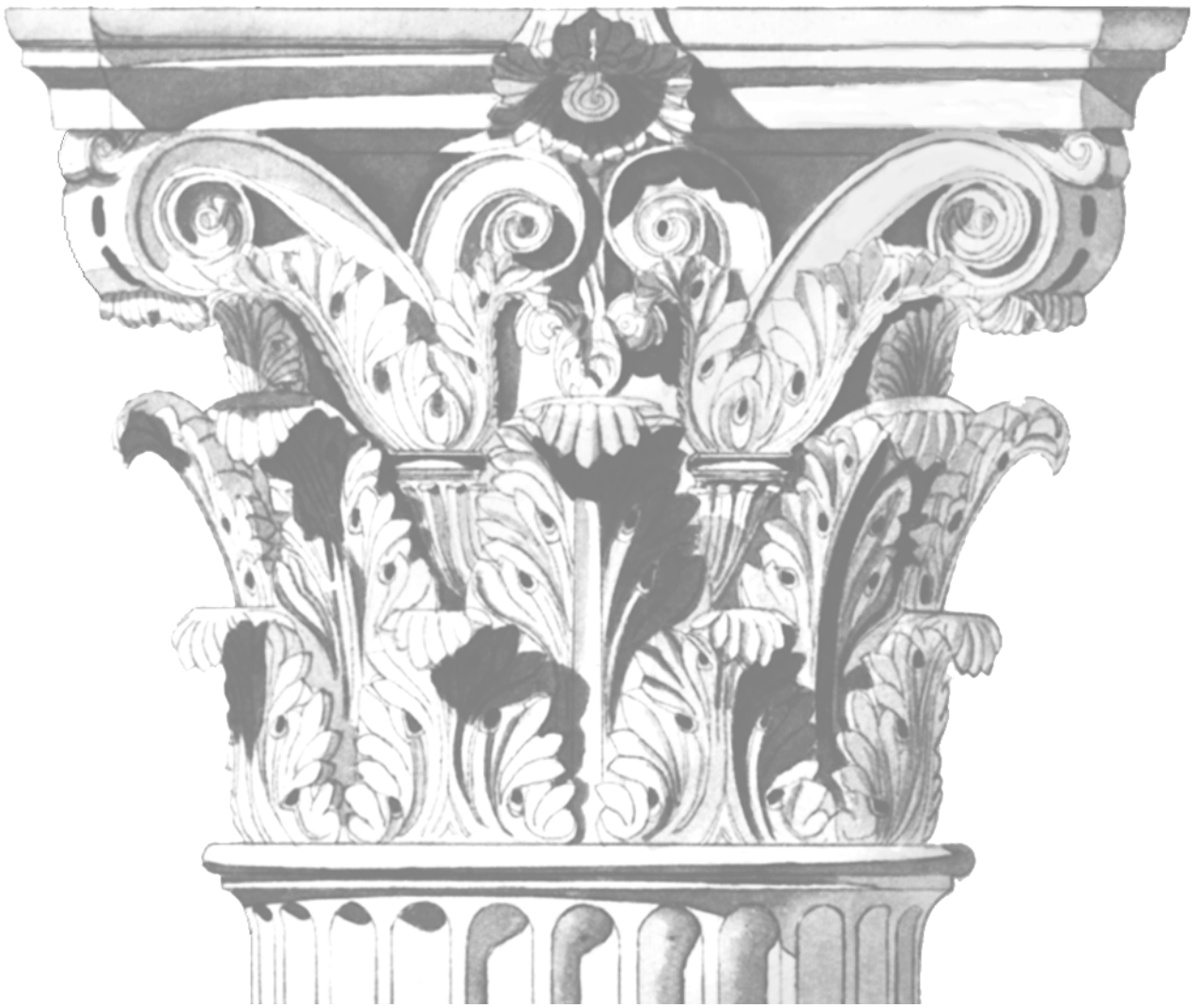

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Abstract

The semi-domesticated reindeer is a northern species of Cervidae that is exposed to extreme seasonal changes in temperature and nutrition in its living environment. The objective of this study was to examine the significance of thermogenic brown adipose tissue (BAT) for the survival of newborn reindeer in the cold during the critical perinatal period. The other main objective was to study the effect of wintertime undernutrition on serum and bone marrow fatty acid composition in yearling reindeer, with particular attention on the proportions of unsaturated and polyunsaturated fatty acids (PUFAs) and their feasibility as indicators of nutritional status.

The results showed that the most of the adipose tissues in newborn reindeer were functionally active BAT. The tissue had specific anatomical locations, specialized cell morphological structure, high aerobic capacity, and tissue-specific mitochondrial 32000 M_r-uncoupling protein (UCP_1) that is considered a rate-limiting factor for thermogenesis. The most readily mobilized fatty acids from BAT triacylglycerols were arachidonic, linoleic, and α-linolenic acids. BAT was most active at birth and during the close perinatal period but its aerobic capacity declined during the first month of life while UCP_1 disappeared and the tissue gradually adopted the histological characteristics of white adipose tissue.

The newborn reindeer had very low proportions of the principal C18-PUFAs, linoleic and α-linolenic acids, in serum lipids. However, the proportions of C18-PUFAs increased during the first few days of life by a rate that suggests a selective retention of these fatty acids from milk lipids. A prolonged restricted feeding of reindeer with lichen during winter and spring induced significant reductions in the proportions of linoleic and α-linolenic acids in serum cholesteryl esters and phospholipids, while proportion of arachidonic acid and serum prostaglandin $PGF_{2\alpha}$ metabolite concentration increased. Plasma leptin and insulin levels decreased in parallel with decreases in feed intake and body weight. In freely ranging reindeer, the proportions of oleic acid and principal C18-PUFAs were significantly reduced in femur bone marrow triacylglycerols as a result of a wintertime undernutrition.

In conclusion, active BAT is the dominant adipose tissue type in the newborn reindeer and it is likely to have a major significance on the thermoregulatory heat production and cold resistance of reindeer during the perinatal period. The changes in the specific PUFA proportions of serum and bone marrow lipids reflect the changes in the nutritional status and suggest that these fatty acids are preferentially utilized during prolonged wintertime undernutrition.

Key words: brown adipose tissue, serum, bone marrow, UCP_1, fatty acids, cold, undernutrition

Soppela, Päivi, Rasvat fysiologisen tilan indikaattoreina vastasyntyneillä ja nuorilla poroilla (*Rangifer tarandus tarandus* L).
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2000
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Tiivistelmä

Puolikesy poro on pohjoinen hirvieläin, joka on altis suurille vuodenaikaisille lämpötilan ja ravinnon muutoksille elinympäristössään. Tämän tutkimuksen tarkoituksena oli selvittää lämmöntuottoon erikoistuneen ruskean rasvakudoksen merkitystä poronvasojen selviytymiselle vasonta-ajan kylmästressistä. Toisena päätavoitteena oli tutkia talviaikaisen aliravitsemuksen vaikutuksia poronvasojen seerumin ja luuytimen lipidien rasvahappokoostumukseen, kiinnittäen erityistä huomiota tyydyttymättömien ja monityydyttymättömien rasvahappojen osuuksiin ja niiden soveltuvuuteen ravitsemustilan indikaattoreiksi.

Tulokset osoittivat, että suurin osa vastasyntyneiden poronvasojen rasvakudoksista oli aktiivista ruskeaa rasvakudosta. Kudoksella oli erityiset anatomiset sijaintipaikat, erikoistunut solumorfologinen rakenne, korkea aerobinen kapasiteetti ja se sisälsi kudosspesifistä mitokondriossa sijaitsevaa 32000 M_r -irtikytkijäproteiinia (uncoupling protein, UCP_1) jota pidetään lämmöntuotolle välttämättömänä tekijänä. Ruskean rasvakudoksen triasyyliglyserolista nopeimmin vapautuvia rasvahappoja olivat arakidoni-, linoli- ja α-linoleenihappo. Ruskea rasvakudos oli erityisen aktiivista ensimmäisinä elinvuorokausina, mutta sen aerobinen kapasiteetti laski nopeasti ensimmäisen elinkuukauden aikana samalla kun UCP_1 hävisi ja kudos muuttui histologialtaan valkean rasvakudoksen kaltaiseksi.

Vastasyntyneellä poronvasalla oli hyvin alhaiset tärkeimpien C18-monityydyttymättömien rasvahappojen eli linoli- ja α-linoleenihapon osuudet seerumin lipideissä. Näiden C18-rasvahappojen osuudet lisääntyivät kuitenkin nopeasti ensimmäisten elinvuorokausien aikana viitaten rasvahappojen valikoivaan pidättämiseen maidon lipideistä. Pitkäaikainen rajoitettu jäkäläruokinta talven ja kevään aikana johti linoli- ja α-linoleenihapon osuuksien merkitsevään vähenemiseen seerumin kolesteroliestereissä ja fosfolipideissä, kun taas arakidonihapon osuus ja seerumin erään prostaglandiinin, $PGF_{2\alpha}$-metaboliitin pitoisuus lisääntyivät. Plasman leptiinin ja insuliinin tasot poronvasoilla laskivat ravinnonoton vähentyessä ja ruumiinpainon laskiessa. Vapaasti laiduntaneilla poroilla reisiluuytimen rasvojen öljyhapon ja tärkeimpien C18-monityydyttymättömien rasvahappojen osuudet olivat talviaikaisen nälkiintymisen seurauksena merkitsevästi alentuneet.

Yhteenvetona voidaan todeta, että aktiivinen ruskea rasvakudos on vallitseva rasvakudostyyppi vastasyntyneillä poroilla ja sillä on todennäköisesti huomattava merkitys vastasyntyneiden poronvasojen lämmöntuotolle ja kylmyyden sietokyvylle. Muutokset tiettyjen monityydyttymättömien rasvahappojen osuuksissa seerumissa ja luuydinten lipideissä heijastavat muutoksia porojen ravitsemustilassa ja viittaavat siihen, että näitä rasvahappoja käytetään valikoivasti pitkän talviaikaisen aliravitsemuksen aikana.

Avainsanat: ruskea rasvakudos, seerumi, luuydin, UCP_1, rasvahapot, kylmyys, aliravitsemus

Soppela, Päivi, Buoiddit fysiologalaš dili indikáhtorin njuoratmisiin ja čearpmahiin (*Rangifer tarandus tarandus* L.).
Biologia instituhtta, Oulu universiteahtta, PL 3000, 90014 Oulu universiteahtta ja Artktalaš guovddáš, Lappi universiteahtta, PL 122, 96101 Roavvenjárga
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Oulu

Beallelojes boazu lea davviguovlluid ealgaealli, mii gártá vuogáiduvvat garra jagiáiggiid liekkasvuođa ja biepmu nuppástusaide eallinbirrasisttis. Dán dutkamuša ulbmilin lei čilget ruškes buoidegođđosa, mii lea spesialiseren liekkasvuođa buvttadeapmái, mearkkašumi misiid birgemii guottetáigge galmmasvuođa dagahan streassas. Nubbin váldoulbmilin lei dutkat dálveáigge jolihisvuođa váikkuhusaid misiid seruma ja ađđama vuodjasivračoakkádussii, nu ahte giddejin earenoamáš fuopmášumi duhtameahttun ja máŋggaláhkai duhtameahttun vuodjasivvraid (PUFAt) ossodagaide ja daid heivvolašvuhtuhtii biebmodili indikáhtorin.

Bohtosat čájehedje, ahte eanaš njuoratmisiid buoidegođđosis lea aktiivvalaš ruškes buoidi. Gođđosis ledje sierranas anatomalaš sajit, spesialiseren seallamorfologalaš ráhkadus, alla suvrradanvuoibmi ja dat sisttisdoalai gođđosii mihtilmas 32000 M_r-luovusgálgiproteinna (uncoupling protein, UCP_1) mii gávdno mitokondrios ja dat adno liekkasvuođa buvttadeamis vealtameahttun dahkkin. Ruškes buoidegođđosa triasylaglyserolas johtilapmosit luovus beassi vuodjasivvrat ledje arakidona,- linola- ja α-linolenasivra. Ruškes buoidegođus lei earenoamážit aktiivvalaš vuosttas eallinjándoriin, muhto dan aerobalaš kapasiteahtta luittii johtilit vuosttas eallenmánotbajiid áigge seammás go UCP_1 jávkkai ja gođus nuppástuvai histologias beales vilges buoidegođđosa láganin.

Njuoratmiesis ledje oalle vuollegis deháleamos C18-máŋggaláhkai duhtameahttun vuodjasivrraid dahjege linola- ja α-linolenasivvraid ossodagat seruma lipidain. Dáid C18-vuodjasivrraid ossodagat lassánedje goittotge johtilit vuosttas eallenjándoriid áigge, dáná dat čujuhedje vuodjasivvraid buoremus retenšuvnna álldu mielkki lipidain. Guhkesáigásaš ráddjejuvvon jeagilbiebman dálvvi ja giđa áigge doalvvui linola- ja α-linolenasvirraid ossodagaid mearkkešahtti njiedjamii seruma kolesterolaesterain ja fosfolipidain, go fas arakidonasivvra ossodat ja seruma dihto prostaglandiinna, $PGF_{2\alpha}$-metabolihta mearri lassánii. Plasma leptiinna ja insuliinna dásit misiin njidne, go biepmu mearri njiejai ja deaddu gahčai. Friddja guhton bohccuin ađavuojaid oljosivrra ja deháleamos C18-máŋggaláhkai duhtameahttun vuodjasivvraid ossodagat ledje dálveáigge jolihisvuođa geažil mealgadit luoitán.

Čoahkkáigeassun sáhttá dadjat, ahte aktiivvalaš ruškes buoidegođus lea ráđđejeaddji buoidegođustiipa njuoratmisiin ja das lea duohtavuođa mielde fuomášahtti mearkkašupmi njuoratmisiid liekkasvuođa buvttadeapmái ja galmmasvuođa gierdamii. Nuppástusat dihto máŋggaláhkai duhtameahttun vuodjasivvraid ossodagain serumas ja ađđama lipidain speadjalastet nuppástusaid bohccuid biebmodilis ja čujuhit dasa, ahte dát vuodjasivvrat geavehuvvojit buoremusat guhkes dálvvi jolihisvuođa áigge.

Čoavddasánit: ruškes buoidegođus, seruma, ađđama lipidat, UCP_1, vuodjasivrrat, galmmasvuohta, jolihisvuohta

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Päivi Soppela

Abbreviations

A	adrenaline
ATP	adenosine triphosphate
BAT	brown adipose tissue
BCFA	branched-chain fatty acid
BHB	β-hydroxybutyrate
BHT	butylated hydroxytoluene
CAT	convertible adipose tissue
CE	cholesteryl ester
COX	cytochrome-c oxidase
DIT	diet-induced thermogenesis
EFA	essential fatty acid
FABP	fatty acid-binding protein
FFA	free fatty acid
GLC	gas-liquid chromatography
ME	metabolizable energy
n-3 PUFA	polyunsaturated fatty acid with a double bond from the third to fourth carbon atom from the methyl end of the carbon chain
n-6 PUFA	polyunsaturated fatty acid with a double bond from the sixth to seventh carbon atom from the methyl end of the carbon chain
NA	noradrenaline
NST	non-shivering thermogenesis
PAGE	polyacryl amide electrophoresis
PG	prostaglandin
PL	phospholipid
PUFA	polyunsaturated fatty acid
SDS	sodium dodecyl sulphate
SFA	saturated fatty acid
TAG	triacylglycerol
TLC	thin-layer chromatography
UCP	uncoupling protein
UCPH	uncoupling protein homologue

UI	unsaturation index
WAT	white adipose tissue

List of original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I Soppela P, Sormunen R, Saarela S, Huttunen P & Nieminen M (1992) The localization, morphology, and respiratory capacity of "brown" adipose tissue in newborn reindeer. Comp Biochem Physiol 101A: 281-293.

II Soppela P, Nieminen M, Saarela S, Keith JS, Morrison JN & MacFarlane F & Trayhurn P (1991) Brown-fat specific mitochondrial uncoupling protein in adipose tissues of newborn reindeer. Am J Physiol 260: R1229-1234.

III Soppela P & Nieminen M (1998) Polyunsaturated fatty acids in serum lipids of reindeer during the close postnatal period. J Comp Physiol B 168: 581-590.

IV Soppela P, Heiskari U, Nieminen M, Salminen I, Sankari S, Kindahl H (2000). The effect of a prolonged undernutrition on serum lipid and fatty acid composition of reindeer calves during winter and spring. Acta Physiol Scand 168: 337-350.

V Soppela P & Nieminen M (2000) The effect of wintertime undernutrition on the fatty acid composition of leg bone marrow fats in reindeer (*Rangifer tarandus tarandus* L.). Manuscript (submitted).

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1 Introduction

The survival of a newborn homeothermic animal greatly depends on how well it is able to respond to the large thermal transition and subsequent heat loss that occurs at birth. Well-developed or precocial species (Blix & Steen 1979) such as the sheep and rabbit are able to keep their body temperature high immediately after birth through the onset of effective metabolic heat production, or non-shivering thermogenesis (NST) (Heim & Hull 1966, Nedergaard *et al.* 1986). Altricial, 'nest-dependent' species such as the rat and 'immature' species such as the hamster are more susceptible to variations of ambient temperature and their capacity for thermoregulatory heat production by NST does not develop until during the first few days or weeks of life (Hissa 1968, Nedergaard *et al.* 1986). It is well established that the main site of NST in the mammalian neonates is brown adipose tissue (BAT) (Heim & Hull 1966, Cannon & Nedergaard 1985, Nedergaard *et al.* 1986) which plays a key role in their postnatal survival in the cold. NST in BAT is stimulated by the cold-induced release of the sympathetic nervous transmitter noradrenaline (NA) (Jansky 1973) and is supported by the activation of thyroid hormones (Bianco & Silva 1987, Trayhurn *et al*. 1993a). In addition to neonatal mammals, BAT plays an important role in rewarming the body at the end of hibernation, and in some adult mammals adapted to remain active in the cold (Cannon & Nedergaard 1985, Trayhurn 1993).

Specific anatomical locations of BAT have been described in different species (Afzelius 1970, Néchad 1986) that confirm the specialization of this tissue for the distribution of heat (Smith & Horwitz 1969). The specialization of BAT for thermogenesis is apparent by its highly organized cellular structure with abundant mitochondria, multilocularly dispersed fat, dense capillarization and sympathetic innervation of adipocytes (Néchad 1986, Lončar 1991). White adipose tissue (WAT) is usually unilocular, with few mitochondria and less visible innervation and vascularization. On the basis of cell morphological features, BAT has been characterized and identified in numerous mammalian species (Néchad 1986). The capacity of BAT for thermogenesis is ultimately dependent on the presence and activity of the tissue-specific 32000 M_r-uncoupling protein, UCP (UCP_1 since 1997, cf. Boss *et al.* 1998) located in the

inner membrane of brown adipocyte mitochondria (Cannon & Nedergaard 1985, Klaus *et al.* 1991). UCP_1 uncouples the chemical energy released in the oxidation of the fatty acids from ATP synthesis, thus releasing it primarily as heat (Nicholls & Locke 1984). The fatty acids are also believed to activate UCP_1 (Nicholls *et al*. 1986, Boss *et al.* 1998, Lowell & Spiegelman 2000), but the nature of this mechanism has yet not been elucidated. As UCP_1 appears only in brown adipocytes, its detection by immunological techniques has been used as a criterion for identifying BAT and differentiating it from WAT in various species, including humans (Cannon & Nedergaard 1985, Klaus *et al.* 1991, Trayhurn 1993). During most of its research history, BAT has been most intensively studied in the altricial rat and other laboratory species. This has led to a situation where much basic knowledge is still lacking about the presence and function of BAT in large precocial species. Lately, large ruminants have been more studied (Casteilla *et al.* 1987, 1989, 1994, Trayhurn *et al.* 1993a, b) but large wild or freely ranging terrestrial species that are exposed to cold in their natural environment have been studied only incidentally.

The reindeer (*Rangifer tarandus*) is a circumpolar northern species of Cervidae that exhibits advanced adaptation to extreme seasonal changes in its arctic and subarctic environments. In Fennoscandia, most reindeer are semi-domesticated and their herding constitutes an important livelihood and basis of Sami culture. Reindeer calves are born under adverse weather conditions during spring when the pastures are usually snow-covered and the ambient temperature frequently falls below 0°C. Reindeer calves are precocial (Blix & Steen 1979) and are able to follow and suckle their mothers within hours of birth. The transition from the uterus to the external thermal environment represents a large thermogradient (30-60°C) and thus an extreme thermoregulatory challenge for a neonatal reindeer. Previous studies have shown that newborn reindeer are capable of effective thermoregulation and they have suggested the presence of BAT and a high capacity for NST (Krog *et al.* 1977, Hissa *et al.* 1981, Markussen *et al.* 1985, Soppela *et al.* 1986), as with another arctic ungulate, the muskoxen (Blix *et al.* 1984). However, the evidence for the presence of BAT in reindeer has been fragmentary and its significance for NST poorly established. The reindeer provides an example of a large freely ranging precocial terrestrial species for whose neonatal survival and reproduction success NST appears crucial. The nutritional condition of a newborn reindeer is also of interest as both the foetal development and calving of reindeer occur in very poor nutritional conditions. Early calf mortality in reindeer is frequently associated with poor nutrition and poor resistance to disease (Eloranta & Nieminen 1986).

For northern freely ranging mammals, winter and early spring are the most challenging periods for survival due to the poor availability of food and its low quality. The major winter diet of Fennoscandian reindeer in many areas, ground lichens (*Cladina* spp.), are rich in carbohydrates but poor in protein content as are most of the other winter feed sources for reindeer (Nieminen & Heiskari 1989). Several studies have shown that reindeer enter a negative energy balance when feeding on natural pastures during winter (McEwan 1968, Reimers *et al*. 1983, Nieminen *et al.* 1984). During the course of winter when

the availability of feed is restricted due to hard digging conditions, the reindeer often enters a state of starvation. The use of adipose tissues has been proposed to play an important role in the wintertime survival of reindeer (Ringberg *et al.* 1981, Larsen *et al.* 1985), especially in the Svalbard reindeer that is the fattest of the subspecies of *Rangifer* (Pond *et al.* 1993). As reindeer are generally lean animals, the importance of adipose tissue as an energy source for their survival during winter has also been questioned (Tyler 1987). The roles of adipose tissues or their fatty acids other than as an energy source have attracted little attention. However, specific fatty acids, in particular polyunsaturated fatty acids (PUFAs) have important physiological and growth-related functions in the body (Innis 1991, Bruckner 1992). The changes in the fatty acid composition of adipose tissues in relation to nutritional condition in freely ranging ungulates, including reindeer, have not been studied.

There is increasing evidence from *in vitro* experiments that the lipolysis and release of fatty acids from triacylglycerols (TAGs) in adipose tissue is not a random process but favors long-chain and unsaturated fatty acids (Gavino & Gavino 1992, Raclot & Groscolas 1993, Raclot *et al.* 1995). These findings and the evidence of the selective mobilization of specific PUFAs from the adipose tissues of weight-cycled rats (Chen *et al*. 1995) and fasting emperor penguins (Grosclas *et al.* 1990) have suggested that unsaturated fatty acids may have special roles in the body during the periods of a negative energy balance. The low interest in the fatty acid composition of ruminant adipose tissues, including that of reindeer is perhaps because they are - due to the effective biohydrogenation of unsaturated fatty acids by rumen microorganisms - mainly saturated (Garton & Duncan 1971, Christie 1981). The striking exception in this respect are leg bone marrow lipids, which contain a high proportion of unsaturated fatty acids in reindeer (Meng *et al.* 1969, Pond *et al.* 1993) and other ruminants (West & Shaw 1975, Turner 1979, Christie 1981), and seem to be preserved until the last phase of undernutrition (Ransom 1965, Nieminen & Laitinen 1986, Davis *et al.* 1987). The deprivation of dietary PUFAs in rodents and humans are reflected as rapid reductions in their circulating levels (Chen & Cunnane 1992, Leichsenring *et al.* 1995). These observations motivate studies also in ruminants as their major serum lipids are enriched with PUFAs (Christie 1981). The changes in the fatty acids of bone marrow TAGs and serum lipids in ruminants appear particularly interesting as potential indicators of undernutrition.

The factors that regulate feed intake, body weight and body fat cycles in ruminants are poorly known. It has been shown that species that have clear seasonal cycles in body weight, such as the reindeer, decrease their feed intake and body weight voluntarily during winter even if they are given high quality rations without limitation (Ryg & Jacobsen 1982, Larsen *et al.* 1985, DelGiudice *et al.* 1987, Suttie & Webster 1995). The effect of a short day-length mediated by hormones such as an insulin-like growth factor (Suttie & Webster 1995) has been proposed to participate in the intrinsic regulation of seasonal feed intake and body weight in highly seasonal species. Recent studies have proposed that the secretory product of adipose tissue, leptin, may also play a role in the control of feed intake and body weight in vertebrates by

providing the brain with a signal about the amount of stored adipose tissue, thereby acting as a satiety factor (Considine & Caro 1997, Trayhurn *et al.* 1998). The exact mechanism of the effect of leptin is not known and is being actively studied (Schwartz *et al*. 2000). The production of leptin is decreased during fasting and increased during overfeeding (Trayhurn *et al.* 1998). As reduction in feed intake and body weight during winter is common in reindeer, this species offers an opportunity to also view the regulatory aspects of the adiposity.

Physiological studies in reindeer are relatively new as the reindeer is not a conventional target of investigation and its viability depends greatly on ecological factors. Due to its large size, long reproduction cycle, and elaborate handling, only a small number of animals can be taken into experiments, and samples have to be collected over several years. However, research in a seasonal and highly adapted species such as the reindeer is important in providing a basis for estimating whether the results obtained in laboratory and domestic species can be generalized to encompass animals in natural conditions. Such studies provide information about the mechanisms underlying or regulating the adaptation of reindeer to its living environment, which can then be implemented in reindeer management. This study was undertaken in order to increase the understanding of the ecophysiological mechanisms which enable reindeer to survive neonatal cold stress and undernutrition during winter.

2 Review of literature

2.1 Ecophysiological characteristics of reindeer

The reindeer (*Rangifer tarandus*) is a species of Cervidae that inhabits the whole northern circumpolar area, spreading from the North America to Eurasia (Banfield 1961). Reindeer herding is a centuries long tradition, a substantial livelihood, a cultural activity, and a way of life among a number of peoples in northern Fennoscandia and Eurasia. The Fennoscandian semi-domesticated reindeer is believed to have descended from the wild Eurasian mountain reindeer (*Rangifer tarandus tarandus* L.) (Siivonen 1975). Reindeer herding obviously developed from wild reindeer hunting, and reindeer have been herded by the Sami people in northern Fennoscandia for probably 1000-1500 years, or even longer (Banfield 1961, Ingold 1980, Eira 1984). The selection process has differentiated the reindeer from its wild ancestor by producing reindeer that are more easy to handle and have some differences in behavior and appearance, but can breed and produce offspring with the wild conspecies. Reindeer graze freely on natural pastures during most of the year and they are gathered only during certain periods of the year for calf branding and slaughtering. During the past few decades, reindeer herding has undergone significant changes, including the establishment of calf slaughter, increased subsistent feeding during winter, antiparasitic treatments, as well as many technical developments. However, the survival of reindeer is still highly dependent on natural conditions. The problems in the survival of reindeer are basically similar to those of other freely grazing northern species, and the success of the animals depends how successfully they calve, grow during summer, and survive over winter.

The reproduction of reindeer is strictly seasonal: the hinds get pregnant in autumn, gestation lasts throughout the winter (ca. 220 days), and the calves are born during spring. There are about 0.7 million semi-domesticated reindeer in Finland, Sweden and Norway, with approximately 300 000 new calves born

every year. The reindeer calves are well developed and capable of effective thermoregulation after birth (Hissa *et al.* 1981, Markussen *et al.* 1985, Soppela *et al.* 1986). The digestion of the newborn calves is essentially monogastric until their forestomachs become used to the fermentation of green vegetation. The nutrition of the calves during the first weeks of life relies on milk that is rich in protein and lipids (Luhtala *et al.* 1968). Reindeer calves grow very quickly during the peak suckling period and their first months (Timisjärvi *et al.* 1982). During summer and autumn, reindeer eat various highly nutritious plant species and mushrooms (Nieminen & Heiskari 1989), accumulate body protein and fat, and increase their body weight. Calves usually accumulate little fat during their first summer and are typically lean (Ringberg *et al.* 1981). The main winter feeds of semi-domesticated Fennoscandian reindeer in many areas are ground lichens (Nieminen & Heiskari 1989). Lichens supply enough energy to maintain the reindeer over winter, and keep water intake and its thermal costs at a low level (Soppela *et al.* 1991) but do not prevent undernutrition in which a negative balance of nitrogen occur (Ryg & Jacobsen 1982).

During winter, reindeer save nitrogen by decreasing the filtration rate of kidneys (Valtonen 1979) and may use adipose tissues as an additional source of energy (Ringberg *et al.* 1981, Larsen *et al.* 1985) but lose about 20 % of their body weight even in normal conditions (Nieminen *et al.* 1984). Pregnant hinds use their body fat stores mainly for foetal growth (White & Luick 1984, Tyler 1987). Similarly to other large northern species that are active throughout the winter (Scholander *et al.* 1950), reindeer are well adapted to tolerating low ambient temperatures, principally because of their prime fur insulation (Timisjärvi *et al.* 1984, Nilssen *et al.* 1984b), and by peripheral cooling of the lower parts of their legs (Irving & Krog 1955) and nasal cavities (Johnsen *et al.* 1985). By the means of various thermal adaptations, reindeer tolerate ambient temperatures as low as -30 °C or lower during winter without increasing their heat production (Nilssen *et al.* 1984b).

2.2 Brown adipose tissue in mammals

The first time BAT was described as a tissue was almost 450 years ago (1551) when Konrad Gessner found it from hibernating marmots. At that time, the function of the tissue was unclear. The function of BAT as a specialized thermogenic tissue and as the main effector of NST was elucidated in the later half of the 20th century (Heim & Hull 1966, Smith & Horwitz 1969, Foster & Frydman 1979), which aroused a high interest in the presence and function of this tissue in cold-adapted, newborn and hibernating animals. The species distribution of BAT is still not defined with certainty but, with the exception of domestic pigs (Trayhurn *et al.* 1989), it seems to be present only in mammals (Klaus *et al.* 1991, Trayhurn 1993). It is generally believed that BAT developed late in the course of evolution in parallel with the development of homeothermy, and more specifically with the capacity for the facultative thermoregulation by NST (Néchad 1986). This characteristic in adult mammals

is considered a key feature in the long-term acclimation to cold. Birds are homeotherms that do not possess BAT (Saarela *et al.* 1989, 1991) but there is evidence that NST does occur in the skeletal muscles of birds, and that the characteristics of avian NST are probably different from those of the mammalian NST (Duchamp *et al.* 1999). Some kind of NST may also occur in lower vertebrates, as findings of a thermogenic tissue 'brain heater' in various species of fish have suggested (Carey 1982). Within mammals, BAT and NST are not limited to placental mammals but have also been found in marsupials (cf. Nicol *et al.* 1997).

There are large differences in the ontogenetic development of BAT in mammals between different species and developmental types. In precocial species, such as the rabbit and humans, BAT is well-developed and functional at birth (Néchad 1986, Nedergaard *et al.* 1986) whereas in altricial species such as the rat, the thermogenic capacity of BAT develops over the first days of life, and it does not develop in the 'immature' hamster until three weeks after birth (Hissa 1968, Nedergaard *et al.* 1986). The cold-induced release of NA from the sympathetic nerve endings is considered necessary for the stimulation of thermogenesis in BAT (Jansky 1973, Nedergaard *et al.* 1986, Obregón *et al.* 1989). In precocial species, the onset of NST at birth also requires the severance of the umbilical cord, or the removal of the inhibitory effect of placental prostaglandins PGE_2 and the onset of another prostaglandin, PGI_2 that lungs start to produce when breathing is established (Ball *et al.* 1995). BAT in precocials develops to a certain degree *in utero,* as shown by the presence of UCP_1 in adipose tissues of bovine and ovine foetuses (Casteilla *et al.* 1987, 1989). The sympathetic nervous system obviously contributes the differentiation of foetal BAT without cold stimulus (Casteilla *et al.* 1994), but also other signals may be involved (Nedergaard *et al.* 1986). The expression of UCP_1 in the adipose tissues of foetal sheep and cattle is very low but increases sharply at birth (Casteilla *et al.* 1987, 1989, Trayhurn *et al*. 1993a), which agrees with the fact that NST in these species peaks at birth (Alexander & Bell 1975, Alexander *et al*. 1975).

During postnatal development, the BAT in precocial species transforms into a tissue that has the characteristics of WAT, while the amount of UCP_1 decreases (Casteilla *et al.* 1989, Trayhurn *et al.* 1993b). These changes are in parallel with the postnatal decrease in the capacity for NST (Gemmel *et al.* 1972, Alexander *et al.* 1975) - a change that also occurs in reindeer (Soppela *et al*. 1986). It has not been clearly established whether the transformation of BAT to WAT is due to the atrophy of brown adipocytes and their replacement by white adipocytes, or due to the transformation of brown adipocytes to white adipocytes. The most important physiological factor maintaining the thermogenic capacity of BAT or the expression of UCP_1 is the cold-induced stimulation of the sympathetic nervous system via adrenergic β_3-receptors (Arch 1989, Himms-Hagen 1991). There is evidence in bovine calves that if raised in cold environments, the disappearance of UCP_1 and its mRNA are delayed (Casteilla *et al.* 1989). In the adults of precocial species, BAT is usually absent. However, the reconvertibility of BAT is, in principle, possible as indicated by the $ß_3$-adrenergic stimulation of the adipose tissues in adult

dogs (Champigny *et al.* 1991).

In the altricial species, BAT is present and active throughout the adult age (Himms-Hagen 1989). In addition to ontogenic growth, their BAT has a special growth that has been characterized by the term 'recruitment' (Nedergaard *et al.* 1986), and which results in the higher mitochondrial content of tissue (Lončar 1991) and a higher amount and activity of UCP_1 in mitochondria (Cannon & Nerdergaard 1985, Klaus *et al.* 1991) and thus increases the proportional significance of BAT in the metabolism of an animal. The main factor that recruits BAT is chronic or regular sympathetic stimulation (Himms-Hagen 1991). In addition to cold, overeating and a high-fat 'cafeteria' diet activate thermogenesis in rodent BAT (DIT, diet-induced thermogenesis) (Rothwell & Stock 1979) and act as a mechanism to burn off excess fat and regulate body weight (Himms-Hagen 1989). Diets containing a high proportion of PUFAs have been found effective in increasing DIT in BAT, and PUFAs have been proposed to stimulate thermogenesis either centrally or peripherally (Nedergaard *et al.* 1983, Sadurskis *et al.* 1995, Oudart *et al.* 1997). Fatty acids are the main fuel for thermogenesis in BAT, and it has been proposed that long-chain fatty acids or their acyl CoAs interact directly with UCP_1 and act as a signal for the activation of thermogenesis (Nicholls *et al.* 1986, Boss *et al.* 1998, Lowell & Spiegelman 2000).

BAT has been considered the only tissue in mammals exclusively capable of producing heat by NST. Recent studies have indicated that WAT and other tissues also contain uncoupling proteins (Boss *et al.* 1998). Three new uncoupling proteins have been found. These have been named UCP_2 (Fleury *et al.* 1997) or UCP homologue, UCPH (Gimeno *et al.* 1997), UCP_3 (Vidal-Puig *et al.* 1997) and UCP_4 (Mao *et al.* 1999). Of these, UCP_2 and UCP_3 are 73 % identical to each other in their amino acid sequence and both are 56 % identical to UCP_1 (Lowell & Spiegelman 2000). UCP_2 has been detected in several tissues of adult humans, including WAT and skeletal muscles, and in mouse BAT (Fleury *et al.* 1997, Gimeno *et al.* 1997), and has been proposed to uncouple oxidative phosphorylation from ATP synthesis in a similar manner as UCP_1 (Fleury *et al.* 1997, Gimeno *et al.* 1997). UCP_3 is abundantly present in skeletal muscles in humans, and it is present in both BAT and skeletal muscles in rodents (Vidal-Puig *et al.* 1997). Neither UCP_2 nor UCP_3 are expressed in avian tissues (Denjean *et al.* 1999). Recent studies have also shown the expression of UCP_1 in rat bone marrow cell line adipocytes (Marko *et al.* 1995). These results suggest that tissues other than BAT may also be capable of producing heat through facultative thermogenesis. However, the uncoupling activity of the newly found UCPs has not yet been established. Their quantitative significance for thermogenesis is still controversial, and it is an active area investigation.

2.3 Roles of white adipose tissue

WAT occurs in virtually all vertebrates, and the physiological basis for its presence has never been investigated thoroughly. Conversely, because of its diverse nature, WAT had been considered to occur almost universally in the animal body and had not been fully recognized as an anatomically organized tissue with site-specific properties until the past few decades (Wasserman 1965, Pond 1978, 1986, 1998, 1999). Although large variability in relative masses of depots and large individual and taxonomical differences are evident, a common pattern of a dozen or so adipose tissue depots can be found in all eutherian and metatherian mammals (Pond 1986). Unilocular white adipocytes are simple by their structure, store large amounts of TAGs during periods of energy excess and deliver fatty acids to other tissues as required. Despite of its simple structure, WAT performs numerous functions in the body that are related but not necessarily restricted with its anatomical locations, such as the provision for lactation (McNamara 1997).

The recently discovered secretory product of white adipocytes, leptin, has been proposed to play a role in the regulation of body weight and the total amount of adipose tissue in the body (Considine & Caro 1997, Schwartz *et al.* 2000). Leptin is expressed by the *ob* gene, which has extensive homology among vertebrates (Considine & Caro 1997). The original 'lipostatic' concept states that leptin is a hormonal substance that circulates in the blood and provides the brain with a signal about the amount of stored adipose tissue, thereby acting as a satiety factor (Kennedy 1953). Fasting and weight loss decrease the level of leptin in circulation, and weight gain and overfeeding increase it (Considine & Caro 1997, Trayhurn *et al.* 1998, Schwartz *et al.* 2000). The exact mechanism of the effect of leptin is not known. The sympathetic nervous system is proposed to play a key role in the regulation of leptin levels, possibly by downregulating leptin production via β_3-adrenoceptors (Trayhurn *et al.* 1998). Reduced blood leptin and insulin levels presumably increase the activity of anabolic neural pathways in the brain to boost appetite and feed intake, and thereby aim to restore energy homeostasis (Schwartz *et al.* 2000). Although WAT seems to be the main site of leptin production, leptin is also produced in other tissues, including BAT and placenta (Trayhurn *et al.* 1998), and may have versatile functions in the body.

Besides leptin, WAT also secretes a large number of other signals that affect energy homeostasis. These include pro-inflammatory cytokines, regulators of lipoprotein metabolism and growth factors, among others (Mohamed-Ali *et al.* 1998). Catecholamines, insulin and the sympathetic nervous system that modulate the adipocyte function also influence efferent signalling of adipose tissue (Mohamed-Ali *et al.* 1998, Trayhurn *et al*. 1998). WAT is thus increasingly being recognised as an active endocrine and paracrine organ, that closely interacts with other organs and tissues, and enables the organism to adapt to a wide range of metabolic challenges.

The lipolysis in WAT is catalyzed by hormone-sensitive lipase and stimulated by catecholamines, NA, and adrenaline (A) mainly via β_1-receptors (Hales *et al.* 1978). The lipolysis can be activated both by circulating

catecholamines and by NA from the sympathetic nerve endings. The sympathetic stimulation of WAT is thought to increase the lipolysis in situations such as exercise, stress and cold exposure (Hales *et al.* 1978, Garofalo *et al.* 1996), but sympathetic stimulation has been considered unlikely during starvation, when a fall in circulating insulin has been considered to be a major stimulus (Hales *et al.* 1978). Recently, it has been shown that prolonged fasting also induces sympathetic activity in WAT (Migliorini *et al.* 1997). The products of the lipolysis in WAT, or glycerol and FFAs are mainly delivered to the other tissues.

2.4 Polyunsaturated fatty acids

Most vertebrates are capable of synthesizing fatty acids containing either no (saturated) or one (monounsaturated) carbon-carbon double bond per molecule, but they cannot produce fatty acids containing two or more double bonds, or PUFAs. The two principal dietary essential fatty acids (EFAs) in vertebrates are linoleic acid (18:2n-6 or 18:2) and α-linoleic acid (18:3n-3) (Noble 1981, Innis 1991, Bruckner 1992). These fatty acids are not interconvertible but they can be further elongated and desaturated by the same enzyme systems into their respective n-6 and n-3 long-chain PUFA derivatives (Bruckner 1992). The principal EFAs and their derivatives are important for the foetal growth and development of young animals by being constituents of cellular membranes (Innis 1991, Bruckner 1992). The n-6 PUFAs possess the ability to eliminate pathogenic bacterial or microbial activity (Bruckner 1992) while very-long chain n-3 PUFAs are vital for the normal development and function of vision and brain (Neuringer & Connor 1986). Certain C20-PUFAs or eicosanoids provide the precursors for prostaglandins and leukotriens that have various physiological functions in the body (Bruckner 1992).

It has long been assumed that lipolysis produces fatty acids in the proportions in which they occur in the TAGs of the adipocytes (Spitzer *et al.* 1966), or are uptaken from the diet (Ekstedt & Olivecrona 1970). Recent studies have shown that proportionally more PUFAs than saturated fatty acids are released from adipose tissues during lipolysis (Gavino & Gavino 1992, Raclot & Groscolas 1993, Raclot *et al.* 1995, Connor *et al.* 1996). In addition, the shorter chain fatty acids (C14-C16) with one double bond are more likely to be hydrolysed than the longer-chain monounsaturated fatty acids. The biochemical mechanism for the preferential mobilization of unsaturated fatty acids is not known. One explanation that has been presented is that TAGs enriched by PUFAs tend to accumulate in the periphery of the TAG droplets and are therefore most susceptible to lipase action (Raclot & Groscolas 1995). So far, there is no evidence to indicate whether all adipocyte or adipose tissue types behave similarly to white adipocytes. However, site-specific differences in the release of unsaturated fatty acids from adipocytes near lymph nodes have been shown (Mattacks & Pond 1997), and there is evidence for the involvement of these fatty acids in immune responses (Pond & Mattacks 1998).

2.5 Ruminant lipids and undernutrition

During the first few postnatal weeks when their major feed is milk, newborn ruminants resemble monogastric animals in their digestive physiology and lipid metabolism (Noble 1981). Thereafter, the specialization to the use of poorly digestible vegetation occurs at a rate that varies between species but occurs in most domestic ruminants by the age of 4-6 weeks (Noble 1981). Ruminants are able to disrupt cellulose of low-quality forages by microbial fermentation in anaerobic conditions of rumen or in forestomachs anterior to the true stomachs (Christie 1981). As a result of fermentation, large amounts of volatile fatty acids are synthesized and they, rather than glucose, are the principal sources for lipid synthesis in ruminants (Ballard *et al.* 1969). The main source of lipid synthesis is acetate and the main sites of lipid synthesis are intestine and adipose tissue, and not the liver as in the non-ruminants (Christie 1981).

Dietary lipids do not directly affect the fatty acid composition of ruminant adipose tissues, as they do in non-ruminants (Christie 1981, Lin *et al.* 1993, Sarkkinen *et al*. 1994). Unsaturated fatty acids in the diet are hydrogenated in the rumen, and as a consequence, they are incorporated only in small proportions in adipose tissues that are characterized with a high proportion of saturated fatty acids (Christie 1981, Garton & Duncan 1971, Pond *et al.* 1993). In addition, the ruminant lipids are synthesized mainly endogenously from the lipid precursors produced by the rumen (Christie 1981). The exception of the low proportion of unsaturated fatty acids in ruminant WAT is the high proportion of unsaturated fatty acids in leg bone marrow (Meng *et al.* 1969, West & Shaw 1975, Turner 1979, Christie 1981, Pond *et al.* 1993). Moreover, there are high proportions of PUFAs in two major lipids of blood, cholesteryl esters (CEs), and phospholipids (PLs) in ruminants (Christie 1981, Noble 1981, 1984), Only a small amount of dietary PUFAs are esterified to circulating TAGs, or occur as the FFAs that are both minor lipids in ruminant blood (Noble 1984). The plasma of newborn ruminants contain very low proportions of the principal PUFAs, 18:2 and 18:3n-3, as compared to adult animals (Christie 1981, Noble 1981), obviously resulting from a low supply of these fatty acids during the foetal development (Elphick *et al.* 1979). Due to the high incorporation of PUFAs in structural lipids, ruminants are believed to withstand low dietary supplies of PUFAs rather well (Noble 1984).

There has been low interest in the fatty acid composition of ruminant adipose tissues as an indicator of nutritional status because they do not seem to clearly respond to dietary changes. There is evidence that unsaturated fatty acids and specific PUFAs are preferentially mobilized from the adipose tissues of non-ruminants such as fasting rodents (Chen *et al.* 1995) and fasting emperor penguins (Groscolas 1990), and this may be a mechanism for sustaining the PUFA metabolism and its related important functions during undernutrition (Chen & Cunnane 1992, Andriamampandry *et al.* 1996). In early stages, the decrease in dietary PUFAs in rodents and humans can be seen as significant reductions in the proportions of the principal PUFAs in serum lipids (Chen & Cunnane 1992, Leichsenring *et al.* 1995). The conditions that strongly decrease the supply of PUFAs - prolonged deprivation of food in particular -

could be expected to decrease the proportions of PUFAs in adipose tissues and serum lipids also in ruminants. The availability of lipids and PUFAs in young animals is important, e.g. for cellular growth (Innis 1991, Bruckner 1992), and their deficiency may impair steroid hormone synthesis (Pond 1998). A low supply of lipid precursors and PUFAs may contribute to retarding growth in freely ranging young ruminants during winter. If significant, the reductions in dietary PUFAs can have also other metabolic consequences.

3 Aims of the study

The main goal of this study was to identify the critical aspects of adipose tissue physiology and lipid metabolism in newborn and young semi-domesticated reindeer (*Rangifer tarandus tarandus* L.). The study focused on the first weeks of life when calves are most susceptible to cold stress and on the first winter when they may be undernourished. The specific objectives were:

1) to examine the functional basis of non-shivering thermogenesis in newborn reindeer by examining the presence of brown adipose tissue and its anatomical location, cellular structure, aerobic capacity, and fatty acid composition;

2) to identify brown adipose tissue and its major developmental stages by using both histological and biochemical criteria, including the expression of tissue-specific uncoupling protein;

3) to study the serum lipid and fatty acid composition in newborn reindeer and their postnatal changes, paying a particular attention to the proportions of polyunsaturated fatty acids and their supply from milk;

4) to study the effects of prolonged undernutrition in reindeer calves on serum lipid and fatty acid composition, in particular on the proportions of polyunsaturated fatty acids, and on the plasma leptin and insulin levels during winter, and

5) to study the effects of wintertime undernutrition on the fatty acid composition of leg bone marrow triacylglycerols in freely grazing calves and adult female reindeer.

4 Material and methods

4.1 Animals

The reindeer (*Rangifer tarandus tarandus* L.) were mostly from the experimental herd of the Finnish Reindeer Herders' Association in Kaamanen near Inari (69°N, 27°E) in the northeastern part of Finnish Lapland. Altogether, 42 reindeer between the ages of 2 weeks *pre partum* and 4.5 months *post partum* were studied for BAT anatomical location, histology and aerobic capacity (I), and fatty acid composition (the results are presented in Table 1). Some of the calves (17) were provided from the nearby reindeer herding co-operatives of Paistunturi, Muddusjärvi and Muotkatunturi. The majority of the calves had died accidentally (29), and 13 calves were slaughtered for the study. The calves that had died accidentally were examined within 48 hours of death and the others were examined immediately. Mainly the same material as in paper I was used to study for the presence of UCP_1 in adipose tissue samples (II). In addition, two red deer calves (*Cervus elaphus*) were provided for the investigations from a herd maintained at the Rowett Research Institute, Aberdeen, Scotland (57°N, 2°W).

The postnatal changes in serum lipid and fatty acid composition were studied in 0-20-day-old calves - 18 in total (III). The calves were kept with their mothers in the calving grounds of the Kaamanen herd. The study on the effects of prolonged experimental undernutrition on serum lipid and fatty acid composition was conducted in Kaamanen with 16 8-month-old reindeer calves (IV). Plasma leptin and insulin levels were also studied in this study (presented here in Fig. 1). The effect of wintertime undernutrition on the fatty acid composition of bone leg bone marrow fats (V) was studied in 34 freely ranging reindeer calves (< 1 y old) and adult females. A total of 16 reindeer in poor condition slaughtered in the Muddusjärvi reindeer herding co-operative (69°N, 27°E) were compared with 18 reindeer in good condition slaughtered in the Poikajärvi reindeer herding co-operative (67°N, 25°E).

4.2 Sampling

The experimental designs and sampling are described in detail in the original papers (I-V). Briefly, the calves in papers I-II were dissected and their adipose tissues were identified visually and sampled (I). The calves in the paper III were blood sampled within 8 h of birth and at about 3, 7, 14 and 20 days *post partum*. The calves were allowed to suckle milk freely from their mothers that grazed freely and were given supplementary concentrates during the calving period (I-III). The mothers were blood sampled and milked at 5-12 days after parturition (III). The calves in paper IV were divided into two groups and fed either with lichen (*Cladina* spp.) or with standard winter feed (Poron-Herkku, Raisio Feed, Finland). The lichen group was fed *ad libitum* for the first 5 weeks, followed by a 40 % restriction of energy for 8 weeks and refeeding for 6 weeks. The control group was given reindeer feed *ad libitum* throughout the experiment. The animals were weighed weekly and blood sampled at about 2-week intervals. The bone marrow samples in paper V were obtained in the common autumn and winter slaughters from the reindeer that had been freely grazing on natural pastures.

The animals were weighed with spring (I-II) or electronic balances (III-IV). The samples of adipose tissues for measuring aerobic capacity and fatty acid analyses were rapidly frozen on dry ice (-79°C) and those samples for the biochemical and molecular biological assays of UCP_1 in liquid nitrogen (-192°C) (I, II). The samples were stored frozen (-40°C) until analyzed. Blood samples were taken from jugular vein (*vena jugularis*) into vacuum serum and plasma tubes (III, IV). The animals were not anesthetized or treated with tranquilizers. For the lipid, other metabolite and fatty acid analyses, the blood samples were centrifuged and the plasma and serum samples were frozen (-40°C) (III, IV). Part of the blood was immediately deproteinized with perchloric acid and frozen for glucose and β-hydroxybutyrate (BHB) measurement (IV). The bone marrow samples were stored at -40°C until analyzed (V). The experiments were approved by the Finnish Game and Fisheries Research Institute's Committee of Animal Experimentation.

4.3 Feed analyses

The feed analyses are described in detail in the original papers (III, IV). The milk total lipid content was quantified using the standard Roese-Gottlieb extraction method; the crude protein content was quantified using the Kjeldahl method (Williams 1984). The gross energy was calculated according to the equation of Perrin (1958) adapted by Oftedal (1984). The fatty acid composition of milk TAGs (III) was analyzed by gas-liquid chromatography (GLC) using the Folch *et al.* extraction method (1957) modified by Moilanen

and Nikkari (1981) (Cf. 4.8).

The crude fat content of lichen (*Cladina* spp.) and concentrates (IV) was determined by acid hydrolysis extraction and the crude protein content by the Kjeldahl nitrogen method (Williams 1984). The amount of metabolizable energy (ME) and digestible fat was calculated by the specific energy and digestibility coefficients for ruminants (Schiemann *et al.* 1971, MAFF 1984). The total lipids of lichen and concentrates (IV) were extracted using acid hydrolysis and their fatty acids were transesterified with CH_3ONa methanol:diethyl ether method (Bannon *et al.* 1982). The methyl esters of the fatty acids were analyzed with gas-liquid chromatography (GLC) using a flame ionization detector and a fused silica capillary column with a temperature program (IV). The peak areas were quantified with a data collection program, and converted to percentage proportions of the sum of all fatty acids (weight-%) using the theoretical correction factors for the detector (Craske & Bannon 1988). The methyl esters of the fatty acids were identified using standard mixtures of methyl esters.

4.4 Anatomical and histological examinations

The precise anatomical location of each adipose tissue depot was determined systematically during autopsies (I). All visible adipose tissue from each location was dissected, fragments of connective tissue and muscles were removed, and fresh weight was determined (I, II). Thin sections (5-7 μm) of adipose tissues for light microscopic examination were stained according to Mayers' haematoxyline and eosine procedure (I). Samples for histofluorescence microscopy were frozen with liquid nitrogen and stored at -70°C. Thin sections (10-15 μm) were cut from frozen samples in a cryostat and treated with a sucrose-potassium phosphate-glyoxylic acid (SPG) (De La Torre 1980) to localize catecholaminergic nerve fibres (I). For transmission electron microscopy, thin sections were prepared from 1-2 mm^3 pieces of adipose tissues fixed with glutaraldehyde-formaldehyde mixture in a phosphate buffer, stained with uranyl acetate, and embedded in epon (I). The amount of mitochondria in adipose tissues (volume-%) was calculated from electron micrographs that were taken from randomly chosen areas of the thin segments (70 nm, magnification 4800 x).

4.5 Measurement of the aerobic capacity of adipose tissue

Succinate dehydrogenase (SDH) and cytochrome-c oxidase (COX) were chosen for marker enzymes of aerobic capacity in adipose tissues (I, II). SDH activity was determined from both the total homogenate and mitochondria (Kinnula *et al.* 1983) using phenazinemethosulphate and 2,6-dichloroindophenol as the electron acceptor system (King 1967). Maximal

activity was obtained by preincubating the samples at 37°C in a potassium succinate mixture (Kimura *et al.* 1967). COX activity was measured polarographically in the same adipose tissue samples that were used to determine the morphometric mitochondrial volume (I). The COX activity was measured in total homogenates at 25°C using a Clark-type oxygen electrode according to the method of Rafael *et al.* (1970) modified by Saarela *et al.* (1989). The COX activity of total homogenate was measured by the spectrophotometric method in paper II (Trayhurn *et al.* 1987).

4.6 Identification of brown adipose tissue by UCP_1 or its mRNA

UCP (UCP_1) was identified from the mitochondrial fraction of adipose tissues by an immunoelectrophoretic assay (II). The mitochondria were separated, and the total mitochondrial protein was measured with Folin and Ciocalteau's phenol method (Trayhurn *et al.* 1987). The mitochondrial proteins were solubilized in sodium dodecyl sulphate (SDS), separated according to molecular weight by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred onto nitrocellulose membranes by electroblotting (Milner & Trayhurn 1990, Milner *et al.* 1989). The membranes were probed with a rabbit anti(-ground squirrel UCP_1) serum (Milner & Trayhurn 1990), and the UCP_1-antibody complex was detected as a goat anti-rabbit immunoglobulin G horseradish peroxidase conjugate. Purified UCP_1 from the axillary BAT of Richardson's ground squirrel (*Spermophilus richardsonii*) was used as a reference protein. Mitochondria isolated from the perirenal BAT of a newborn lamb (*Ovis aries*) was also used as a reference. The sensitivity of the immunoblotting procedure for UCP_1 was 50-100 ng.

The perirenal adipose tissue of newborn red deer was probed for the presence of the mRNA for UCP_1 (northern blot) by using a 27-mer oligonucleotide (MacFarlane & Trayhurn 1990). The oligonucleotide was specific to a highly conserved region of the UCP_1 gene, and its sequence was obtained from comparing rat and cattle cDNA probes. The total RNA was extracted from the frozen tissue by a guanidinium isothiocyanate-phenol method (Chomczynski & Sacchi 1987), separated by agarose gel electrophoresis and transferred to a nitrocellulose membrane (Immobilon-N, Millipore) by capillary blotting. The 27-mer oligonucleotide was labeled with ^{32}P, incubated with the Immobilon-N membrane (Sambrook *et al.* 1990), and exposed after hybridization to autoradiographic film.

4.7 Blood biochemical analyses

The serum total lipids in paper III were determined with a spectrophotometric assay (Zöllner & Kirsch 1962) and serum TAG and total cholesterol were determined with enzymatic assays (Allain *et al.* 1974, Tiffany *et al.* 1974).

Serum phospholipids (PL) were determined with an enzymatic method from serum choline (Takayama *et al.* 1977).

A colorimetric method was used to measure blood glucose (Hyvärinen & Nikkilä 1962) and an enzymatic method was used to measure the blood BHB (Hansen & Freier 1978) in paper IV. The total lipid concentration of serum was determined by a colorimetric method (Epstein *et al.* 1972). Enzymatic, colorimetric methods were used to determine serum TAGs (Wahlefeld 1974), total cholesterol (Allain *et al.* 1974), and FFAs (Shimizu *et al.* 1980). The choline containing PLs were determined by an enzymatic method (Takayama *et al.* 1977). Serum glycerol was measured with a direct colorimetric procedure that utilizes a quinonamine chromogen system in the presence of glycerol lipase, peroxidase and glycerol phosphate oxidase. The prostaglandin $F_{2\alpha}$ metabolite (15-ketodihydro-$PGF_{2\alpha}$) concentration was measured from plasma using a radioimmunoassay (Kindahl *et al.* 1976, Granström & Kindahl 1982).

The plasma leptin concentration was assayed using a multi-species leptin radioimmunoassay kit (Linco Research, Inc., Cat. # XL-85K, Saint Louis, MO, USA). The antibody used in the kit has been raised against human leptin in the guinea pig but shows broad cross reactivity to the leptin molecules of many, but not all, species. 125I-Human leptin was used as a label reagent. The validity test for reindeer showed a linear correlation between the label and sample concentration. The unit of measure was $ng \cdot ml^{-1}$ as Human Equivalent ($ng \cdot ml^{-1}$ HE). A sensitive rat insulin radioimmuno kit from Linco Research (Cat. # SRI-13K, Linco, St. Louis, MO, USA) was used to measure plasma insulin. The kit utilizes an antibody made against rat insulin. The validity test of insulin for reindeer showed a linear correlation between the label and sample concentration.

4.8 Fatty acid analyses

Serum, milk, bone marrow, and BAT lipids (III, V, and Table 1) were extracted with methanol-chloroform using the method of Folch *et al.* (1957) and modified by Moilanen and Nikkari (1981). Serum TAGs, PLs and cholesteryl esters (CEs), milk TAGs, and bone marrow TAGs as well as BAT TAGs and BAT FFAs were separated using thin-layer chromatography (TLC), and their fatty acids were transesterified with H_2SO_4/methanol. Butylated hydroxytoluene (BHT, 0.005 %) was used as an antioxidant in the extraction of bone marrow and BAT lipids. The methyl esters of the fatty acids were analyzed with GLC using a flame ionization detector and a fused silica capillary column with a temperature program (III, V). The peak areas were quantified with an integrator and the methyl esters of fatty acids in each chromatogram were identified using retention times and standard mixtures of methyl esters. The results are presented as percentages of the sum of all fatty acids identified (weight-%).

The serum lipids in paper IV were extracted with dichloromethane-methanol (Folch *et al.* 1957). The PL and CE fractions were separated by TLC and

transesterified to methyl esters with acidic methanol (Stoffel *et al.* 1959). The methyl esters of fatty acids were analyzed by GLC using a flame ionization detector and a fused silica capillary column with a temperature program. The percentage proportions of the fatty acids were determined with a data collection program and normalized to 100-weight-%. The methyl esters of fatty acids were identified with standard mixtures of methyl esters and by analyzing the fractions from the $AgNO_3$ thin-layer chromatographic separations. As a control for the method, a pooled human serum sample was analyzed with each sample series (III, IV).

Fatty acids are designated by shorthand nomenclature of chain length: the number of double bonds, where n-x refers to the position of the last double bond relative to the terminal methyl end. All the data refer to the relative abundance of the fatty acids in the respective lipid fraction. The unsaturation index (UI), a measure of the degree of the unsaturation of tissues, was calculated as (% monoenoic + 2 (% dienoic) + 3 (% trienoic)...etc.) fatty acids. The fatty acid composition of BAT FFAs was compared with that of the BAT TAGs from which they, according to a hypothesis, had originated *in vivo*. The mobilization of FFAs from TAGs was evaluated according to Raclot & Groscolas (1993) by dividing the proportion of FFA by the proportion of TAG (% FFA · % TAG^{-1}), i.e. calculating the ratio of relative mobilization. A ratio greater, equal, or lower than one shows that the fatty acid is, respectively, more, equally, or less mobilized than the total fatty acids.

4.9 Statistical analyses

In papers I and III the groups were compared with the one-way analyses of variance (ANOVA), and as *a posteriori* tests either the LSD-test (I) or Student-Newman-Keuls test (III) were used. The immunoblotting results in paper II are semiquantitative and they were not tested. Repeated-measures ANOVA was used in paper IV and complemented with one-way ANOVA and the paired t-test. One-way ANOVA and the paired sample t-test were used in paper V. Simple regression analyses were performed with the method of least squares. Correlation coefficients were calculated with Pearson's correlation test.

5 Results

5.1 Anatomy, histology and aerobic capacity of adipose tissue

Adipose tissue with the characteristics of BAT in newborn reindeer had specific anatomical locations and wide distribution in the body (I). The largest of BAT were located in the perirenal-abdominal (32 %), inter(pre)scapular (18 %) and peri- and substernal locations (12 %). There was more BAT in locations within body cavities than outside them (66 vs. 34 %). The total amount of BAT in the newborn reindeer was on average 1 % of the body weight (60g/5kg). The adipose tissue of the foetal (ages 7 and 7.5 months) and older reindeer (1-4.5 months) was located in sites corresponding to those in the newborn reindeer. The calves aged 1 month (20 kg) had more subcutaneous and visceral adipose tissue than the newborns. The foetal reindeer had approximately 2 % adipose tissue of their total body weight and the calves aged 1 month had 0.2-1.6%.

Most adipose tissues of the newborn reindeer had the typical characteristics of BAT: abundant mitochondria, multilocular lipid vacuoles, and a dense network of capillaries. The adipose tissue in the foetal reindeer resembled BAT, whereas the adipose tissue in the older calves had the histological characteristics of WAT; adipocytes were almost unilocular and contained few mitochondria. Fluorescence microscopy showed green fluorescent staining to be typically located around the adipocytes in the newborn reindeer and mainly around arteries in the young reindeer. Based on histological features, the majority of the adipose tissues of the newborn reindeer were concluded to be BAT, except the subcutaneous adipose tissue in the occipital, orbital and caudal areas. During the first month of life, there was a progression in the BAT of the newborn reindeer towards the general histological characteristics of WAT.

The SDH and COX activity was highest after birth and decreased to an almost undetectable level during the first month (I, II). The morphometric mitochondrial volume was respectively highest in the newborn reindeer (40 %) and lowest in the calves aged 1 month (1-3 %). There was a significant positive

correlation between mitochondrial volume and the COX activity of adipose tissues ($r= 0.848$, $P<0.001$).

5.2 Identification of brown adipose tissue by UCP_1 or its mRNA

The results of the biochemical identification of BAT using the presence of UCP (UCP_1) as a criterion (II) were consistent with the results obtained by conventional anatomical and histological methods (I). Immunoreactivity consistent with UCP_1 was detected in most adipose tissue depots in the neonatal reindeer, i.e. perirenal-abdominal, inter(pre)scapular, sternal, intralumbar, vertebral, tracheal, inguinal, and omental locations, indicating that these tissues represent functional BAT (II). Only in the adipose tissue taken from the coronary groove was no immunoreactivity for UCP_1 detected. Strong immunoreactivity for UCP_1 was found also in adipose tissues taken from the inter(pre)scapular and perirenal locations of the foetal reindeer (ca. 2 weeks prepartum).

Immunoreactivity for UCP_1 was clearly evident during the first few days post partum, less so at 1 month of age and not at all at the age of 2 months or thereafter. The oldest reindeer studied were 3.5, 4.5 and 16 months old. The comparative analyses in the newborn red deer indicated the presence of both UCP_1 and its mRNA in perirenal adipose tissue at both ages studied, 2 h and 2 days.

5.3 The fatty acid composition of brown adipose tissue

There were no site-specific differences in the fatty acid composition of BAT TAGs between perirenal, inter(pre)scapular, peristernal and intralumbar locations in the newborn reindeer, and thus, perirenal BAT was chosen as a representative tissue (Table 1). The most striking feature of the fatty acid composition of BAT TAGs in the newborn reindeer was a high proportion of oleic acid, 18:1 (46.7 %) (Table 1). Other major fatty acids (each > 10 %) in the perirenal BAT were the palmitic (16:0) and stearic acids (18:0), and the most common minor fatty acids (each > 1%) were the myristic (14:0), 18:2 and palmitoleic acids (16:1).

The proportions of most fatty acids in BAT FFAs were significantly different from those of BAT TAGs (Table 1). In particular, the proportions of PUFAs such as 18:2, 18:3n-3 and 20:4n-6 were significantly higher in FFAs than in TAGs. The proportion of 18:1 in BAT FFAs was 1.5 times higher than the corresponding proportion in TAGs, and the proportions of 18:2 and 18:3n-3 were three and two-fold higher in FFAs than in TAGs. The proportion of 20:4n-6 was almost eight times higher in the FFAs than in TAGs. In accordance with their high proportions in BAT FFAs, 20:4n-6, 18:2 and 18:3n-3 had the highest relative mobilization from BAT TAGs, or 8.1, 4.2 and 2.5, respectively

(Table 1). There were no site-specific differences in the proportions of FFAs, neither were there any differences in their relative mobilizations between different BAT depots.

Table 1. The fatty acid composition of triacylglycerols (TAG) and free fatty acids (FFA) in the perirenal BAT of newborn reindeer (wt-%, mean ± SE), and the relative mobilization of FFAs from TAGs (% FFA · % TAG^{-1}) from which they, according to a hypothesis, had originated in vivo. Significant differences in the proportions of fatty acids between the two lipids are indicated with asterisks: $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ (paired t-test).

Fatty acid	TAG	FFA	Statistical difference	Relative mobilization
14:0	2.41 ± 0.31	1.68 ± 0.45	ns	0.71 ± 0.17
ai[a]-15:0	0.10 ± 0.02	0.13 ± 0.03	ns	1.33 ± 0.28
15:0	0.34 ± 0.11	0.14 ± 0.03	ns	0.56 ± 0.14
i[b]-16:0	0.11 ± 0.02	0.09 ± 0.01	ns	0.86 ± 0.15
16:0	26.18 ± 1.28	11.17 ± 1.13	**	0.43 ± 0.05
16:1 sum	1.42 ± 0.26	2.70 ± 0.14	**	2.05 ± 0.21
ai[a]-17:0	0.38 ± 0.05	0.33 ± 0.01	ns	0.95 ± 0.14
17:0	0.75 ± 0.07	0.35 ± 0.02	**	0.48 ± 0.05
17:1	0.22 ± 0.02	0.40 ± 0.02	**	1.89 ± 0.27
18:0	18.42 ± 3.01	6.40 ± 0.58	*	0.39 ± 0.07
18:1	46.70 ± 2.17	67.33 ± 2.46	**	1.46 ± 0.11
18:2	2.18 ± 0.47	6.13 ± 0.88	*	4.18 ± 1.98
18:3n-6	0.11 ± 0.02	0.02 ± 0.01	*	0.21 ± 0.15
18:3n-3	0.22 ± 0.05	0.45 ± 0.08	*	2.54 ± 0.75
18:4n-3	0.34 ± 0.10	0.46 ± 0.11	*	2.02 ± 0.80
20:4n-6	0.12 ± 0.01	0.90 ± 0.05	***	8.08 ± 1.07
SFA	48.10 ± 2.85	20.01 ± 1.85	**	0.43 ± 0.06
BCFA	0.59 ± 0.09	0.55 ± 0.05	ns	1.00 ± 0.15
MUFA	48.35 ± 2.27	70.44 ± 2.59	**	1.48 ± 0.11
PUFA	2.96 ± 0.61	7.97 ± 0.98	**	3.67 ± 1.48
n-3 PUFA	0.56 ± 0.14	0.91 ± 0.18	*	2.22 ± 0.79
n-6 PUFA	2.40 ± 0.49	7.05 ± 0.91	**	3.93 ± 1.56
N	5	5		5

Symbols: [a]ai = anteiso, methyl branched at the n-2 position, [b]i = iso, methyl branched at the n-1 position and ns = not significant.

5.4 Postnatal changes in serum lipid and fatty acid composition

The concentration of serum lipids was low in the newborn reindeer (<8 h) but the total lipids and cholesterol increased significantly during the first few days and PLs increased during the first week after birth (III). The principal serum lipid in the newborn reindeer was PLs whereas in the older calves the principal serum lipid was total cholesterol followed by PLs and TAGs.

The fatty acid composition of serum CEs of the newborn calves was significantly different from that of their mothers, especially the proportion of C18-PUFAs. The proportion of CE-18:2 in the newborn reindeer was only one fifth of the proportion in their mothers (11 vs. 49 %), and the proportion of 18:3n-3 was one sixth (0.4 vs. 2.3 %). The proportions of both of these principal PUFAs increased rapidly and significantly during the first few days of life: the proportion of CE-18:2 increased from 11 to 34 %, and the proportion of CE-18:3n-3 from 0.4 to 1.3 % during the same time (III). By the age of two weeks, the proportions of CE-18:2 and CE-18:3n-3 in the calves were at the same level as in the mothers.

The proportions of 18:2 and 18:3n-3 in serum PLs were also significantly lower in the newborn reindeer than the mothers. In addition, the proportions of long-chain PUFAs, 20:4n-6 and docosapentaenoic acid (22:5n-3), were significantly lower in the calves than in the mothers, but the proportion of docosahexaneoic acid (22:6n-3) was higher. The proportion of the principal C18-PUFAs increased significantly in serum PLs of the calves during the first few days after birth. The fatty acid composition of serum TAGs in the newborn reindeer resembled that of the milk TAGs. The proportions of oleic acid (18:1) and palmitic acid (16:0) were the dominant fatty acids in both. The proportion of 18:2 was 3 % of all TAG fatty acids of the milk and 2 % of its energy content. There were significant positive correlations between all serum lipids and milk TAGs.

5.5 The effect of undernutrition on serum lipid and fatty acid composition, plasma leptin, and insulin

Changes in body weight and in serum energy metabolites, FFA, and glycerol reflected the development of a negative energy balance in the reindeer fed with lichen (IV). The concentrations of major serum lipids, cholesterol, and PLs in the reindeer calves fed with lichen decreased significantly already during the *ad libitum* period (by 50 and 44 %, respectively). The proportion of major PUFA, or 18:2 in serum CEs decreased from 58 to 38 % during the *ad libitum* period ($P<0.01$), and to 29 % during the restriction ($P<0.01$). The proportion of 18:2 in PLs decreased from 28 to 16 % during the *ad libitum* period ($P<0.01$) and further to 13 % during the restriction ($P<0.01$). Also, 18:3n-3 in the CEs and PLs decreased significantly during the *ad libitum* and restriction periods. The decreases in major lipids and 18:2 were reversed during refeeding.

The control group, which was fed high-quality concentrates *ad libitum*, gained weight most of the spring but showed similar although slower decreases in the major serum lipids and PUFAs compared with lichen group. The body weight gain of the calves in the control group stopped in late January and February.

During the refeeding period, the compensatory growth of the lichen group started quickly. Although the mean daily weight gain during that period was even faster than in the control group (290 vs. 210 $g \cdot d^{-1}$), body weight did not reach that of the control group during the entire refeeding period, i.e. between April and June. The results indicated that feeding calves on lichen during winter led to the retardation of growth and significant reductions in major serum lipids and their principal C18-PUFAs (IV).

Plasma $PGF_{2\alpha}$ metabolite was used in the present study as a stress index. Large variations were found, but in general $PGF_{2\alpha}$ concentrations increased in the lichen group with the advance of undernutrition and were significantly higher than those of the control group.

Parallel with decreases in daily ME intake and body weight, plasma leptin and plasma insulin also decreased in the lichen group during the *ad libitum* period (Fig. 1). Insulin remained low throughout the 8 weeks restriction period, whereas leptin slightly increased by the end of the period. The leptin level of the lichen group was significantly lower than that of the control group during both the *ad libitum* and the restriction periods ($P<0.05$). During the refeeding period, the insulin level of the lichen group increased significantly, but leptin remained unchanged (Fig. 1). The plasma leptin of the control group decreased significantly during a period in midwinter (January-February, Fig. 1) when the body weight gain of these animals was depressed.

5.6 The effect of undernutrition on leg bone marrow fatty acid composition

Significant reductions were found in the proportions of the major monounsaturated fatty acid (MUFA), or 18:1, and in 18:2 and 18:3n-3 in the femur TAGs of the undernourished reindeer slaughtered in winter when compared with the reindeer slaughtered in good condition in autumn (V). As a result of these changes, the unsaturation index (UI) of the femur TAGs was reduced by 11 % in the both calves and hinds. Similarly, there were significant reductions in the proportions of 18:1 and 18:2 in the metatarsal TAGs in the undernourished hinds, but only in 18:2 in the calves. The UI of the metatarsal TAGs of the hinds was reduced by 7 % but that of the calves remained unchanged. The results suggest selective mobilization of 18:1 and the principal C18-PUFAs from bone marrow TAGs in the undernourished reindeer during winter.

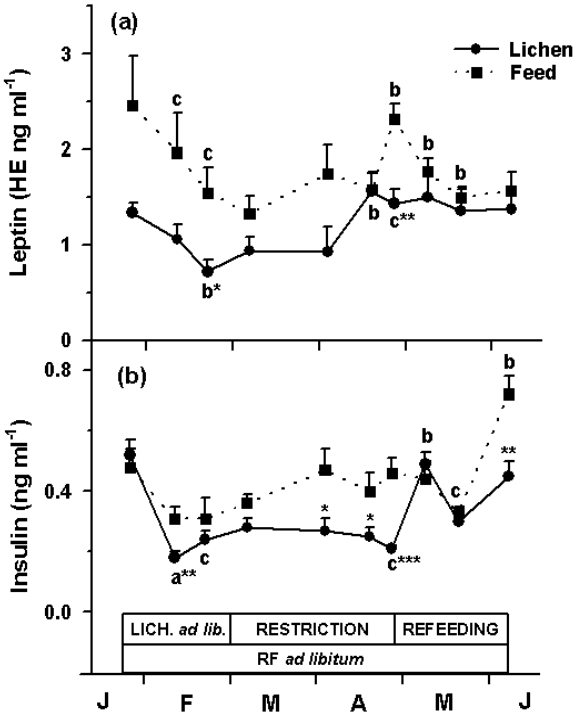

Fig. 2. Changes in the concentrations of plasma leptin (A) and insulin (B) in the calves fed with lichen at different levels ( ), and in the calves fed with reindeer feed (RF; control group) *ad libitum* during winter and spring. Significant changes within the groups during each experimental period are indicated by superscript letters: ^{a}P<0.001 and ^{b}P<0.01 and ^{c}P<0.05 (repeated-measures ANOVA, difference contrast). The leptin profile of the lichen group was significantly different than that of the control group during the *ad libitum* and restriction periods (P<0.05). The insulin profile of the lichen group was significantly different than that of the control group during the restriction and refeeding periods (P<0.01). Differences between the groups at different points were tested by one-way ANOVA. Significant differences found are indicated with asterisks: *P<0.05, **P<0.01 and ***P<0.001, placed above the point of the lichen group.

5.7 The major changes in specific unsaturated fatty acids of tissue lipids

The major reductions in the specific unsaturated fatty acids of various tissue lipids observed in the studies (Section 5.3 and IV-V) are summarized in Table 2. In addition to these changes, major increases occurred in both 18:2 and 18:3n-3 in serum CEs and PLs in suckling reindeer calves during the close perinatal period (III).

Table 2. Summary of the major reductions in the specific unsaturated fatty acids of various tissue lipids in reindeer calves under different physiological constraints in this study.

Tissue lipids	Physiological constraint	Fatty acids reduced/ mobilized	Potential use of fatty acids (of them all)
Brown fat TAGs	Thermogenesis	18:2 18:3n-3 20:4n-6	Substrate Activation of UCP_1
Serum CEs	Undernutrition	18:2 18:3n-3	EFA metabolism
Serum PLs	Undernutrition	18:2 18:3n-3	EFA metabolism
Bone marrow TAGs	Undernutrition	18:1 18:2 18:3n-3	Oxidation Cellular growth Immune responses

6 Discussion

6.1 Presence and significance of brown adipose tissue

The results of the present study showed that the majority of adipose tissue in the newborn reindeer is functional BAT. The adipose tissue in the newborn reindeer showed the typical cell morphological features of BAT characterized in various species (Néchad 1986). The appearance and amount of mitochondria was paid particular attention, but the multilocularity of fat was not used as a criterion as it varies greatly in both BAT and WAT (Cannon & Nedergaard 1985, Néchad 1986, Trayhurn 1993). In addition to the high mitochondrial volume, evidence for the typical spot-like sympathetic nerves around adipocytes, for high aerobic capacity and for the presence of brown fat-specific UCP (UCP_1) all support the conclusion that most of the regularly prominent adipose tissue in the newborn reindeer represents active BAT. Altogether, the results support the conclusion that BAT has a fundamental significance for the NST of the newborn reindeer and their survival in the cold during close postnatal period.

BAT was present as distinct depots in more than ten different locations in the body, corresponding largely to the locations described in other newborn ruminants, including lambs (Gemmel *et al.* 1972, Alexander & Bell 1975), bovine calves (Alexander *et al.* 1975) and muskoxen (Blix *et al.* 1984) and other large precocial species (Néchad 1986). The strategic distribution of BAT within the main body cavities and close to vital organs and blood vessels supports the distribution of heat produced by BAT both locally and on a whole-body basis. The proportional size and importance of BAT depots varies greatly between species (Nedergaard *et al.* 1986). The newborn reindeer had a substantial depot of BAT in the perirenal-abdominal region (1/3 of all) which is typical for ruminants (Alexander & Bell 1975, Alexander *et al.* 1975). This depot is obviously one of the major sites of NST in newborn reindeer as shown by its highest aerobic capacity. The third largest BAT depot located on both

sides of sternum beneath the pectoral muscles in the newborn reindeer has not previously been described in ruminants. All the depots classified as BAT occurred regularly in the data. However, the subcutaneous adipose tissue depots, which had no cellular morphology of BAT but resembled common WAT, were present only in some of the newborn reindeer.

Although BAT was the dominant type of adipose tissue in the newborn reindeer, its proportion of the body weight was only 1-2 %. This figure agrees with earlier findings in newborn ruminants and other large precocial species (Alexander & Bell 1975, Alexander *et al.* 1975). In small rodents such as guinea pigs, by contrast, the proportion of BAT can be 5 % of body weight (Néchad 1986). These species usually have considerably more WAT than ruminants. For comparison, the newborn human child has about 1-2 % of BAT but as much as 15-20 % of the WAT of their body weight (Lean & James 1986). In spite of the small amount of BAT, the thermogenesis of BAT contributes significantly to the metabolism and heat production of an animal. The thermogenic capacity of BAT (500 $W \cdot kg^{-1}$) is about four times that of muscles and about 300 times that of other tissues (Girardier 1983). Thus, stimulation of 1 g of BAT can double the basal metabolic rate of a rat and 30-60 g of BAT can increase the basal metabolic rate of a human child by 110-170 % (Lean & James 1986). In newborn reindeer, NA-induced NST results in a three-fold increase in maximal heat production at +10°C (Soppela *et al.* 1986). The distribution of heat is intensified by the lively blood circulation of BAT (Alexander *et al.* 1973).

During the first weeks of life, the BAT of the newborn reindeer transformed into a tissue with the general histological characteristics of WAT. These changes included the disappearance of mitochondria and spot-like symphathetic innervation while the adipocytes accumulated lipids and became gradually unilocular (I). Simultaneously, the aerobic capacity of the tissue decreased. A similar histological development has been reported in the lamb (Gemmel *et al.* 1972) and the bovine calf (Alexander *et al.* 1975) and it appears characteristic of precocial species during the postnatal period in a natural environment (Nedergaard *et al.* 1986). The changes both at cellular and biochemical levels are strikingly matched with a fall in the capacity for NA-induced thermogenesis during the weeks of life in reindeer (Soppela *et al.* 1986). The reduction in the capacity for NST is likely to reflect a decrease in the demand for thermoregulatory heat production**.** With age, both insulation and the surface to volume ratio improve and this diminishes the demand for extra heat. The postnatal inactivation of BAT and its conversion to WAT-like tissue can be delayed or reversed by the stimulation of the tissue by its sympathetic innervation which can be activated by cold exposure (Gemmel *et al.* 1972).

As the newborn reindeer had only little WAT, but this tissue appeared during later life to the identical locations as BAT, it is possible that these two tissues have the same origin or that they develop from the same preadipocytes. They can thus represent different forms of the same tissue, as has been suggested in the goat (Trayhurn *et al.* 1993b). Very little is known about the ontogeny of interconversion of the two types of adipose tissues.

The immunoblotting studies indicated that almost all adipose tissues in the newborn reindeer had immunoreactivity consistent with UCP_1 and were thus 'brown' by their nature (II). The results are in line with those results obtained by conventional methods, except for the coronary adipose tissue which had no UCP_1 and resembled BAT only by its cellular morphology (I). Identified BAT depots in the reindeer calves agree with the depots identified by the presence of UCP_1 in bovine calves and lambs (Casteilla *et al.* 1987, 1989, Trayhurn *et al.* 1993a), and goat kids (Trayhurn *et al.* 1993b). However, fewer depots were reported in these species than in the reindeer. In the present study, there were no subcutaneous adipose tissue samples for the immunoblotting studies of UCP_1 as it was so rare (I). Subcutaneous depots have been judged 'white' based on the missing immunoreactivity for UCP_1 in the newborn calf and lamb (Casteilla *et al.* 1987) but 'brown' based on the existence of UCP_1 in lambs and kids (Trayhurn *et al.* 1993a, b). Such contradictory results suggest that the tissues were not necessarily homologous. It is also possible that the 'phenotype' of this tissue varies according to its functional requirements.

Coronary adipose tissue was the only depot in the newborn reindeer that did not display UCP_1, or the definite biochemical criteria of BAT. Therefore, this depot is probably not essential for thermogenesis. Distinct coronary WAT is also found in adult reindeer, and this tissue exists in significant amounts in the Svalbard reindeer (Pond *et al.* 1993). The very few previous studies of the cardiac adipose tissue in mammals (Marchington *et al.* 1989, Marchington & Pond 1990) have suggested that this depot may have a special function to fuel cardiac muscle and/or mop up dangerous excesses of fatty acids in the blood. Well-developed thoracic BAT is characteristic to hibernators (Néchad 1986) that need to warm the heart from a very low beat when arousing from hibernation.

Developmental studies indicate that UCP_1 is present in foetal reindeer by late gestation, about two weeks *pre partum.* However, the precise stage at which the protein appears *in utero* was not determined. UCP_1 is detectable in the bovine calf at 80 days *pre partum* and its expression increases toward the end of gestation (Casteilla *et al.* 1987, 1989). Foetal reindeer therefore clearly have the potential for BAT thermogenesis and the development of the tissue *in utero* presumably ensures that the calf is well prepared for the large change in ambient temperature (30-60°C) that occurs at birth. The results from the experiments in which perirenal adipose tissues of newborn red deer were probed for the mRNA for UCP_1 indicate that the gene coding for the protein is strongly expressed around the time of birth. This result is in line with the findings for other newborn ruminants (Casteilla *et al.* 1987, 1989, Trayhurn *et al.* 1993 a, b) and rabbit (Rozon *et al.* 1989).

UCP_1 was present in most adipose tissues of reindeer during the first days after birth but there was a rapid loss of protein thereafter and protein was not evident at the age of 2 months (II). Similar results have also been found in other ruminants (Casteilla *et al.* 1987, 1989, Trayhurn *et al.* 1993b). The disappearance of UCP_1 confirms that the histologically recognizable conversion of BAT to WAT-like tissue clearly occurs at the functional level. The rates of the postnatal disappearance of UCP_1 in different depots were not

examined here in reindeer. In the goat kid, the disappearance of UCP_1 begins in subcutaneous adipose tissues (Trayhurn *et al.* 1993b). In the present study, the oldest animals were 6 month-old calves and adult reindeer that had undergone partial cold acclimatization during autumn and winter. No evidence for the presence of UCP_1 was found in their adipose tissues. This suggests that BAT does not reappear in older calves or in adult reindeer in natural conditions.

A prolonged, cold-mimicking β_3-adrenergic stimulation has not been found to re-induce UCP_1 in several adipose tissues of 1-year old reindeer calves although the sympathetic nervous system and lipolysis have been activated (Soppela, Trayhurn & Nieminen, unpublished observations). These findings suggest that there may be a strong inhibitory factor for UCP_1 gene expression in the adipose tissues of reindeer during winter that blocks the reconversion of BAT and dictates the 'normal' function of WAT. Reindeer are principally well adapted to even extreme cold due to their prime insulation (Scholander *et al.* 1950, Nilssen *et al.* 1984b) and their thermoregulatory costs appear to be substituted by heat generated from activity (Nilssen *et al.* 1984a). Therefore, reindeer do not probably need thermogenesis based on BAT, or such use of adipose tissues might be even wasteful. The reconvertibility of BAT in reindeer and similar large mammals in adult age is still an open question.

Anatomical and histological methods in the studies of BAT have been largely overruled by specific biochemical and molecular biological methods based on identifying UCP_1 and its mRNA. The present results show that active BAT can adequately be identified by a combination of electron and fluorescence microscopy supported by aerobic measurements. However, other, less active, fatty forms of BAT cannot be separated from WAT without the identifying UCP_1 or its mRNA (Trayhurn 1993). Accurate determination of the anatomical locations and the systematic description of adipose tissues is important for comparison of the biochemical properties of these tissues. This is particularly important in studies of adipose tissues which, exceptionally among the tissues of birds and mammals has largely missed the description and anatomical definitions that are foundation of comparative studies and the interpretation of any tissue (Wasserman 1965, Pond 1978, 1986, 1999).

One of the most interesting observations in the anatomy of the adipose tissues in reindeer was that the locations of BAT in the newborn reindeer and the locations of WAT in the older calves and adults were homologous. This supports the view of Lončar (1991) that there may basically exist only one type of adipose tissue in large mammals, convertible adipose tissue (CAT) which is 'brown' in the newborns and 'white' in the older animals, depending on the biochemical characteristics of its mitochondria. However, it is important to note that adults may have also many other adipose tissue depots in addition to those present in newborns. At least adult semi-domesticated reindeer and Svalbard reindeer have many large superficial depots (Pond *et al.* 1993) that were not present here in the newborn reindeer.

6.2 Postnatal changes in serum lipid composition

The results in paper III showed very low concentrations of serum lipids and low proportions of serum C18-PUFAs in newborn reindeer. The proportion of the principal serum n-6 PUFA, or 18:2 in adult domestic ruminants is normally highest in CEs (Christie 1981). In the newborn reindeer the proportion of CE-18:2 was only one-fifth of the corresponding proportion of their mothers. Also, the proportion of 18:3n-3 was very low in the newborn calves. Both 18:2 and 18:3n-3 are considered as dietary EFAs for vertebrates and crucial for the normal growth and development (Innis 1991, Bruckner 1992). The low proportions of C18-PUFAs found in the newborn reindeer match the results in other newborn ruminants (Christie 1981, Noble 1981) and agree with the limited free transfer of long-chain fatty acids across the placenta in ruminants (Christie 1981, Elphick *et al.* 1979). The low proportions of C18-PUFAs in the newborn reindeer also suggest that there is an immediate requirement for these fatty acids from the diet during the period after birth.

Interestingly, 20:4n-6 and other long-chain PUFAs like 22:6n-3 were present in quite moderate proportions in the serum PLs of the newborn reindeer. It is possible that these fatty acids had been preferentially transported across the placenta from maternal circulation. In the human foetus, the long-chain PUFAs are preferentially transported across the placenta by a specific plasma membrane fatty acid -binding protein system (FABP) (Dutta-Roy 1997) and such a protein has been identified in sheep (Campbell *et al.* 1994). There is preliminarily evidence of such a fatty acid-binding system also in reindeer (Soppela *et al.* 1999). The capability of the placenta for the preferential transportation of long-chain PUFAs and their synthesis would be particularly important for foetal reindeer, as these fatty acids and their precursors are low in the major winter diet of reindeer, lichen (IV). An adequate supply of long-chain PUFAs is critical for the foetus as these fatty acids, particularly 22:6n-3, are essential for the development of brain and retina (Neuringer and Connor 1986).

The low postpartum serum lipid levels and their rapid rise in the reindeer during the neonatal period agree with those documented in other species (Noble 1981, Stammers *et al.* 1987) and reflect the sudden change that occurs at birth; from the use of carbohydrates as a major energy source to the use of lipids. The reindeer calves get about 60 % of their milk energy as lipids, with the majority of the milk TAGs consisting of saturated fatty acids (SFAs) which obviously serve primarily as a source of energy. The low proportion of PUFAs, like that of 18:2 in milk TAGs (3 wt-%), probably reflects its low proportion in reindeer adipose tissues (Garton & Duncan 1971). The fatty acids of the maternal adipose tissue are an essential source of milk fatty acids, regardless of the energy balance of the mother (McNamara 1997). The proportion of milk 18:2 was in the same range found during peak lactation in reindeer or other ruminants (Luhtala *et al.* 1968, Oftedal 1984) but much less than in non-ruminants such as horses (20 %, Stammers *et al.* 1987) or humans (5-15 %, Jensen 1996). Despite their low supply in diet, the proportion of the principal PUFAs, mainly 18:2, increased significantly in serum CEs and PLs during the first few days after birth. The results agree with those obtained in freely

suckling bovine calves and lambs (Noble 1981), and suggest a highly efficient absorption and retention mechanism of 18:2 and other principal PUFAs from milk lipids. This mechanism may include an increased activity of plasma lecithin-cholesteryl acyl transferase (LCAT), an enzyme that has a high affinity to 18:2 (Noble 1981) and contributes to CE synthesis in the neonatal lamb (Christie 1981). Such a mechanism may confirm a particular survival advantage for reindeer during the critical postnatal development by ensuring that calves get sufficient amounts of specific PUFAs for their rapid growth and development.

In addition to their roles in cellular growth and the provision of the immune system (Bruckner 1992, Pond 1998), certain PUFAs such as 18:2 and 18:3n-3 may play also a role in the activation of thermoregulatory heat production, or NST in BAT (Nedergaard *et al.* 1983, Sadurskis *et al.* 1995, Oudart *et al.* 1997). As newborn reindeer possess very little WAT (I), they are more dependent on mother's milk as a primary fatty acid source for thermogenesis than species that can use also their WAT fatty acids. The proportion of 18:2 and other PUFAs measured here in the BAT TAGs of the newborn reindeer were not particularly high, but closely resembled that of the milk TAGs. However, when the relative mobilization of different fatty acids (% FFA · % TAG^{-1}) was estimated, 20:4n-6, 18:2 and 18:3n-3 had the highest relative mobilizations from BAT TAGs, respectively (Soppela, unpublished). The high mobilization of these PUFAs from BAT TAGs suggests that they are metabolized rapidly, and thus their continuous supply from the diet may be essential for thermogenesis (Table 2). The present results support the view that the lipolysis and release of fatty acids from adipose tissues is not a random process but favors long-chain and unsaturated fatty acids (Raclot & Groscolas 1993, Raclot *et al.* 1995, Connor *et al.* 1996). Since 18:2, 18:3n-3 and 20:4n-6 are minor fatty acids in the BAT TAGs of newborn reindeer, they are probably not the principal substrates of thermogenesis. However, they may act as stimulators of the NST mechanism. FFAs or their acyl derivatives are believed to act as signals for the activation of UCP_1 in BAT mitochondria (Nicholls *et al.* 1986, Boss *et al.* 1998, Lowell & Spiegelman 2000). Knowledge about this mechanism is still obscure, and to the best of my knowledge, the significance PUFAs for the activation of UCP_1 has not been studied.

The first few days *post partum* appear, on the basis of the present results, the most critical period in respect to the serum PUFA status of newborn reindeer. The low proportions of the principal PUFAs in serum lipids of the newborn reindeer suggests that the calves may be less protected against cold stress and infections if there are disturbances in the lactation or if the improvement of the serum PUFA status is delayed. During summer, high ambient temperatures combined with a dense insect plague are likely to greatly challenge the immune systems of the calves. Reindeer calves are sensitive to heat stress, as shown by a rise in body temperature and an increase in heat production and heart rate at high temperatures (>+20°C) (Soppela *et al.* 1986). High ambient temperatures (>+30°C) have been found to severely disturb the improvement of PUFA status in tissues of growing ruminants (Noble 1981). Since reindeer calving peaks in mid-May in Finnish Lapland, most of the calves probably reach high PUFA

levels by midsummer. Improvements in the milk PUFA supply could benefit the survival and well being of newborns during the critical postnatal period. However, the manipulation of the maternal diet by including PUFAs would require the protection of the feed lipids from rumen biohydrogenation.

6.3 The effects of undernutrition on lipid composition and metabolism

The prolonged undernutrition in reindeer calves on lichen led to significant decreases in body weight, serum lipids and their principal PUFA proportions (IV). The decreases in body weights agree with the development of negative energy balance in reindeer feeding on lichen in captivity (Soveri *et al*. 1992) and on natural pastures during winter (Reimers *et al*. 1983, Nieminen *et al.* 1984). The body weight losses were comparable with those in reindeer in natural conditions during a normal winter (Timisjärvi *et al.* 1982), and can be taken to represent moderate undernutrition.

Consistent with previous studies (Ryg & Jacobsen 1982, Larsen *et al.* 1985, Suttie & Webster 1995), body weight gain also stopped in late January and February in the control group, although they had an unlimited access to high quality food. This implies that factors other than feed, such as the effects of a short day-length (cf. Suttie & Webster 1995) or low ambient temperatures may also regulate the feed intake and body weight of reindeer during winter. The present results of the parallel decreases in serum leptin and insulin levels with decreasing body weights imply that they may also contribute to the regulation of appetite and the body weight cycle in reindeer (Soppela, Saarela, Heiskari & Nieminen, unpubl.) as in other species (Considine & Caro 1997, Schwartz *et al*. 2000). The leptin production of white adipocytes decreases with fasting and cold exposure (Trayhurn *et al.* 1998). The present results support the view that insulin may regulate leptin secretion (Schwartz *et al*. 2000). Decrease in insulin was not apparently so high that it would have affected on degradation of muscle proteins, as shown by results of a parallel study in the same animals (Pösö *et al.* 1999).

Serum total lipids, cholesterol, and PL concentrations decreased sharply in the reindeer fed with lichen during the *ad libitum* period, and remained low throughout the restriction period (IV). The reductions in total lipids and cholesterol agree with those found during winter in reindeer and other ungulates (Nieminen *et al.* 1984, Larsen *et al.* 1985, DelGiudice *et al.* 1987, Soveri *et al.* 1992). They most probably reflect the small supply of lipid precursors, or volatile fatty acids from the diet. The availability of volatile fatty acids such as acetate is crucial for the *de novo* synthesis of cholesterol and other lipids in ruminants (Bell 1981), as they are generally not obtained from the diet as in non-ruminants. The serum lipid concentrations here were so much reduced that they resembled those found in newborn reindeer during the immediate perinatal period (III). Interestingly, serum cholesterol and PLs also decreased in the control group, but more slowly than in the lichen group, or

between January and April. Because the control calves gained weight during most of this period, or from February on, their lipid decreases are likely to reflect their increased lipid requirements rather than seasonal factors. Lipids are required for the synthesis of cell membranes and lipids-based hormones. Cholesterol, for example, is a precursor of steroid hormones such as testosterone, which is a well-known growth booster (Ganong 1987).

The most striking observation in the present study was a large decrease in the proportions of the 18:2 and 18:3n-3 in serum CEs and PLs in the reindeer fed with lichen during the *ad libitum* and restriction periods (IV). The reductions of n-6 PUFAs, including the 18:2 we observed in the reindeer calves in CEs, were strikingly similar to those found in children who suffered from severe energy-protein malnutrition (Leichsenring *et al.* 1995). The decreases in the proportion of PL-18:2 in the reindeer also resembled those reported in the serum PL of fasting rats (Chen & Cunnane 1992). The present results also fit the overall picture that the principal PUFAs of serum total lipids in freely grazing reindeer are lowest during winter (Väyrynen *et al.* 1980). Although the proportions of 18:2 and 18:3n-3 in serum CEs and PLs were decreased, this does not seem to represent a classical EFA deficiency (cf. Bruckner 1992) because the level of 20:4n-6 was increased. The reason for the increase of 20:4n-6 is unclear, but it may be related to the parallel increase in one of its C20-derivative, plasma prostaglandin $PGF_{2\alpha}$. The immediate precursor of PG synthesis is free 20:4n-6 and other C20-PUFAs called eicosanoids (Bruckner 1992). Prostaglandins are a group of local hormones involved in various physiological functions in the body (Bruckner 1992).

The levels of serum PUFAs in non-ruminants such as humans depend on their availability in the diet (Sarkkinen *et al.* 1994). This relationship is much more complicated in ruminants, because rumen microorganisms disrupt and modify dietary lipids, thus decreasing their availability to the host animal (Christie 1981, Noble 1984). However, the most probable explanation for the decreased proportions of the principal serum C18-PUFAs in reindeer is that their low dietary supply did not meet their bodial requirements (Table 2). The fatty acid analysis confirmed that the lichen species (*Cladina* spp.) commonly used by reindeer during winter are low in 18:2 and other PUFAs. The rumen protozoa can decrease the absorption of dietary PUFAs by incorporating them into their cellular membranes (Harfoot & Hazlewood 1988) and this may have happened in the reindeer in the present study. The principal C18-PUFAs in serum CEs and PLs decreased during winter and spring also in the control calves, which obtained more PUFAs including 18:2 from their feed than the lichen group. The reductions in the control calves were obviously due to the high PUFA requirements for growth in these animals that were not fully satisfied by the diet.

In general, the reindeer calves appeared to tolerate deprivation of the dietary essential PUFAs, such as 18:2, rather well. How do they cope with this situation? Reduced feed intake, and subsequently lowered resting metabolic rates (Nilssen et al. 1984b), may decrease the immediate needs for PUFAs. In addition, the depletion of PUFAs from the adipose tissues presumably plays a role in providing PUFAs for circulation. The preferential release of PUFAs

from adipose tissues (e.g. Raclot *et al.* 1995) can be beneficial for the formation of the vital PUFA-enriched metabolites during the deprivation of dietary PUFAs (Chen & Cunnane 1992, Andriamampandry *et al.* 1996). It has been proposed that the high level of circulating FFAs (Hasselblatt *et al.* 1971) or high proportions of PUFAs in membranes (Andriamampandry *et al.* 1996) may affect positively the body protein sparing during starvation. Adipose tissues in reindeer and other ruminants contain small amounts of PUFAs (Garton & Duncan 1971, Christie 1981), and may constitute a relatively poor source of PUFAs during undernutrition. Besides to their own tissues, one potential source of PUFAs in ruminants can be degenerated rumen microbes. Rumen protozoa, for example, contain a moderate proportion of 18:2 of dietary origin (Harfoot & Hazlewood 1988). The rumen microbial population may also be able to synthesize the principal PUFAs *de novo* and thus provide them to the host animal. However, there is no evidence for such a synthesis in the rumen, and PUFAs in ruminal microbes are believed to be the result of the exogenous uptake of these fatty acids (Harfoot & Hazlewood 1988).

When the undernourished calves were refed, their serum PUFA proportions were rapidly reversed to the levels reported in well-nourished adult domestic ruminants (Christie 1981). The mechanism for the rapid compensatory increase in serum PUFAs is unclear, but it refers to the existence and activation of a similar efficient retention mechanism as in newborn calves (III). Such a mechanism, again, can provide a great survival value for the reindeer.

Bone marrow TAGs are the last to be mobilized when ungulates starve (Ransom 1965, Nieminen & Laitinen 1986, Davis *et al.* 1987, Wolkers *et al.* 1994). Previous studies had shown a high proportion of unsaturated fatty acids such as 18:1 especially in the distal parts of the legs in the conspecies of reindeer and other ungulates (Meng *et al.* 1969, West & Shaw 1975, Turner 1979, Pond *et al.* 1993), and also in the extremities of other terrestrial species (Käkelä & Hyvärinen 1996). Whether this characteristic is maintained in undernourished reindeer is interesting because the high degree of unsaturation and low melting point of the extremities are related (Irving *et al.* 1957) and are believed to maintain the fluidity of the fats in the cold (Meng *et al.* 1969, West & Shaw 1975, Turner 1979). The results indicated that undernutrition in freely ranging reindeer during winter is accompanied with significant reductions in the proportions of 18:1, 18:2 and 18:3n-3 in bone marrow TAGs. The proportions of 18:1 decreased in both the femur and metatarsal bone marrow of the hinds, but only femur TAGs in the calves (V). These results suggest either selective mobilization of 18:1 or its decreased synthesis during the development of undernutrition.

The mobilization hypothesis fits well with the evidence that unsaturated fatty acids are preferentially released from adipose tissue TAGs during lipolysis (Gavino & Gavino 1992, Raclot & Groscolas 1993, Raclot *et al.* 1995, Connor *et al.* 1996). The released 18:1 would be used for fuel or other purposes such as for the formation of new cells (Table 2). The fatty acid composition of femur marrow closely resembles that of the adipose tissue in reindeer, with 18:1 being the major fatty acid (Garton & Duncan 1971, Pond *et al.* 1993). There is evidence that 18:1 is released from subcutaneous adipose tissues of another

ruminant, sheep, during prolonged fasting (Christie 1981). The use of bone marrow TAGs as a source of energy for the whole body consumption probably occurs during the last stage of starvation when the major shift from lipid to protein catabolism occur (Thouzeau *et al.* 1997). Another explanation for the lower 18:1 proportions in bone marrow TAGs would be lower synthesis of 18:1 or its precursor 18:0 on a low plane of nutrition during winter.

The decreased proportions of 18:2 and 18:3n-3 in the bone marrow TAGs of the undernourished hinds and calves are likely to indicate the increased mobilization of these fatty acids during undernutrition because they cannot be synthesized in the body (Bruckner 1992) and they are low in lichens (IV). The selective reductions of these PUFAs support the view that PUFAs are most preferentially mobilized from adipose tissues during lipolysis (Raclot & Groscolas 1993, Raclot *et al.* 1995, Connor *et al.* 1996). The reductions of 18:2 and 18:3n-3 in the bone marrow TAGs of the undernourished reindeer resemble the selective reductions in the proportions of these fatty acids in the adipose tissue TAGs in growing rats as a response to fasting and subsequent refeeding (Chen *et al.* 1995). Similar reductions in 18:2 and 18:3n-3 have been found in total lipids of WAT in undernourished reindeer (Soppela & Nieminen, unpublished observations). The limited PUFAs may not have been used primarily as an energy source (Table 2). Instead, the PUFAs may have been used for the synthesis of cellular phospholipids in lymphopoiesis and hematopoiesis (Dorshkind 1990, Gimble *et al.* 1996) or they may have participated in the provision of the immune system also by providing the precursors for eicosanoid synthesis (cf. Pond 1999). The striking similarity in the reductions of 18:2 and 18:3n-3 in the major serum lipids (IV) and bone marrow lipids (V) suggests that within each tissue and age group, 18:2 and 18:3n-3 may be used as possible 'biomarkers' of the undernutrition in reindeer.

The most striking difference in the fatty acid composition of bone marrow fats as related to age of reindeer was the lower proportion of 18:1 in the calves as compared with the hinds, especially in the metatarsal TAGs (ca. ¾), which obviously reflect the differences in their nutrition or metabolism. The activity of the desaturase that converts 18:0 to 18:1 is usually lower in young than in adult ruminants (Christie 1981). The low proportion of 18:1 in the calves suggests that either the desaturation of 18:1 is lower in the calves than the adults or that the biosynthesis of its precursor, 18:0, is lower. The calves may also have allocated their 18:1 for the growth of the other tissues than adipose tissues. The low 18:1 proportion of the calves implies that the legs of the calves may have less fluid fats than the legs of the adults.

Surprisingly, the proportion of 18:1 in the metatarsal TAGs in the undernourished calves was similar to that in the calves in good condition. Calves are usually considered more sensitive to nutritional constraints than adults (Soveri *et al.* 1992). An explanation for the stability of 18:1 in the metatarsal TAGs in the calves may be that it was selectively retained, and this could be a mechanism by which the calves could maintain a certain degree of unsaturation and hence the fluidity of the fats in the distal parts of their legs.

Altogether, the reductions in the UI of the bone marrow TAGs of the undernourished reindeer were not large. However, taking into account the

relative stability of the fatty acid composition of the ruminant storage fats (Christie 1981), they can be considered prominent. The results of the apparently selective reductions of 18:1 and the principal C18-PUFAs in reindeer during prolonged winter undernutrition agree with the hypothesis of Irving *et al.* (1957) that while the high proportion of the unsaturated fatty acids in the bone marrow of arctic mammals is useful in the cold, it is probably not an exclusive adaptation to the cold climate. The maintenance of tissue fluidity is important not only for maintaining leg bone marrow adipocytes soft in the cold; it also enables their fatty acids to be mobilised. The capacity to withdraw fatty acids from bone marrow to support general metabolism seems to be a special feature of ungulates, especially ruminants. In rabbits, and probably also in rodents, bone marrow lipids are not depleted even in severe starvation (Bathija *et al.* 1979).

If advanced, the reduction of the degree of unsaturation of the bone marrow TAGs may raise the melting point of the fats and impair their fluidity. The reductions in the PUFAs in reindeer suggest that bone marrow adipocyte TAGs may have special functions in providing these fatty acids to the other tissues. In total, the results show that the characteristic fatty acid composition of the bone marrow TAGs may be modified during prolonged undernutrition according to local or systemic requirements.

7 Conclusions

This study shows that the reindeer, as most other precocial mammals, have functional BAT at birth. The great majority of the adipose tissues of newborn reindeer are functionally 'brown', occupy specific anatomical locations in the body and have a highly specialized cellular apparatus for heat production. Consequently, BAT is likely to provide a major functional basis for NST in newborn reindeer and contribute substantially to their survival in the cold.

BAT is most active in reindeer at birth and during the first days, but its capacity for thermogenesis declines during the first month while it adopts the histological characteristics of WAT.

The low proportions of linoleic acid and other C18-PUFAs in the serum lipids of newborn reindeer indicate an immediate and high requirement for the principal C18-PUFAs during the period just after birth. Although the proportion of 18:2 is low in the mothers' milk, its proportion increases in the serum lipids of the calves during the immediate perinatal period at a rate that refers to the existence of a highly efficient retention mechanism.

As newborn reindeer possess very little WAT, they seem to depend on their diet or mother's milk as a primary source of fatty acids for thermogenesis in BAT. The high relative mobilization of 20:4n-6, 18:2 and 18:3n-3 from BAT TAGs suggest that these fatty acids are metabolized rapidly, and that their continuous supply may be essential for thermogenesis. Thermogenesis using BAT can thus be rather expensive in terms of dietary PUFAs, which are also required for growth.

The prolonged feeding of reindeer calves with lichen during winter and spring, even *ad libitum*, leads to significant reductions of body weight and major serum lipids and their principal C18-PUFA proportions. The calves appear to tolerate a low dietary supply of PUFAs rather well. However, the reductions in serum lipids and PUFAs in the calves fed with high-quality concentrates *ad libitum* show that early growth greatly increases dietary PUFA requirements. Decreases in serum leptin and insulin levels with decreases in body weights imply that these factors may contribute to the regulation of appetite and the body weight and body fat cycle.

The results of the apparently selective reductions of 18:1 and the principal

C18-PUFAs in bone marrow TAGs in reindeer as a result of winter-time undernutrition suggest that specific unsaturated fatty acids are preferentially mobilized from bone marrow adipocytes and used according to either systemic or local requirements.

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