Ornis Fennica 76:151–165. 1999

Regulatory, cellular and molecular aspects of avian muscle nonshivering thermogenesis

Claude Duchamp, Florence Marmonier, Frédérique Denjean, Joël Lachuer, Tristram P. D. Eldershaw, Jean-Louis Rouanet, Anne Morales, Roger Meister, Christophe Bénistant, Damien Roussel & Hervé Barré

Duchamp, C., Marmonier, F., Denjean, F., Lachuer, J., Eldershaw, T. P. D., Rouanet, J.-L., Morales, A., Meister, R., Bénistant, C., Roussel, D. & Barré, H., Lab. Physiologie des Régulations Energétiques, Cellulaires et Moléculaires, UMR 5578 CNRS-Univ. Cl. Bernard Lyon 1, Fac. Sciences, Bat 404, 43, Bld du 11 Nov. 1918, F-69622 Villeurbanne, France. E-mail: duchamp@cismsun.univ-lyon1.fr

Received 14 August 1998, accepted 10 August 1999

Regulatory, cellular and molecular aspects of muscle avian nonshivering thermogenesis are reviewed. The endocrine control of muscle NST may involve an interaction between several hormones including glucagon, catecholamines and thyroid hormones. The metabolic, central, vascular or genomic actions of these hormones have been investigated at several levels, *in vivo* and *in vitro* using perfused avian muscle preparations. Two main thermogenic processes may account for avian muscle NST. The first one, which is based on an uncoupling of mitochondrial oxidations and phosphorylations, is supported by experimental data using isolated mitochondria, perfused skeletal muscle and efficiency of locomotor activity in vivo. The second mechanism, which involves an increased ATP-dependent sarcoplasmic reticulum Ca^{2+}-cycling, is supported by biochemical analysis of the activity and expression of SR proteins. The molecular basis of the two processes have been investigated and fatty acids or their derivatives may play an important role in their control. Muscle NST is fuelled by coordinated increases in fatty acid supply from adipose tissue, cellular uptake by lipoprotein lipase, and intracellular fatty acid transport capacity by small cytosolic proteins fatty acid-binding protein (FABP). The control at the gene level of the functional adaptations of skeletal muscle during cold acclimation should now be investigated.

1. Introduction

Survival of endotherms in the cold critically depends on their ability to sustain elevated levels of heat production for long periods in order to compensate for heat losses to the environment and keep their body temperature constant. In the short-term, most endotherms rely primarily on shivering for regulatory thermogenesis. The metabolic processes activated by muscle contraction (myosine- and cation-ATPases, mitochondrial synthesis of ATP) indeed generate heat because of their rather low efficiency. In the long-term, regulatory thermogenesis can also be achieved through the development of nonshivering thermogenesis (NST), a process which generates heat independently

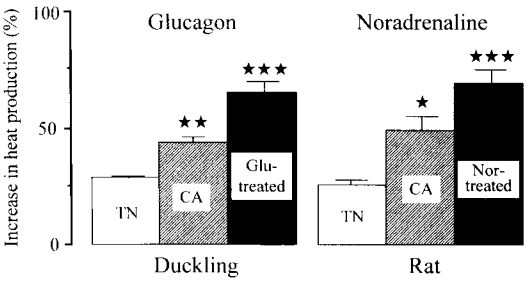

Fig. 1. Comparison of the relative thermogenic effects of glucagon (360 μg.kg^{-1}) in ducklings and noradrenaline (200 μg.kg^{-1}) in rats. Animals reared at thermoneutrality (TN) are compared with cold (4°C, 3–5 wks) acclimated animals and animals kept at thermoneutrality but receiving daily intraperitoneal injections of either glucagon in ducklings or noradrenaline in rats. Duckling data are from Barré et al. (1987) and rat values from Leblanc and Pouliot (1964). Values are expressed as percentage of increase above resting metabolic rate at thermoneutrality. Means ± SEM. ** $P < 0.01$ vs TN; *** $P < 0.001$ vs TN.

from contractile activity and which is considered to be the main characteristic of cold-acclimation (Jansky 1973).

In small mammals, NST has been mostly associated with brown adipose tissue (BAT), a specialized heat-producing tissue. It is now well recognized that BAT NST is based on an uncoupling of the mitochondrial respiratory chain by the proton-translocator uncoupling protein (Nicholls et al. 1986) and is mainly under the control of noradrenaline released by sympathetic nerves innervating BAT. The importance of BAT in mammalian NST led to the generalisation that without BAT there was no NST.

Birds are endotherms which do not posses BAT (Johnston 1971, Barré et al. 1986a, Olson et al. 1988, Saarela et al. 1989, 1991). Although cold-acclimated birds have a multilocular fat tissue (Barré et al. 1986a, Olson et al. 1988, Saarela et al. 1989) with an increased blood flow after cold stimulation (Duchamp & Barré 1993), this tissue does not have the number of mitochondria, the Krebs cycle enzymes, the cytochromes (Barré et al. 1987a, Olson et al. 1988, Saarela et al. 1989), the uncoupling protein (Saarela et al. 1991, Denjean et al. submitted), nor the sympathetic innervation of true BAT (Saarela et al. 1989). Multilocularity of adipocytes may in fact be a sign of intense lipolytic activity of the tissue to deliver fatty acids for thermogenesis in other tissues.

Despite their lack of BAT, a few species of birds including chickens, ducklings and penguin chicks can develop regulatory nonshivering thermogenesis (NST) after prolonged exposure to cold (El Halawani et al. 1970, Barré et al. 1986a, Duchamp et al. 1989). This mechanism contributes to the increased thermogenic capacity (Barré et al. 1985) and improved cold endurance of these birds (Barré et al. 1987b). This non-BAT dependent cold-induced NST is primarily of skeletal muscle origin as estimated by the measurement of regional blood flow with radioactive microspheres and the arteriovenous difference in oxygen content across leg skeletal muscles in the absence of shivering assessed by simultaneous electromyographic recordings (Duchamp & Barré 1993). Because avian NST differs from that of mammals with respect to the tissue sites, its regulation and biochemical mechanisms are most probably different. Recent data on the regulatory, cellular and molecular mechanisms of avian muscle NST will be reviewed.

2. Endocrine stimulation of avian NST

2.1. Role of glucagon

2.1.1. Effect on thermogenesis

On the basis of its marked thermogenic and lipolytic effects in birds (Freeman 1971, Barré & Rouanet 1983, Barré et al. 1986b, 1987b), glucagon appears as a potential mediator of avian NST. Glucagon-induced thermogenesis is increased by cold acclimation (Duchamp et al. 1993) and by chronic injections of glucagon to ducklings kept at thermoneutrality (Barré et al. 1987b). This is very much in parallel with what is observed in mammals with noradrenaline (Leblanc & Pouliot 1964, Fig.1). However, glucagon-induced thermogenesis were not observed in all species, as in 2-day-old chicks and adult pigeons, and may depend on the age and acclimation status of the animal (Hohtola et al. 1977, Barré et al. 1986b). Nevertheless, plasma glucagon concentration is increased in cold acclimated (CA) ducklings (Barré et al. 1986b) and glucagon-treated ducklings kept at thermoneutral-

ity develop NST (Barré et al. 1987b). It was postulated that glucagon may trigger muscle NST by stimulating the release of fatty acids (FAs) from a multilocular adipose tissue differentiated for lipolytic activity (Barré et al. 1986a, Bénistant et al. 1998). Released FAs may then affect the respiration of muscle mitochondria which show a higher sensitivity to their uncoupling effect in CA ducklings (Barré et al. 1986c). As reflected by *in vivo* measurements of muscle blood flow and arteriovenous differences in oxygen content, in the absence of shivering activity assessed by electromyography, muscle NST can be stimulated by exogenous glucagon (Duchamp et al. 1993). However, these experiments are unable to distinguish whether the action of glucagon is indirect through lipolysis or direct on myocytes.

An *in vitro* perfused muscle preparation initially developed chickens (Eldershaw et al. 1997) has been used in ducklings to provide insights to the humoral control of muscle thermogenesis (Marmonier et al. 1997). The results obtained with this *in vitro* preparation showed that glucagon does not directly stimulate muscle oxygen uptake. Glucagon showed marked vasodilatory effects in noradrenaline preconstricted perfused muscles of CA ducklings suggesting an enhanced effect of glucagon on the vascular system after cold-acclimation. The action of glucagon as a potential mediator of muscle NST is therefore likely to be indirect possibly via its vasomotor, metabolic and neurogenic actions. The vasodilatory effect of glucagon may enhance muscle thermogenesis through a rise in blood flow increasing the supply of hormones, substrates and/or hormones to the tissue. Alternatively, the vasodilatory effects of glucagon may act to potentiate the thermogenic effects of other hormones, such as endogenous catecholamines, by lowering the thermogenic down-effects of excessive vasoconstriction (Eldershaw et al. 1997). Besides its cardiovascular effects, glucagon also has marked effects on the mobilization of lipids and carbohydrates which could possibly modulate muscle thermogenesis. Finally, recent data from our laboratory indicate that glucagon injection stimulates the endogenous release of catecholamines in ducklings (Filali-Zagzouti et al. unpublished data) suggesting that part of the glucagon effect *in vivo* may be mediated by catecholamines (see below). This is in keeping with the activation of muscle sympathetic nerve activity described in man (Takayama et al. 1995). Clarification of these different possibilities obviously merits investigation.

2.1.2. Effect on inhibition of shivering

The control of shivering activity is of primary importance for the survival of many birds in the cold and possibly for the development of NST. Several hormones, including noradrenaline, adrenaline, serotonin and dopamine, have been shown to alter shivering activity, when injected in the central nervous system, by modulating the central control of shivering (Hissa & Rautenberg 1974, Hohtola et al. 1989). Glucagon may also be involved in the modulation of shivering activity by acting through its interaction with specific membrane receptors. Indeed, peripheral injection of glucagon induces a rapid suppression of shivering in ducklings or penguin chicks exposed acutely to cold, before any stimulation of peripheral heat production (Barré & Rouanet 1983, Barré et al. 1987b). Further, intracerebroventricular (ICV) perfusion of glucagon markedly suppressed shivering and associated regulatory thermogenesis in ducklings acutely exposed to cold (Montaron et al. 1995). At thermoneutrality, there was no effect of the ICV injection of the same dose of glucagon on heat production. There may therefore be a specific action of glucagon on the central command of shivering. Glucagon may act via interactions with specific membrane receptors which have been characterised in various areas of the duck brain, including the neocortex, the basal ganglia, and the dorsomedial nucleus of the hypothalamus (Montaron et al. 1994). However, it cannot be excluded that the glucagon effect is indirectly mediated by an activation of noradrenaline release by sympathetic nerves. Noradrenaline infusion into the preoptic area of the hypothalamus has indeed been shown to cause an immediate and complete inhibition of shivering in pigeons (Hissa & Rautenberg 1974, Hohtola et al. 1989). The precise mechanisms of the central effects of glucagon and the origin of the endogenous peptide acting centrally remain unknown.

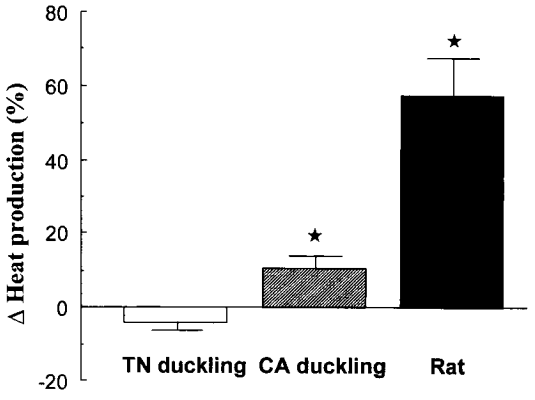

Fig. 2. *In vivo* thermogenic effects of catecholamines in thermoneutral (TN) or cold-acclimated (CA) ducklings and in rats. Ducklings were infused with adrenaline (1 μg.kg^{-1}min^{-1}, n = 5) and rats with noradrenaline (8 μg.kg^{-1}min^{-1}, n = 4) at thermoneutrality (25°C). Means ± SE. *P < 0.05 vs basal metabolic rate (ANOVA). Values are expressed as percentage of changes from resting metabolic rate. Duckling data are from Marmonier et al. 1997.

2.2. Role of catecholamines

There have been a variety of accounts describing thermogenic actions of catecholamines in birds, both *in vivo* (Barré & Rouanet 1983, Sutter & MacArthur 1989, Hissa et al. 1975a) and *in vitro* (Hissa et al. 1975b). Further, the catecholamine-induced calorigenesis is more pronounced after cold-acclimation in ducklings (Marmonier et al. 1997, Fig. 2) and in pigeons above thermoneutrality (Hissa et al. 1975a). However, such calorigenic action is not invariably found (Chaffee & Roberts 1971) and is in general much lower than the calorigenic action of catecholamines in rats (Fig. 2) or that of glucagon in birds (Barré & Rouanet 1983). Further, they are usually observed only at or above thermoneutral ambient temperatures, although significant effects have been noted in the cold using king penguin chicks (Barré & Rouanet 1983). In the cold, and usually in birds actively shivering, injection of exogenous catecholamines is associated with an inhibition rather than a stimulation of heat production (Hissa 1988). With this in mind, it is therefore puzzling to observe increased plasma catecholamine levels in CA chickens (Lin & Sturkie 1968), and increased sympathetic nervous system activity and catecholamine turnover in a number of cold-exposed birds (El Halawani et al. 1970, Koban & Feist 1982). It may be postulated that in the experiments using injections of exogenous catecholamines in cold exposed birds, the doses used may have favoured central inhibition of shivering (Hissa 1988). In birds exposed to cold and actively shivering, this central inhibitory effect may have masked underlying (if any) calorigenic effects of these hormones. Another important parameter to take into account is age as shown in juvenile coots (Sutter & MacArthur 1989). The dose used may also be to consider since higher doses of catecholamine resulted in much higher inhibitory effects on thermogenesis (Hissa & Palokangas 1970).

The perfused muscle model was used to address whether catecholamines may be involved in the control of muscle NST in birds. We have subsequently shown a marked thermogenic effect of catecholamines on duckling skeletal muscle *in vitro* as reflected by the dose-dependent increases in muscle oxygen uptake ($\dot{M}O_2$) (Marmonier et al. 1997, Fig. 3). The stimulatory effect of noradrenaline was observed at low doses (1–10 nM) which are within the physiological circulating levels of hormones observed in birds (Hart et al. 1989). Higher doses may reflect those occurring at sympathetic synapses (review in Clark et al. 1995), since local concentrations near sympathetic nerve terminals may be significantly higher than reported plasma values. Similar results were obtained after noradrenaline infusion in perfused chicken (Eldershaw et al. 1997) and rat hindlimb (review in Clark et al. 1995). The maximal noradrenaline-induced increase in $\dot{M}O_2$ in TN ducklings (+ 31% over the basal) was comparable to that observed in chickens (+ 35% over the basal) but not as pronounced as in the rat hindlimb (+60–80%). Thus the stimulation of skeletal muscle sympathetic nerve activity and hence elevation of skeletal muscle noradrenaline concentration in cold-exposed redpolls (Koban & Feist 1982) may have implications for the control of regulatory thermogenesis.

Most interestingly, cold-acclimation and chronic glucagon treatment, two experimental regimes inducing duckling muscle NST *in vivo* (Barré et al. 1986a, 1987b, Duchamp & Barré, 1993, Duchamp et al. 1993), were associated with increased thermogenic response of perfused skeletal mus-

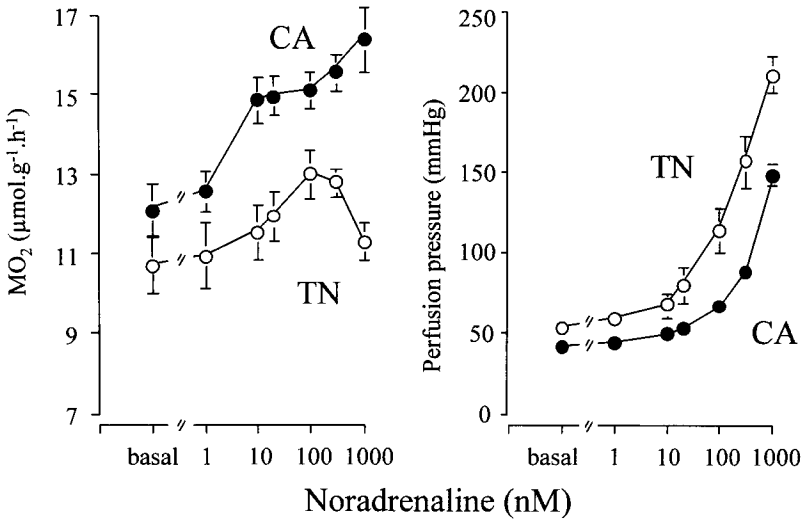

Fig. 3. Effects of norepinephrine on muscle oxygen uptake ($\dot{M}O_2$) and perfusion pressure in thermoneutral (TN) and cold-acclimated (CA) ducklings. Leg muscles were perfused at 0.47 ml.min^{-1}.g^{-1} with a Krebs-Ringer bicarbonate buffer without red blood cells at 25°C. Values are means ± SEM from 5–6 experiments. In each group, norepinephrine induced a significant increase in both $\dot{M}O_2$ and Pressure for concentrations ≥ 10 nM ($P < 0.05$, ANOVA). When not visible, bar errors are within the symbols. Adapted from Marmonier et al. (1997).

cle to noradrenaline. In addition, very high doses of noradrenaline, which become inhibitory in thermoneutral ducklings muscle, are still stimulatory in that of CA or glucagon-treated animals (Marmonier et al. 1997, Fig. 3). The mechanisms underlying this increased thermogenic effect after cold-acclimation remain to be investigated. Changes in the vascular effects of catecholamines may partly be involved by account of the observation that vasoconstriction induced by noradrenaline was less marked in CA than in control ducklings (Marmonier et al. 1997, Fig. 3). Such less sensitive vasoconstriction to noradrenaline may possibly be related to an altered expression of the various types of adrenergic receptors and/or the increased density of the vascular tree after cold acclimation (Duchamp et al. 1992).

A thermogenic effect of noradrenaline at the muscle level *in vitro* suggests that catecholamines have the potential to mediate some thermogenic effect *in vivo*. Yet, these findings contrast with many studies which did not find any thermogenic effect of catecholamines in birds (review in Chaffee & Roberts 1971). These apparent incongruence of these data might be related to factors such as differences in species, age of the animal, ambient temperature, dose of catecholamine used, mode of injection, or to the combined effects of several hormonal actions *in vivo*. Most presumably, the variability of published findings is related to major interactions between the vascular and metabolic actions of catecholamines. Indeed, the use of high hormone concentrations may result in responses corresponding to the inhibitory part of the *in vitro* dose-response curve associated with excessive vasoconstriction. The response to adrenaline *in vivo* clearly depends on the cold- acclimation status of the bird as well as the dose used, with high doses inhibiting rather than stimulating thermogenesis (Marmonier et al. 1997). The potential for catecholamine-induced muscle NST shown *in vitro* may therefore be expressed *in vivo* when vasomotor action is reduced such as in CA ducklings. In support of this hypothesis, it was shown that a reduction in vasoconstriction induced by glucagon increases the thermogenic effect of noradrenaline in perfused chicken muscles (Eldershaw et al. 1997). Further, changes in the population of adrenergic receptors on vascular cells and/or association with increased plasma glucagon may favor increased perfusion of skeletal muscles in CA ducklings (Duchamp & Barré 1993) in association with increased plasma levels of hormones as observed in CA

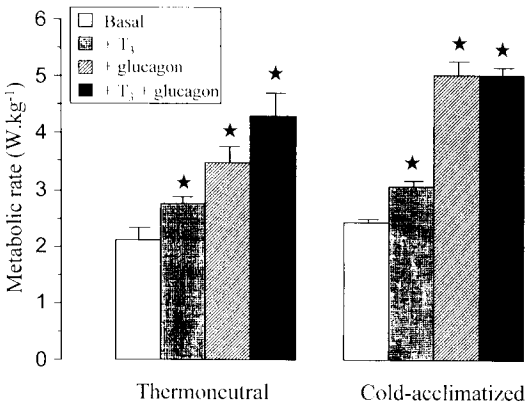

Fig. 4. Thermogenic effects of T_3 and glucagon in winter-acclimatized king penguin chicks and in chicks reared at thermoneutrality for 3 wk. T_3 (35 μg.kg^{-1}min^{-1}) or saline were infused intravenously. Glucagon was infused (0.05 μg.kg^{-1}min^{-1}) 20 h after the infusion of either saline (bars labelled glucagon) or T_3 (bars labelled T_3 + glucagon). Experiments were performed at thermoneutrality (+10°C). Values are means ± SEM. * $P < 0.05$ vs basal (ANOVA).

chickens.

At this point, the mechanism of action of the catecholamines at the muscle level is unclear but recent in vitro evidences of our lab (unpublished data) indicate that ATP consuming mechanisms may be involved.

2.3. Role of thyroid hormones

Thyroid hormones are generally involved in the process of cold-acclimation. During cold exposure, the thyroid gland is classically stimulated and indeed CA ducklings have higher circulating levels of total 3,5,3'-triiodo-L-thyronine (T_3) than thermoneutral controls (3.3 ± 0.2 vs 2.6 ± 0.1 nM). In birds, thyroid hormones play a particularly important role in the development of homeothermy (Saarela et al. 1990) and muscle development (Kumegawa et al. 1980) of chicks. For instance, these hormones have an essential role in avian muscle differentiation and myosin expression during the first weeks of growth. Furthermore T_3 is well known to control muscle fibre typing, oxidative capacity and mitochondrial biogenesis, parameters which have all been shown to be altered or increased in ducklings growing in the cold (Barré et al. 1986c,1987a Duchamp et al. 1991, 1992). Finally, and although these studies were done in mammals only, T_3 is known to uncouple oxidative phosphorylation at low doses (Hafner et al. 1988), and to regulate the expression of proteins involved in Ca^{2+} uptake and release by the sarcoplasmic reticulum (Arai et al. 1992). It is interesting to note that these mechanisms have been implicated in avian muscle NST (see below).

The possible thermogenic effect of T_3 was investigated *in vivo* at thermoneutrality in winter-acclimatized king penguin chicks known to exhibit NST (Duchamp et al. 1989). It was found (Fig. 4) that infusion of T_3 at a high dose (35 μg.kg^{-1}.min^{-1} iv) induced a slight thermogenic effect (+ 25% over the basal, maximum effect 15–20h after the infusion). A similar effect was observed in chicks acclimated to thermoneutrality for 3 wks, a period sufficient to reverse the effects of cold-adaptation. The significance of such long delay in the peak response to elevated T3 is unclear but may presumably be associated with stimulation of gene expression and protein synthesis. This contrasted with the rapid response to exogenous glucagon. Further, a higher calorigenic response was observed with a low dose of glucagon (0.05 μg.kg^{-1}.min^{-1} iv) in winter-acclimatized (+ 110%) than in thermoneutral chicks (+ 70%). Most interestingly, the thermogenic action of glucagon in thermoneutral chicks was potentiated by a previous perfusion of T_3 20h earlier (+ 50% of the effect without T_3). These results indicate a marked interaction between T_3 and glucagon, possibly because T_3 controls the expression of glucagon receptors. Experiments in progress should shortly clarify the possibility.

We recently investigated the potential involvement of T_3 in the control of diet-induced thermogenesis in birds. Two experimental lines have been obtained by divergent selection for high or low food efficiency (review in Gabarrou et al. 1997). For a given body weight cockerels with a low food efficiency exhibit a higher food intake than the efficient line (+ 49–76%) but an increased DIT (+2.5%). After a meal plasma T_3 levels are higher in the inefficient than in the efficient line. Injections of iopanoic acid (an inhibitor of peripheral conversion of thyroxine to T_3) markedly decreased plasma T_3 in both lines and abolished the differ-

ences in meal-induced heat production (Gabarrou et al. 1997). These results suggest that T_3, mainly originating from peripheral conversion of thyroxine to T_3, is involved in the DIT of the inefficient line. The precise mechanisms of this rather rapid action of T_3 now remain to be determined.

2.4. Involvement of these hormones in controlling regulatory NST

Regulatory NST is typically employed at temperatures below the thermoneutral zone and its intensity should be reflective of the severity of the cold challenge. Therefore, the hormones listed above which all show thermogenic effects in birds should undergo both rapid changes in plasma concentrations and effects during an acute cold exposure to be considered as playing a putative role in the control of avian regulatory NST. According to these criteria, the above data are suggestive of the involvement of glucagon and catecholamines in the control of avian regulatory NST. To be fully conclusive, it should however be checked whether the cold-induced changes in the levels of these hormones is compatible with the magnitude of the NST observed in vivo. The case of thyroid hormones is less clear as there is a long delay in the peak response to exogenous T_3. The involvement of thyroid hormones in avian NST is therefore likely to concern long-term adaptive changes in the thermogenic tissues (e.g. control of gene expression). However, relatively short-term effects of T_3 on diet-induced heat production are observed when endogenous plasma T_3 levels are reduced pharmacologically in cockerels (Gabarrou et al. 1997). One possibility is that there may be differences in effects between endogenously produced and exogenously injected hormone.

3. Mechanisms of avian muscle NST

Proposed mechanisms for avian NST include those based on fatty acid-induced loose-coupling of mitochondrial respiration (Barré et al. 1986), and those involving an increased Ca^{2+} cycling by the sarcoplasmic reticulum (Dumonteil et al. 1994).

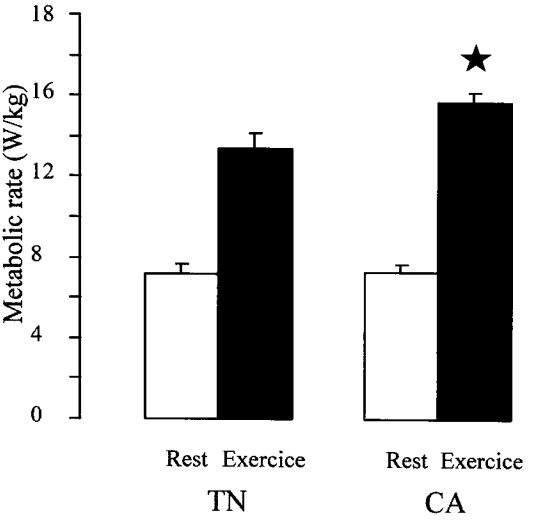

Fig. 5. Increase in metabolic rate associated with imposed exercise (walk at 540 m.h^{-1}) at thermoneutrality in thermoneutral (TN) or cold-acclimated (CA) ducklings. Values are means ± SEM of 12–14 experiments. In both groups of ducklings, exercise significantly increased metabolic rate but the increase was higher in CA than in TN ducklings (*P < 0.05, ANOVA).

3.1. Uncoupling of oxidative phosphorylations

Loosely coupled isolated mitochondria have been described in skeletal muscles of CA ducklings (Barré et al. 1986c), glucagon-treated ducklings (Barré et al. 1989) and winter-acclimatized king penguin chicks (Duchamp et al. 1991). These results are not an artefact inherent in the mitochondrial isolation procedure since similar results were obtained using an histochemical detection of the degree of coupling (Duchamp et al. 1992). Further, such mitochondrial defects in coupling is supported by studies both at the organ level and *in vivo*. The successive use of specific inhibitors (oligomycin and cyanide) on perfused skeletal muscles of ducklings suggested that the higher oxygen uptake of resting muscle in CA ducklings was related to a higher contribution of an uncoupled mitochondrial respiration (Eldershaw et al. unpublished data).

In vivo we recently assessed the efficiency of locomotor activity in ducklings (Denjean, unpublished data). As shown in Fig. 5, an imposed walk

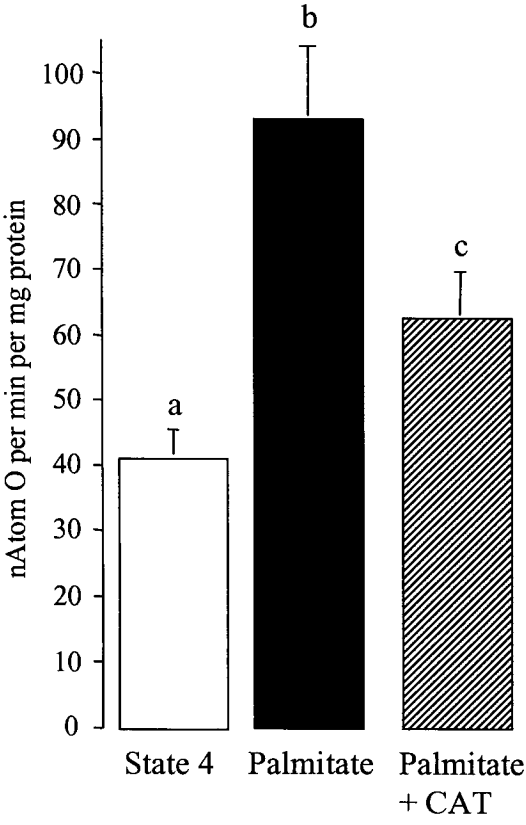

Fig. 6. Effect of carboxyatractylate (CAT) on the uncoupling effect of palmitate (20 μM) on intermyofibrillar mitochondria from cold-acclimated ducklings. Columns with different letters (a, b, c) are significantly different ($P < 0.05$, ANOVA). State 4 is the respiration rate of isolated mitochondria limited by the availability of ADP. In this experiment, state 4 was first measured, then palmitate was added to stimulate respiration by its uncoupling effect. Finally, CAT was added. Values are means ± SEM.

in a rotating wheel at thermoneutrality (25°C) increased metabolic rate significantly in both TN and CA ducklings. However, the energy cost of this imposed exercise was higher (+ 36%, $P < 0.05$) in CA than in control ducklings (Fig. 5) suggesting a lower efficiency of mitochondrial ATP generation and thus a defect in the coupling between oxidations and phosphorylations.

The mechanisms of such uncoupling have been investigated *in vitro* with isolated mitochondria. Muscle mitochondria from CA ducklings show a higher responsiveness to the loose coupling effect of non esterified fatty acids (FAs) (Barré et al. 1986). Addition of albumin to the incubation medium, restore a normal coupling of muscle mitochondria. Similar results have also been observed in glucagon-treated ducklings exhibiting NST (Barré et al. 1989). Such loose-coupling differs from that observed in BAT mitochondria, because the BAT-specific uncoupling protein-1 (UCP-1) is not expressed in CA ducklings (Denjean et al. submitted). Similarly, no immunoreactive UCP-1 was detected in various tissues of winter-acclimatized birds (Saarela et al. 1991). In addition, recent experiments from our laboratory failed to provide evidence for the expression in avian tissues of proteins similar to the other members of the mammalian UCP family (UCP-2 and UCP-3) (Denjean et al. submitted). In the potential absence of mammalian-like UCP in avian mitochondria, the mechanism of the fatty acid-induced loose coupling may possibly involve interaction of FAs with the ATP/ADP antiport (Andreyev et al. 1989). Indeed, the use of carboxyatractylate, a specific inhibitor of the ATP/ADP antiport, abolished a large part, but not all, of the uncoupling effect of palmitate on mitochondrial respiration (Fig. 6). Such a defect of energy coupling in muscle mitochondria may in fact be detrimental for all the ATP-consuming processes. Thus the capacity of muscle mitochondria to synthesise ATP was investigated and it was shown that even at the maximal uncoupling effect of high doses of palmitate, muscle mitochondria of CA ducklings were still able to synthesise ATP at half their maximal rate (Roussel et al. 1997). The ATP-consuming processes can therefore be fuelled even if NST mechanisms are occurring at the mitochondrial level. The molecular mechanisms of the FA-effect in avian mitochondria and of the adaptive changes induced by cold-acclimation and their control require experimental clarification.

3.2. Increased Ca^{2+} cycling

ATP-consuming processes such as cation cycling are potential mechanisms for muscle NST. We have shown that cold acclimation of ducklings induces an increase in skeletal muscle sarcoplasmic reticulum (SR) Ca^{2+}-ATPase and Ca^{2+} release channel/ryanodine receptor densities (Dumonteil et al. 1993, Fig. 7). Thus an increase in ATP-de-

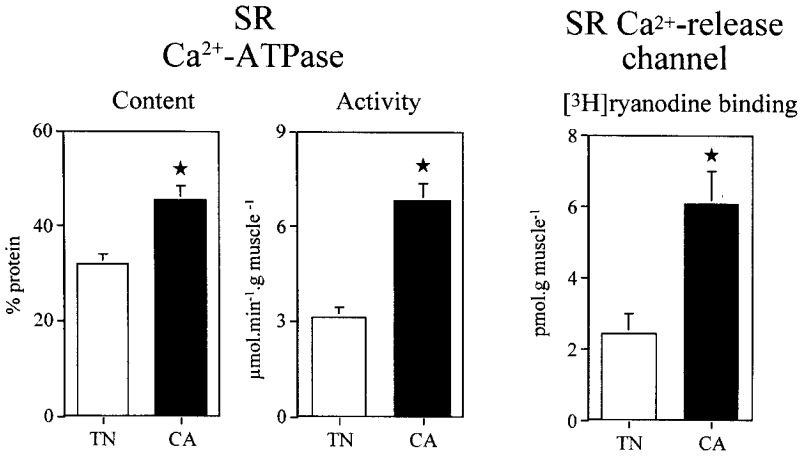

Fig. 7. Increase in sarcoplasmic reticulum (SR) Ca^{2+}-Atpase activity and content, and SR Ca^{2+}-release channel in skeletal muscles from cold-acclimated (CA) compared with thermoneutral(TN) ducklings. Values are means ± SEM. *P < 0.05 vs TN. Adapted from Dumonteil et al. 1993.

pendent calcium cycling between the SR and the cytosol could contribute to muscle NST in CA ducklings. Regulation of this Ca^{2+} cycling may occur via some fatty acid related metabolites. Indeed, palmitoyl carnitine and palmitoyl coenzyme A activated the Ca^{2+} release channel at concentrations ≥ 20 µM while palmitic acid was without effect (Dumonteil et al. 1994). Further, induction of Ca^{2+} release was observed with long-chain (C ≥ 14) but not with short-chain acyl carnitines (C ≥ 12). These results are consistent with the accumulation of long-chain acyl carnitines in duckling skeletal muscle during cold acclimation (Dumonteil et al. 1994). There may therefore be specific interactions of acyl carnitines with the Ca^{2+} release channel, although the underlying mechanisms are not known.

The time course of expression of the SR Ca^{2+}-ATPase (SERCA, sarco(endo)plasmic reticulum Ca^{2+}-ATPase) has been investigated in both control and CA ducklings. $^{45}Ca^{2+}$ uptake and [^{3}H]ryanodine binding measurements with skeletal muscle homogenates showed that a cold acclimation period of ~ 4 wk was required to observe a substantial increase in SERCA activity and Ca^{2+} release channel content (Dumonteil et al. 1995). Such a time course correlates well with NST development, which requires 4–5wk of cold exposure in ducklings (Barré et al. 1986a). Immunoblot analysis of muscle homogenates revealed that the SERCA1 (specific of fast fibres) level was increased after 4 wk of cold acclimation while the decrease with age of SERCA2a (specific of slow oxidative fibres) content was delayed in cold acclimating birds. The persistence of SERCA2a may be related to shivering thermogenesis in slow oxidative fibres in the early stage of the cold acclimation period. The increase in SERCA1 content in parallel with the development of NST suggests that a Ca^{2+}-dependent NST may preferentially occur in fast-twitch fibres (Dumonteil et al. 1995). The mechanisms underlying this fibre specific regulation of SERCA isoforms remain uninvestigated.

4. Fatty acid mobilisation and supply to thermogenic tissues

FAs play a major role in avian NST by serving both as substrates for respiration and as stimulators of NST mechanisms. Indeed, the uncoupling of mitochondrial oxidative phosphorylation (Barré et al. 1986c) or the activation of ATP-consuming SR Ca^{2+} cycling through their long-chain related metabolites (Dumonteil et al. 1994) depend on FAs. The capacity to supply FAs to thermogenic tissues could therefore comprise a number of controlled and limiting steps for cold thermogenesis. This aspect has been recently investigated in CA ducklings.

4.1. Adipose tissue lipolysis

Mobilisation from the sites of storage, i.e. adipose tissue, may be the first limiting step in fatty acid mobilisation. On the basis of the multilocul-

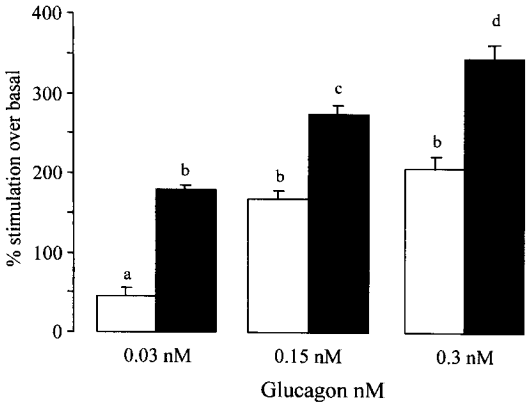

Fig. 8. Glucagon-induced lipolysis in adipose tissue fragments from thermoneutral (open bars) or cold-acclimated (dark bars) ducklings. Values are means ± SEM and are expressed as percentage of stimulation above basal lipolytic activity. Columns with different letters are significantly different ($P < 0.05$, ANOVA). From Bénistant et al. 1998.

arity of adipocytes and the increased density of capillaries with abundant microvilli in adipose tissue of CA ducklings, it was suggested that an intense lipolysis may take place in the cold (Barré et al. 1986a). This hypothesis was tested *in vitro* by measuring the lipolytic activity of incubated tissue fragments (Bénistant et al. 1998). It was higher (202 ± 9 vs 130 ± 14 nmol glycerol per 100 mg tissue per hour, + 55%, $P < 0.01$) in CA than in TN ducklings. Glucagon stimulated lipolysis in both groups of ducklings in a dose-dependent manner, but the lipolytic response to increasing doses of glucagon was higher ($P < 0.01$) in CA than in TN ducklings (Fig. 8). Interestingly, in order to obtain the same relative stimulation of lipolysis (around + 170% over the basal), a dose of 0.15 nM was needed in TN controls while only 0.03 nM was required in CA ducklings. These results indicate an increased responsiveness and a higher glucagon sensitivity of adipose tissue from CA ducklings. Interestingly, similar increases in adipose tissue responsiveness and sensitivity to glucagon were also observed in winter-acclimatized king penguin chicks (George et al. unpublished data). Data in ducklings are consistent with the observation that adipose tissue blood flow is much higher in CA than in TN ducklings after either cold or glucagon stimulation (Duchamp & Barré 1993, Duchamp et al. 1993), possibly reflecting a more intense rate of lipid mobilisation in CA ducklings *in vivo*. Such stimulation *in vivo* may be related to the higher plasma glucagon level in CA than in TN ducklings (Barré et al. 1986b). It should now be investigated whether the cold-induced enhanced adipocyte response to glucagon involves an increased density of glucagon receptors, as shown in CA rats (Uehara et al. 1986), or is in fact related to other mechanisms.

4.2. Tissue uptake of FAs

Tissue uptake of FAs may be the second limiting step in FA supply to thermogenic tissues. This process can be modulated by the activity of endothelial lipases, lipoprotein lipase (LPL) and hepatic lipase, all of which are rate limiting step enzymes enabling the extraction of FA from triacylglycerol and phospholipids of lipoproteins (Kinnunen et al. 1983). For instance, it is well known that BAT NST is fuelled by a marked LPL up-regulation (Radomski & Orme 1971). We have recently shown an increased *in vitro* activity of endothelial lipases in red gastrocnemius muscle and liver of CA ducklings relative to thermoneutral controls (Bénistant et al. 1998). Total endothelial lipase activity expressed per organ (Fig. 9) was higher in red gastrocnemius muscle (+ 80%) and liver (+ 55%) of CA ducklings whereas no change occurred in pectoralis muscle and adipose tissue. Similar results were also obtained in winter-acclimatized king penguin chicks (Bénistant et al. unpublished data). Part of the cold-induced rise in LPL activity may be related to the slight increase in the proportion of slow oxidative fibres observed in CA ducklings (Duchamp et al. 1992) on account of the higher specific (per g muscle) LPL activity found in red than in white muscles. Because LPL activity is generally high in tissues that are recruited in the cold (Radomski & Orme 1971), present results suggest that red skeletal muscles rich in slow-oxidative fibres may play an important role in duckling thermogenesis during cold-acclimation. The increased hepatic lipase activity in the cold is consistent with the higher oxidative capacity of this tissue in CA ducklings (Barré et al. 1987a), suggesting a possible role for liver in cold-induced thermogenesis (Duchamp & Barré 1993).

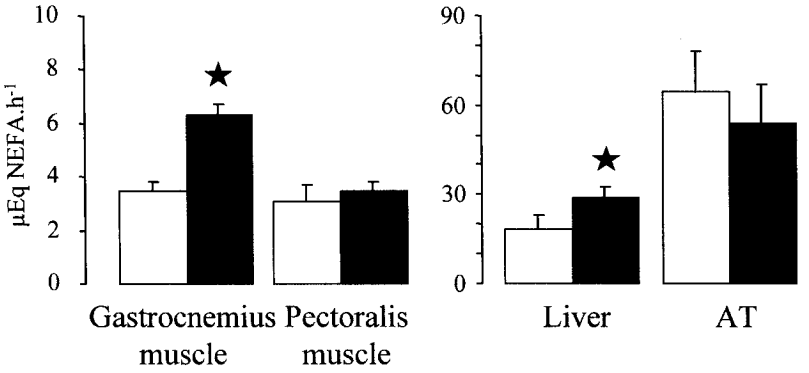

Fig. 9. Effect of cold-acclimation on skeletal muscle and adipose tissue (AT) lipoprotein lipase and hepatic lipase activity in ducklings. Thermoneutral ducklings (open columns) are compared with cold-acclimated ducklings (dark columns). Values are means ± SEM (n = 6 per group). * $P < 0.05$ vs TN (Student's *t*-test). The activity of the enzyme is measured by the amount of non-esterified fatty acids released from a triglyceride emulsion per hour. Adapted from Bénistant et al. 1998.

4.3. Intracellular transport of FAs

Intracellular transport of FAs may also represent an important step for fuelling cold thermogenesis. Within the cells, small (12–16 kDa) and abundant cytoplasmic FA-binding proteins (FABPs) have been involved in the cytoplasmic trafficking of FA and hydrophobic molecules (Glatz et al. 1984, Miller et al. 1988). Although their physiological function is yet to be unequivocally established, FABPs may be an important and limiting determinant of FA transport (Storch et al. 1996) to membranes and organelles for energy storage or expenditure. We have recently shown that the FA-binding capacity of a fraction of cytosolic protein weighing 12–18 kDa was increased with cold-acclimation in both red gastrocnemius muscle (+46%) and liver (+74%) (Bénistant et al. 1998). We then purified a cytosolic protein with a high capacity to bind FA and a molecular weight of 15.4 kDa, similar to mammalian muscle FABPs. Specific antibodies were raised against duckling FABP and used in Western blots on skeletal muscle cytosolic proteins. Muscle FABP content was found to be respectively high, intermediary and low in heart, gastrocnemius and pectoralis muscles (Fig 10). There was a higher FABP content (+ 37%) in the cytosol of the red gastrocnemius muscle of CA ducklings which may well account for the cold-induced rise in FA-binding capacity of the 12–18kDa protein fraction. It should be noted that the rise in FABP content only affected oxidative muscles rich in slow oxidative fibres while there was no change in FG- and FOG-rich skeletal muscles, suggesting that additional FABPs may only be required in the most oxidative fibres preferentially recruited in the cold. Enhanced thermogenic capacity of slow oxidative fibres has already been observed in CA ducklings (Duchamp et al. 1992). The combined effects of (i) increased FA supply from adipose tissue, enhanced tissue FA-uptake by LPL, (ii) increased intramuscular transport by FABP (present study), and (iii) increased muscle oxidative capacity (Barré et al. 1987a) and blood flow (Duchamp & Barré 1993), may thus favour lipid oxidation and thermogenesis in red skeletal muscles of CA ducklings. Similarly, increased FABP content paralleled increased lipid oxidation in muscle of cold exposed fishes (Londraville & Sidell 1995). Furthermore, a dramatic increase in the heart-type FABP was also observed in BAT of CA rats (Daikoku et al. 1997) in conjunction with the stimulation of the thermogenic capacity of this tissue.

In liver, the increased FA uptake through hepatic lipase was also positively linked to an increase in the (12–18 kDa) FA-binding capacity, which may be related to a putative hepatic FABP by analogy with mammals. These data therefore suggest an increased intrahepatic trafficking of FA towards either resynthesis of triglycerides or lo-

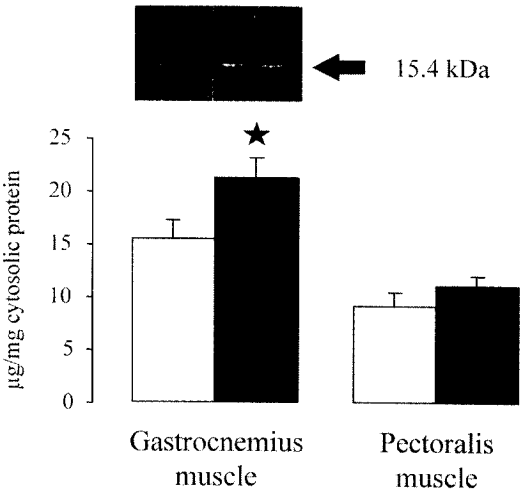

Fig. 10. Effect of cold-acclimation on skeletal muscle fatty acid binding protein (FABP) content as determined by western blots using specific antibodies against duckling FABP. Typical immunoblots obtained from gastrocnemius cytosols of two thermoneutral (left side of upper panel) and two cold-acclimated (right side of upper panel) ducklings are shown. Calibration of immunoblot intensity with known amounts of muscle FABP enabled us to determine the amount of FABP present in cytosols isolated from skeletal muscles of thermoneutral (open bars) or cold-acclimated (dark bars) ducklings (lower panel). Values are means ± SEM (n = 6 per group). * $P < 0.05$ vs thermoneutral controls (Student's *t*-test).

cal oxidation which may parallel an increased aerobic metabolism as indicated by the higher hepatic blood flow observed in CA ducklings (Duchamp & Barré 1993).

5. Conclusions

We have therefore reviewed a number of recent data from our laboratory and elsewhere showing various aspects of the regulation and the cellular and molecular mechanisms of avian NST. A comprehensive scheme emerges from these experiments exploring different aspects at several levels of integration. Avian NST cannot be regarded as a simple extrapolation of what is known in mammals although some aspects may be shared by the two Classes. No single thermogenic mechanism nor a unique humoral activator has yet emerged, but rather a likely combination of several mechanisms. This may be due to the fact that birds do not possess a specialised thermogenic tissue such as brown fat. It is however obvious that the development of muscle NST in avian species may have an impact on their daily energy budget and that this aspect should be taken into account in the future. Several aspects still remain unknown, or should be clarified, before we can draw a complete picture of avian regulatory NST. In particular, the mechanisms controlling skeletal muscle gene expression and leading to the development of NST in certain circumstances in birds should be elucidated.

Acknowledgements: This work was supported by grants from the Centre National de la Recherche Scientifique, Université Claude Bernard Lyon 1, the Région Rhône-Alpes, and the Institut Français pour la Recherche et la Technologie Polaire (Programme R131).

Selostus: **Lintujen lihaskudoksen nonshivering-termogeneesin säätelyn solubiologinen ja molekulaarinen perusta**

Kirjoittajat ovat havainneet kasvavilla pesäjättöisillä linnunpoikasilla uuden, lihasperäisen lämmöntuottojärjestelmän, joka toimii vanhastaan tunnetun lihasvärinälämmöntuoton ohella. Tähän lihaksen nonshivering-termogeneesin (NST, eilihasvärinäperäinen lämmöntuotto) endokriiniseen kontrollijärjestelmään kuuluu useita hormoneja, mm. glukagoni, katekoliamiinit ja kilpirauhashormonit. Näiden hormonien vaikutusta aineenvaihduntaan, keskushermostoon, verenkiertoon ja geenien toimintaan on tutkittu linnuilla monella eri tasolla: koko eläimillä sekä eristetyillä lihaspreparaateilla. Tutkimuksissa on löydetty lintujen lihaskudoksesta kaksi NST-mekanismia. (1) Lihassolun mitokondrioista on löydetty oksidatiivisesta fosforylaatiosta (ATP:n synteesi) irtikytkeytynyttä energiantuottoa. Uncoupling-tapahtumassa syntynyttä energiaa ei sidota kemiallisesti ATP:hen kuten normaalisti tapahtuu, vaan se vapautuu mitokondrion ulkopuolelle lämpönä. Tämän mekanismin olemassaoloa tukevia tuloksia on saatu sekä eristettyjä mitokondrioita että perfusoituja lihaksia tutkimalla ja myös mittaamalla liikeaktiivisuuden energiataloutta elävillä linnuilla. (2) Toinen mekanismi liittyy ATP:stä riippuvaisen sarkoplasmaattisen kalvoston kalsiumin

kierrätyksen lisääntymiseen. Tätä on tutkittu epäsuorasti kalsiumia sitovien proteiinien esiintymisen ja aktiivisuuden avulla. Kirjoittajat pohtivat tässä katsauksessa näiden kahden mekanismin merkitystä lihaskudoksen NST:n kannalta. Molempien tapahtumien molekulaarista perustaa tutkiessaan he havaitsivat, että rasvahapot tai niiden johdannaiset osallistuvat kyseisen lämmöntuottomuodon säätelyyn. Toisaalta lihaksen NST käyttää energianlähteenään rasvakudoksesta saatavia samaisia rasvahappoja, jotka otetaan lihassoluun lipoproteiinilipaasi -entsyymin avulla. Solujen sisällä rasvahappojen kuljetukseen osallistuvat soluliman rasvahappoja sitovat proteiinit (FABP). Kirjoittajat esittävät, että seuraavaksi tulisi tutkia kylmäakklimoidun eläimen luurankolihaksen toiminnallista sopeutumista sääteleviä geenejä.

References

Andreyev, A. Y., Bondareva, T. O., Dedukhova, V. I., Mokhova, E. N., Skulachev, V. P., Tsofina, L. M., Volkov, N. I. & Vygodina, T. V. 1989: The ATP/ADP-antiport is involved in the uncoupling effect of fatty acids on mitochondria. — Eur. J. Biochem. 182: 585–592.

Arai, M., Otsu, K., MacLennan, D. H., Alpert, N. R. & Periasamy M. 1992: Effect of thyroid hormone on the expression of mRNA encoding sarcoplasmic reticulum proteins. — Circ. Res. 69: 266–276.

Barré, H, Géloën A., Chatonnet J., Dittmar A. & Rouanet J. L. 1985: Potentiated muscular thermogenesis in cold-acclimated Muscovy duckling. — Am. J. Physiol. 249: R533–R538.

Barré, H. & Rouanet J. L. 1983: Calorigenic effect of glucagon and catecholamines in king penguin chicks. — Am. J. Physiol. 244: R758–R763.

Barré, H., Bailly, L. & Rouanet, J.-L. 1987a: Increased oxidative capacity in skeletal muscles from cold-acclimated ducklings: a comparison with rats. — Comp. Biochem. Physiol. 88B: 519–522.

Barré, H., Berne, G., Brebion, P., Cohen-Adad, F. & Rouanet, J. L. 1989: Loose-coupled mitochondria in chronic glucagon-treated hyperthermic ducklings. — Am. J. Physiol. 256: R1192–R1199.

Barré, H., Cohen-Adad, F. & Rouanet, J. L. 1987b: Two daily glucagon injections induce nonshivering thermogenesis in Muscovy ducklings. — Am. J. Physiol. 252: E616–E620.

Barré, H., Cohen-Adad, F., Duchamp, C. & Rouanet, J. L. 1986a: Multilocular adipocytes from Muscovy ducklings differentiated in response to cold acclimation. — J. Physiol. London 375: 27–38.

Barré, H., Géloën, A., Mialhe, P. & Rouanet, J. L. 1986b: Effect of glucagon on bird thermogenesis. — In: Assenmacher, Y. & Boissin, J. (eds), Endocrine Regulations as Adaptive Mechanisms to the Environment: 395–401. Paris, CNRS.

Barré, H., Nedergaard, J. & Cannon, B. 1986c: Increased respiration in skeletal muscle mitochondria from cold-acclimated ducklings: uncoupling effect of free fatty acids. — Comp. Biochem. Physiol. 85B: 343–348.

Bénistant, C., Duchamp, C., Cohen-Adad, F., Rouanet, J. L. & Barré, H. 1998: Increased in vitro fatty acid supply and cellular transport capacities in cold-acclimated ducklings (Cairina moschata). — Am. J. Physiol. 275: R683–R690.

Chaffee, R. R. J. & Roberts, J. C. 1971: Temperature acclimation in birds and mammals. — Annu. Rev. Physiol. 33: 155–202.

Clark, M. G., Colquhoun, E. Q., Rattigan, S., Dora, K. A., Eldershaw, T. P. D., Hall, J. L. & Ye, J. M. 1995: Vascular and endocrine control of muscle metabolism. — Am. J. Physiol. 268: E797–E812.

Daikoku, T., Shinohara, Y., Shima, A., Yamazaki, N. & Terada H. 1997: Dramatic enhancement of the specific expression of the heart-type fatty acid binding-protein in rat brown adipose tissue by cold exposure. — FEBS Lett. 410: 383–386.

Duchamp, C. & Barré, H. 1993: Skeletal muscle as the major site of nonshivering thermogenesis in cold acclimated ducklings. — Am. J. Physiol. 265: R1076–R1083.

Duchamp, C., Barré, H., Delage, D., Rouanet, J. L., Cohen-Adad, F. & Minaire, Y. 1989: Nonshivering thermogenesis and adaptation to fasting in king penguin chicks. — Am. J. Physiol. 257: R744–R751.

Duchamp, C., Barré, H., Rouanet, J. L., Lanni, A., Cohen-Adad, F., Berne, G. & Brebion, P. 1991: Nonshivering thermogenesis in king penguin chicks. I. Role of skeletal muscle. — Am. J. Physiol. 261: R1438–R1445.

Duchamp, C., Chatonnet, J., Dittmar, A. & Barré H. 1993: Increased role of skeletal muscle in the calorigenic response to glucagon of cold-acclimated ducklings. — Am. J. Physiol. 265: R1134–R1091.

Duchamp, C., Cohen-Adad, F., Rouanet, J. L., & Barré H. 1992: Histochemical arguments for muscular non-shivering thermogenesis in muscovy ducklings. — J. Physiol. London 457: 27–45.

Dumonteil, E., Barré, H. & Meissner G. 1993: Sarcoplasmic reticulum Ca^{2+}-ATPase and ryanodine receptor in cold-acclimated ducklings and thermogenesis. — Am. J. Physiol. 265: C507–C513.

Dumonteil, E., Barré, H., & Meissner G. 1994: Effects of palmitoyl carnitine and related metabolites on the avian Ca^{2+} ATPase and Ca^{2+} released channel. — J. Physiol. London 479: 29–39.

Dumonteil, E., Barré, H., & Meissner G. 1995: Expression of sarcoplasmic reticulum Ca^{2+} transport proteins in cold-acclimating duckings. — Am. J. Physiol. 269: C955–C960.

El Halawani, M. E., Wilson, W. O. & Burger, R. E. 1970: Cold-acclimation and the role of catecholamines in

body temperature regulation in male Leghorns. — Poultry Sci. 49: 621–632.

Eldershaw, T., Duchamp, C., Ye, J., Clark, M. G. & Colquhoun, E. Q. 1997: Potential for nonshivering thermogenesis in perfused chicken (Gallus domesticus) muscle. — Comp. Biochem. Physiol. 117: 545–554.

Freeman, B. M. 1970: Thermoregulatory mechanisms of the neonate fowl. — Comp. Biochem. Physiol. 33: 219–230.

Gabarrou, J. F., Duchamp, C., Williams, J. & Gereart, P. A. 1997: A role for thyroid hormones in the regulation of diet-induced thermogenesis in birds. — British J. Nutr. 78: 963–973.

Glatz, J. F., Baerwaldt, C. C. F. & Veerkamp J. H. 1984: Diurnal variation of cytosolic fatty acid-binding protein content and of palmitate oxidation in rat liver and heart. — J. Biol. Chem. 259: 4295–4300.

Hafner, R. P., Nobes, C. D, McGown, A. & Brand, M. D. 1988: Altered relationship between protonmotive force and respiration rate in non-phosphorylating liver mitochondria isolated from rats of different thyroid hormone status. — Eur. J. Biochem. 178: 511–518.

Hart, B., Stanford, G., Ziegler, M., Lake, C. & Chernow B. 1989: Catecholamines: Study of interspecies variations. — Crit. Care Med. 17: 1203–1222.

Hissa, R. 1988: Controlling mechanisms in avian temperature regulation: a review. — Acta Physiol. Scand. 132: 1–148.

Hissa, R. & Palokangas R. 1970: Thermoregulation in the titmouse (Parus major L.). — Comp. Biochem. Physiol. 33: 941–953.

Hissa, R., Pyörnilä, A. & Saarela S. 1975a: Effect of peripheral norepinephrine on thermoregulation in temperature-acclimated pigeon. — Comp. Biochem. Physiol. 51C: 243–247.

Hissa, R., Rantala, K. & Jeronen E. 1975b: Effect of norepinephrine on the oxygen uptake in vitro by the pigeon. — Gen. Pharmac. 6: 303–307.

Hissa, R. & Rautenberg W. 1974: The influence of centrally applied noradrenaline on shivering and body temperature in the pigeon. — J. Physiol. London 238: 421–435.

Hohtola, E., Hissa, R. & Saarela, S. 1977: Effect of glucagon on thermogenesis in the pigeon. — Am. J. Physiol. 232: E451–E455.

Hohtola, E., Saarela, S., Harjula, R. & Hissa, R. 1989: Cardiovascular and thermoregulatory responses to intra-hypothalamically injected neurotransmitter in the pigeons. — J. Therm. Biol. 14: 41–45.

Jansky, L. 1973: Nonshivering thermogenesis and its thermoregulatory significance. — Biol. Rev. 48: 85–132.

Johnston, D. W. 1971: The absence of brown adipose tissue in birds. — Comp. Biochem. Physiol. 40A: 1107–1108.

Kinnunen, P. K. J., Virtanen, A. & Vaino P. 1983: Lipoprotein lipase and hepatic endothelial lipase: their role in lipoprotein metabolism. — Atheroscl. Rev. 11: 65–105.

Koban, M. & Feist, D. D. 1982: The effect of cold on norepinephrine turnover in tissues of seasonally acclimatized redpolls (Carduelis flammea). — J. Comp. Physiol. 146: 137–144.

Kumegawa, M., Hikeda, E., Hosoda, S. & Takuma, T. 1980: In vitro effets of thyroxine and insulin on myoblasts from embryo skeletal muscle. — Dev. Biol. 79: 493–499.

Leblanc, J. & Pouliot, M. 1964: Importance of noradrenaline in cold adaptation. — Am. J. Physiol. 207: 853–856.

Lin, Y. C. & Sturkie, P. D. 1968: Effect of environmental temperatures on the catecholamines of chickens. — Am. J. Physiol. 214: 237–240.

Londraville, R. L. & Sidell, B. B. 1995: Purification and characterisation of fatty acid-binding protein from aerobic muscle of the Antarctic icefish Chaenocephalus aceratus. — J. Exp. Zool. 273: 190–203.

Marmonier, F., Duchamp, C., Cohen-Adad, F., Eldershaw, T. P. D. & Barré, H. 1997: Hormonal control of thermogenesis in perfused muscle in Muscovy ducklings. — Am. J. Physiol. 273: R1638–R1648.

Miller, W. C., Simoneau, J. A., Veerkamp, J. H. & Pette, D. 1988: Fatty acid-binding proteins in the three types of rat skeletal muscle. — Proc. Soc. Exp. Biol. Med. 189: 183–188.

Montaron, A., Moyse, E. & Barré, H. 1994: Radioautographic demonstration and localisation of glucagon receptors in duck brain. — Brain Res. 663: 121–130.

Montaron, A., Rouanet, J. L. & Barré, H. 1995: Inhibition of shivering thermogenesis by centrally applied glucagon in Muscovy ducklings. — Brain Res. 702: 49–54.

Nicholls, D. G., Cunningham, S. A. & Rial E. 1986: The bioenergetic mechanisms of brown adipose tissue mitochondria. — In: Trayhurn, P. & Nicholls, D. G. (eds.), Brown adipose tissue: 52–85. Edward Arnold, Baltimore.

Olson, J. M., Dawson, W. R. & Camilliere, J. J. 1988: Fat from black-capped chickadees: avian brown adipose tissue? — Condor 90: 529–537.

Radomski, M. W. & Orme, T. 1971: Response of lipoprotein lipase in various tissues to cold exposure. — Am. J. Physiol. 220: 1852–1856.

Roussel, D., Rouanet, J. L. & Barré H. 1997: Maximal loose coupling by free fatty acids does not abolish ATP production of skeletal muscle mitochondria. — In: Johannsen, B. N. & Nielsen, R. (eds), Thermal Physiology 1997: 231–234. The August Krogh Institute, Copenhagen.

Saarela, S., Hissa, R., Pyörnilä, A., Harjula, R., Ojanen, M. & Orell, M. 1989: Do birds possess brown adipose tissue ? — Comp. Biochem. Physiol. 92A: 219–228.

Saarela, S., Keith, J. S., Hohtola, E., & Trayhurn, P. 1991: Is the "mammalian" brown fat-specific mitochondrial uncoupling protein present in adipose tissue of birds? — Comp. Biochem. Physiol. 100B: 45–49.

Saarela, S., Petäjä-Repo, U. & Hissa, R. 1990: Monoamines, thyroid hormones, and energy reserves in developing

capercaillie chicks (Tetrao urogallus: Tetraonidae). — Comp. Biochem. Physiol. 97A: 353–360.

Storch, J., Herr, F. M., Hsu, K. T., Kim, H. K., Liou, H. L. & Smith, E. R. 1996: The role of membranes and intracellular binding proteins in cytoplasmic transport of hydrophobic molecules: fatty acid-binding proteins. — Comp. Biochem. Physiol. 115B: 333–339.

Sutter, G. C., & MacArthur, R. A., 1989: Beta-adrenergic sensitivity in juvenile american coots (Fulica americana): evidence of nonshivering thermogenesis. — Comp. Biochem. Physiol. 93C: 105–110.

Takayama, S., Nakajima, Y., Toma, S. & Sakamoto, T. 1995: Increased muscle sympathetic nerve activity after glucagon administration in man. — Journal of the Autonomic nervous System 54: 171–175.

Uehara, A., Habara, Y. & Kuroshima A. 1986: Effect of cold acclimation on glucagon receptors of rat white adipocytes. — Jpn. J. Physiol. 36: 891–903.