

**THERMOGENIC MECHANISMS  
DURING THE DEVELOPMENT  
OF ENDOTHERMY IN  
JUVENILE BIRDS**

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# **Marjonieni, Kyösti, Thermogenic mechanisms during the development of endothermy in juvenile birds**

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## ***Abstract***

The use of regulatory and obligatory heat production mechanisms were studied in juvenile birds during the development of endothermy.

The development of shivering thermogenesis was studied in the pectoral and gastrocnemius muscles of the altricial domestic pigeon and in three precocial galliforms (Japanese quail, grey partridge and domestic fowl). The development of shivering was the determinant for the beginning of endothermy. Homeothermy also necessitated avoidance of excess heat loss by insulation and behavioural thermoregulation. In the precocial species, shivering thermogenesis was present in the leg muscles of the youngest age groups (1-2 d) studied. Breast muscles contributed shivering from the second post-hatching week. In the altricial pigeons, significant thermogenesis was apparent later than in the precocials, at the age of 6 d. In contrast to the precocials, the pectoral muscles of the altricials were the most significant heat production tissues. In newly-hatched partridges and pigeons, incipient shivering did not result in significant heat production.

The ability to produce heat in cold by putative nonshivering thermogenesis was studied in Japanese quail chicks and domestic ducklings. In both species, three-week cold acclimation resulted in morphometric and physiological changes, but there was no clear evidence of nonshivering thermogenesis. The lack of NST was evident because an increase in shivering amplitude at least in one of the muscles studied paralleled an increase in oxygen consumption. Consequently, shivering thermogenesis was probably the only mode of regulatory heat production.

The amplitudes of shivering EMGs measured during cold exposure were dependent on the coexistence of postprandial thermogenesis or exercise. Japanese quail chicks were able to substitute shivering thermogenesis partially with postprandial heat production when nourished. Bipedal exercise both inhibited shivering in pectorals directly via inhibitory neural circuits and stimulated it indirectly via decreased body temperature. Because of increased heat loss, exercise was not used as a substitute for shivering.

Shivering is a flexible mode of thermogenesis and its magnitude can be adjusted according to the magnitude of obligatory thermogenesis. The adjustment works towards energy saving by avoidance of the summation of different modes of heat production. The prerequisite for successful adjustment of shivering is adequate insulation, whose role in preventing excessive heat loss is pronounced during exercise. It is concluded that the energetics of posthatching thermoregulation includes the potential for optimizations in energy use in order to avoid dissipation of waste energy as heat.

***Keywords:*** thermoregulation, ontogeny, shivering, nonshivering thermogenesis, postprandial heat production, exercise



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## Abbreviations

BAT	brown adipose tissue
BMR	basal metabolic rate
DIT	diet-induced thermogenesis
DRT	digestion-related thermogenesis
EMG	electromyogram
FFA	free fatty acid
$I_h$	index of homeothermy
HIF	heat increment of feeding
K	thermal conductance
LCT	lower critical temperature
MR	metabolic rate
MU	motor unit
MUAP	motor unit action potential
MUAPT	motor unit action potential train
NST	nonshivering thermogenesis
PMR	peak metabolic rate
RER	respiratory exchange rate
RMR	resting metabolic rate
SDA	specific dynamic action of feeding
STT	shivering threshold temperature
$T_a$	ambient temperature
$T_b$	body temperature
TNZ	thermoneutral zone
UCP	uncoupling protein
$U_{mrv}$	mean rectified value (voltage)
$U_{rms}$	root mean square value (voltage)
$\dot{V}$	flow rate
$\dot{V}O_2$	oxygen consumption



## List of original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals and some original unpublished data.

- I Marjoniemi K, Hohtola E, Putaala A & Hissa R (1995) Development of temperature regulation in grey partridge (*Perdix perdix*). *Wildl Biol* 1:39-46.
- II Marjoniemi K & Hohtola E (1999) Shivering thermogenesis in leg and breast muscles of galliform chicks and nestlings of domestic pigeon. *Physiol Biochem Zool* 72:484-492.
- III Marjoniemi K & Hohtola E (2000) Does cold-acclimation induce nonshivering thermogenesis in juvenile birds? Experiments with Pekin ducklings and Japanese quails chicks. *J Comp Physiol* 170B:537-543.
- IV Marjoniemi K (2000) The effect of short-term fasting on shivering thermogenesis in Japanese quail chicks (*Coturnix coturnix japonica*): indications for a significant role of diet-induced/growth related thermogenesis. *J Therm Biol* 25:459-465.
- V Marjoniemi K, Saarela S & Hohtola E (2001) Shivering thermogenesis during forced bipedal exercise in three-week-old Japanese quail chicks (*Coturnix coturnix japonica*). Manuscript, submitted.

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# 1 Introduction

Mammals and birds are homeothermic and capable of sustaining their high body temperature in a cold environment. Endothermic homeothermy results both from aerobically supported high resting heat production rates in virtually all soft tissues, from thermoregulatory heat production and from prevention of excessive heat loss by insulation with fur or plumage (Ruben 1995). In contrast, the body temperature of poikilothermic animals corresponds passively with ambient temperature, ectothermic poikilothermy characterizing animals which have insufficient endogenous heat production for thermoregulation and which gain their internal heat from the environment.

The high resting metabolic rates of mammals and birds are thought to have evolved as a by-product of aerobically supported sustained activity. This sustained activity has been a beneficial characteristic in, for example, hunting a prey or escaping a predator, and it has been favoured by natural selection. Sustained activity was also a precondition for the postural change from sprawled to limb-supported upright posture in which continuous muscle twitches are utilized (Heath 1968). A high resting metabolic rate and high body temperature have not been advantageous features as such and indeed their maintenance has been a costly process in terms of energy consumed. Thus selection has not favoured high and controlled body temperature but it has certainly encouraged the sustained activity that such a temperature enables.

In juvenile mammals and birds, the ability to thermoregulate is rarely comparable to that found in adults. Smaller body size and weaker insulation lead to greater heat loss in cold, and regulatory heat production has a smaller capacity to increase body temperature. This thesis deals with thermoregulation in cold among young birds, especially with the development of heat production.

## **2 Endothermy and thermoregulation**

### **2.1 Ontogeny of endothermy**

#### ***2.1.1 Maturity of hatchlings***

The development of homeothermy is closely related to the maturational state of a hatchling. Newly-hatched chicks of different species of birds vary markedly in the maturity of many anatomical, physiological and behavioural aspects. In regard to hatchling maturity and the pattern of post-hatching development, birds are divided into two main groups: precocial and altricial. In addition, the terms “semiprecocial” and “semialtricial” are commonly used to name intermediate forms of the two main groups. Different further subdivisions are made by Nice (1962), Skutch (1976) and Starck (1993). The latter, for example, divides altricials and precocials into two and three subgroups, respectively, and names the most developed precocials as superprecocials (family Megapodidae). The precocial–altricial classification has also been adapted for mammals.

At hatching, precocial chicks have downy coats and well-developed nervous and muscular functions (e.g. Starck 1993). The chicks leave the nest soon after hatching, and they are frequently exposed to ambient temperatures below the thermoneutral zone. This is enabled by an ability to increase heat production soon after hatching (Matthew 1983). Semiprecocials also have downy plumage and open eyes, and they are able to walk outside the nest shortly after hatching. However, they differ from precocials in that they stay within the nest or nesting area for a longer time while food is brought to them by their parents (Starck 1993). The thermoregulatory ability of semiprecocials is comparable to that of precocials.

Altricial nestlings hatch totally or almost without plumage and eyes closed, and they exhibit little motor activity other than begging (Ricklefs 1973, Starck & Ricklefs 1998a). Altricials are totally unable to survive without parental feeding and heating. The body



temperature of altricials corresponds passively to changes in ambient temperature, indicating that altricials are poikilothermic and lack thermoregulatory heat production. Semialtricials have somewhat better insulative down, may hatch with their eyes open and show more movement activity.

Precociality and altriciality represent two different strategies for the allocation of energy to the reproduction. On the one hand, precocial eggs and chicks require large energy commitments from parents, the post-hatching energy investments going mainly into protection activity (Dunn 1980). Altricial species, on the other hand, require extensive energy input from the parents in the form of feeding and heating. Due to these different strategies, the stage of development at hatching, the post-hatching maturation speed, and the ability to move and thermoregulate, all vary considerably between the chicks of the two main patterns.

More quantitative bases for the classification of hatchling maturity have also been used. Carey *et al.* (1980) classified hatchlings based on the yolk content of the newly laid egg, a positive correlation being found between the hatchling maturity and the proportion of yolk in the egg. Yolk content is related to hatchling maturity because the development of the most mature forms requires a larger amount of energy in the form of yolk (Whittow & Tazawa 1991). Ricklefs (1983) and Starck and Ricklefs (1998a) presented the use of water content or the inversely dry matter content of a tissue as an index of the tissues functional maturity and more comprehensively as a way of describing the state of maturation of hatchlings. Lean dry matter content increases with age in nearly all tissues and species. Furthermore, it is usually well correlated with other functional measures such as enzyme activities in skeletal muscles, and it is also inversely correlated with growth rate.

### ***2.1.2 Post-hatching development***

Most juvenile birds have only a limited capability to maintain their body temperature in a cold environment. Small body size and weak insulation expose chicks to heat loss. Because of their limited ability to increase heat production, chicks tolerate ambient temperatures only slightly lower than the lower critical temperature (LCT). The post-hatching development of thermoregulation appears in the ability to sustain constant body temperature at gradually decreasing ambient temperatures and for a longer time period. The ability to both produce extra heat and to prevent heat loss during a cold spell are the basic factors of thermoregulation (for a review, see Visser 1998). In newly-hatched birds, the thermoneutral zone (TNZ) is narrow and in practice there may be just one thermoneutral temperature rather than a zone (e.g. Mathiu *et al.* 1991). During post-hatching development, the zone gets broader due to the development of insulative plumage and increased metabolism. The lower critical temperature, which determines the lower end of the thermoneutral zone, can be estimated from the equation

$$\text{LCT} = T_b - \text{RMR} / K, \quad (1)$$

where  $K$  is the minimum thermal conductance and RMR is the resting metabolic rate. Consequently, high RMR and low  $K$  results in low LCT. To maintain body temperature

unchanged in ambient temperatures lower than the LCT, the metabolic rate (MR) must be increased by means of regulatory thermogenesis equalling heat loss:

$$\text{MR} = K(T_b - T_a). \quad (2)$$

The magnitude of peak metabolic rate (PMR) and the time-period that it can be sustained for are important factors in regulatory thermogenesis. Although the opportunities for heat production and prevention of heat loss are limited, juvenile birds can partly compensate for these limitations by behavioural thermoregulation and the good endurance of hypothermia. Chicks also benefit from their low body temperature, which is lower than that of adult birds. The low body temperature entails smaller thermal gradient and consequently smaller heat loss to the surroundings.

Even though the embryos of some precocial species are capable of slightly increasing heat production in response to acute cold exposure (e.g. Steen & Gabrielsen 1988, Whittow & Tazawa 1991), the escape from the egg shell in hatching is the major step enabling increased ventilation, heat production by aerobic metabolism and thermoregulation (Mathiu *et al.* 1991). During internal and external pipping, the mechanism used for gas exchange passes from the chorioallantoic membrane to the lungs. Internal pipping occurs when the embryo accesses the egg air cell by piercing the chorioallantoic membrane and the inner shell membrane with its beak. In external pipping, the embryo breaks the eggshell with its egg tooth.

In precocial chicks, the capability for significant regulatory thermogenesis usually appears within a few hours of hatching, after the hatching down has dried. The prerequisite for the regulatory thermogenesis is the maturity of the skeletal muscles and the neuronal control of these muscles. In altricial birds, the required maturation level is achieved only during post-hatching development and therefore regulatory thermogenesis is not possible until several days after hatching. Precocial chicks benefit from the early attainment of homeothermy since it permits independent food seeking for longer foraging periods, thus enabling increased energy intake. However, this early homeothermy generally incurs higher metabolic costs for the chicks and thus limits their growth rate. The growth rate of precocial birds is 3–4 times slower than in altricial chicks of the same asymptotic body mass (Ricklefs 1979). High functional maturity seems to be incompatible with high growth rate. In altricial nestlings, energy is allocated mainly to growth at the cost of the maturity needed for thermoregulation.

In most precocial and semiprecocial birds, the mass-specific resting metabolic rate in thermoneutrality shows a swift increase during the first post-hatching days or week (Koskimies 1962, Freeman 1967, Palokangas & Hissa 1971, Bernstein 1973, Blem 1978, Hissa *et al.* 1983, Matthew 1983, Spiers *et al.* 1985, Klaassen & Bech 1992, Sutter & MacArthur 1992, Visser & Ricklefs 1993, Dietz *et al.* 1995). After the maximum is reached, the mass-specific resting metabolic rate decreases, finally approaching the level existing in adult birds. At the inflexion point, chick mass is approximately 25% of adult mass (Weathers & Siegel 1995). The physiological basis for the biphasic pattern is somewhat unclear. The increasing phase of the pattern is probably linked to an increase in the oxidative capacity of organs, an increase in the relative proportion of metabolic active tissues, the increasing mass and function of gut and heart, and the absorption of metabolically inactive residual yolk (Dietz *et al.* 1995). The subsequent decreasing phase

may be related to a decrease in the proportion of metabolically active tissue, due for example to the deposition of fat (Weathers & Siegel 1995). However, since the oxidative capacity of muscle tissue increases continuously during development (Choi *et al.* 1993, Dietz & Ricklefs 1995, Dietz & Ricklefs 1997), the heat production capacity of metabolically active tissue probably does not decrease after the inflexion point (Dietz *et al.* 1995). In altricial nestlings, the mass-specific resting metabolic rate usually increases linearly to the adult level (Weathers & Siegel 1995, Visser 1998). There is some evidence, however, that suggests that a biphasic pattern can also exist in large-sized altricials, e.g. in white-necked ravens, *Corvus cryptoleucus* (Mishaga & Whitford 1983).

The heat produced by the resting metabolism can only be used to a minor extent in sustaining a constant body temperature in ambient temperatures below LCT. The peak metabolic rate is more essential for this purpose. In newly-hatched altricials, the PMR equals the resting metabolic rate, indicating both the lack of regulatory thermogenesis and the fact that nestlings are poikilothermic (Weathers & Siegel 1995). Furthermore, cold exposure strongly reduces heat production when body temperature decreases. At some point in post-hatching development, nestlings attain the ability to increase heat production in response to cold exposure. For example, in bank swallows (*Riparia riparia*), this ability appears suddenly at the age of 8 days (Marsh & Wickler 1982) and in European starlings, *Sturnus vulgaris*, at 6 days of age (Ricklefs & Webb 1985). Newly-hatched precocial chicks, when exposed to moderate cold of 20°C, are able to increase their heat production 1.4–5 times above the resting metabolic rate (Visser 1998). During subsequent development, the increase in the PMR results from maturation and increase of body mass. In particular, the increase of muscle mass and the muscle-mass-specific heat production are important determinants.

Two major muscle groups can be found in birds, namely leg and flight muscles. Flight or breast muscles (*m. pectoralis* and *m. supracoracoideus*) constitute the largest and most energy-consuming tissues in adult volant birds (Marsh & Dawson 1989, Butler 1991). For most newly-hatched precocials and semiprecocials, the leg muscles are the principal site of regulative thermogenesis because of their larger mass and higher level of maturity in comparison to the breast muscles. For example, young galliforms seem to rely primarily on leg muscles in thermogenesis although they can fly at a relatively early age (Choi *et al.* 1993). Aulie and Grav (1979) calculated that even in 2-week-old bantam chicks, the total respiratory capacity of the leg muscles is 3.4 times higher than that of the pectorals. However, some exceptions may occur among precocials. In Procellariiformes, chicks have relatively large breast muscles whose dry matter content is similar to that of their leg muscles (Visser 1998). This finding possibly indicates that breast muscles also have a significant role in thermogenesis. During precocial post-hatching development, the breast muscles gradually grow larger in mass and assume the principal task in heat production. In newly-hatched capercaillie (*Tetrao urogallus*), the pectoral muscles comprise only 2.5% of the total heat production, but at the age of 80–105 d the pectorals already produce 21% of the total heat (Saarela *et al.* 1990). In altricial species, the breast muscles are important heat producing tissues when thermogenesis in cold appears, even though their mass may be smaller than (Morton & Carey 1971, Olson 1994) or equal to the mass of the leg muscles (Marsh & Wickler 1982, Ricklefs & Webb 1985).

At hatching, thermal conductance is dependent on the insulative structures and the size of the chick. In precocial chicks, minimum thermal conductance either decreases steadily

during post-hatching development (Hissa *et al.* 1983, Spiers *et al.* 1985, Sutter & MacArthur 1992, Gdowska *et al.* 1993) or most rapidly within the first post-hatching week (Eppley 1984, Ricklefs *et al.* 1984). The two latter studies showed that a significant decrease in conductance may occur without apparent plumage growth. The change is obviously due to an increase in the effectiveness of the vasomotor control of peripheral circulation. In altricial nestlings, the thermal insulation of the nest and huddling enable effective homeothermy for the whole brood even before the completion of plumage growth and before the individual nestling is homeothermic outside of the nest (e.g. Diehl & Myrcha 1973, Clark 1982, Mayer *et al.* 1982).

The development of temperature regulation can be described using the index of homeothermy,  $I_h$ , which represents the ability to maintain body temperature during cold exposure in relation to initial or adult body temperature:

$$I_h = (T_{final} - T_a)/(T_{initial} - T_a). \quad (3)$$

Theoretically, in totally poikilothermic chicks, the value of the index is 0 and in adult birds 1. The prerequisite for the use of the index is the standardization of both temperature and duration of cold exposure (Ricklefs 1987). In altricial species, the homeothermic index shows a three-phasic development that can be illustrated with a steep sigmoid curve (Shichun *et al.* 1979, Weathers 1996). In the first phase, the index remains unchanged. In the second, a rapid increase occurs, and in the third phase, the index levels off after a slight increase. These phases correspond to the three stages that Morton & Carey (1971) have observed in the homeothermic development of nestling Passeriformes. The first stage is a period of maximal growth when the nestling is totally dependent on parents as the heat source. The second stage is a rapid transition phase, and the third is a period of thermal independence when feather growth continues and mass increase ceases. In precocial turkeys (*Meleagris gallopavo*), guinea fowls (*Numida meleagris*) (Dietz & van Kampen 1994) and Muscovy ducklings (*Cairina moschata*) (Harun *et al.* 1997), the homeothermic index shows a quantum leap during first two post-hatching days and thereafter only a small increase occurs. In newly-hatched precocial shorebirds, the index increases with body size, partly reflecting increased thermal inertia with increasing body mass (Visser & Ricklefs 1993).

## 2.2 Thermogenic mechanisms

All living tissues produce heat as a by-product of the vital life-supporting metabolism. This obligatory minimum heat production associated with the cost of living is commonly estimated in animals by measuring the basal metabolic rate (BMR). The BMR does not include the costs of growth, feeding, processing of food, activity or thermoregulation since it is measured from resting, awake and postabsorptive animals at a thermoneutral temperature. The resting metabolic rate is another measure of obligatory heat production. It can be measured from animals resting in a thermoneutral environment but not being in a postabsorptive state. A substantial proportion (25–40%) of metabolic rate of free-living animals is attributed to the BMR (Hulbert & Else 2000).

Rolfe and Brown (1997) quantified various processes composing mammalian BMR. For the sum metabolism of various tissues, ~10% was estimated as being attributable to non-mitochondrial oxygen consumption and ~20% to oxygen consumption for maintaining the mitochondrial membrane potential against the leak of protons. The remaining 70% of oxygen consumption is used for: mitochondrial oxidative phosphorylation to provide energy for protein synthesis (~20–25%), maintenance of transmembrane  $\text{Na}^+$  (~20–25%) and  $\text{Ca}^{2+}$  (~5%) gradients, gluconeogenesis (~7%), ureagenesis (~2.5%), actinomyosin ATPase (~5%) and the rest for activities such as substrate cycling and nucleic acid synthesis. Although large differences exist in the magnitude of the BMR among different vertebrate classes, the percentage composition seems to be similar (Hulbert & Else 2000).

Obligatory thermogenesis is independent of short-term changes in the ambient temperature. In contrast, (thermo)regulatory thermogenesis occurs at temperatures below the thermoneutral zone in response to acute cold exposure. The purpose of regulatory thermogenesis is to increase heat production in cold to sustain body temperature despite increased heat loss. Regulatory thermogenesis can occur in muscle in the form of shivering thermogenesis, and in brown adipose tissue and possibly in some other tissues too as nonshivering thermogenesis.

### ***2.2.1 Shivering thermogenesis***

Shivering thermogenesis is regarded as “an increase in the rate of heat production during cold exposure due to increased contractile activity of skeletal muscles not involving voluntary movements and external work” (IUPS Thermal Commission 2001). Shivering is initiated and maintained by the neuronal system since there is no evidence of enhancement or maintenance of shivering by any blood-borne humoral factors (Mercer & Hammel 1993). Shivering is initiated by the same  $\alpha$ -motoneurons that act in voluntary muscle contractions and all shivering thermogenesis, as voluntary contractions, can be blocked by curare poison. The difference exists in the motor control, which in voluntary muscle contractions comes from the motor nuclei of the CNS, while in shivering the motor commands originate in the thermosensitive and integrating part of the CNS. Incipient shivering progresses from thermoregulatory muscle tone to micro-vibrations and eventually to clonic contractions or tremor of both flexor and extensor muscles. True shivering occurs only in mammals and birds (Heath 1968, Kleinebeckel & Klussmann 1990, Ruben 1995). At least in warm-acclimated individuals, it is the only means of regulatory heat production. The term “shivering” has also been used to describe the mechanism some insects use to elevate their thoracic temperature during pre-flight warm-up (e.g. Esch & Goller 1991). As in vertebrate shivering, this mechanism involves the use of muscle contractions for heat production without external work, but it is under the command of a completely differently organized nervous system. In addition, its biochemical basis for heat production may include separate metabolic routes.

The initiation of shivering requires cold stimuli, which may be effective at various sites of the body. For example, cooling of the skin, internal organs, hypothalamus, midbrain brain stem or spinal cord induce shivering in mammals (e.g. Cabanac 1975,

Simon *et al.* 1986). In birds, cooling of the hypothalamus does not stimulate shivering thermogenesis but in contrast, it may lead to the inhibition of shivering (Rautenberg *et al.* 1972, Snapp *et al.* 1977, Simon-Oppermann *et al.* 1978, Mercer & Simon 1984). Extrahypothalamic centers of the brain and spinal cord (Rautenberg *et al.* 1972, Inomoto and Simon 1981, Martin *et al.* 1981, Østnes & Bech 1992) and thermosensors in the peripheria, like skin cold-receptors, have a dominant role in eliciting shivering (Necker 1977, Simon *et al.* 1986, Østnes & Bech 1998).

The energetic efficiency of skeletal muscle work ranges between 20–25% (Wilkie 1960, Prompero *et al.* 1969, Gibbs & Gibson 1972, Tappy & Guenat 2000). Thus muscles liberate substantial amounts of heat as a by-product of the coupling of chemical energy into mechanical work. During shivering, virtually all the chemical energy of fuels consumed is transformed into heat. Thermogenesis in muscle is initiated by  $\alpha$ -motoneuronal stimulation following depolarization of muscle cell membrane and the release of  $\text{Ca}^{2+}$  from intracellular stores of the sarcoplasmic reticulum.  $\text{Ca}^{2+}$  results in activation of myosin ATPase and myofibrillar cross-bridge cycling. The increase of ADP in turn accelerates the mitochondrial oxidative phosphorylation increasing the combustion of fuels.

Ion pumping forms another process of heat production during muscle activity. The process involves restoration of the normal polarized state of the sarcolemma and removal of  $\text{Ca}^{2+}$  from the cytosol. The former is done by  $\text{Na}^+$ - $\text{K}^+$  ATPase and the latter principally via  $\text{Ca}^{2+}$  ATPase activity of the sarcoplasmic reticulum. Both processes in turn accelerate mitochondrial respiration by altering the phosphorylation state ratio. Some of the free  $\text{Ca}^{2+}$  in the cytosol is actively taken up by the mitochondria. In the mitochondria,  $\text{Ca}^{2+}$  stimulates respiration to enhance the rate of ATP production to match demand (McCormack *et al.* 1990). During prolonged heat production in muscle cells, ~25% of heat released is related to splitting of ATP into ADP and inorganic phosphate residue, and ~75% to the regeneration of ATP in mitochondrial oxidative phosphorylation (Hochachka 1974). Thermogenic reactions in muscle are reviewed in detail by Hochachka (1974), Himms-Hagen (1976), Homsher and Kean (1978), Woledge *et al.* (1985) and Block (1994).

Shivering closely resembles the normal isometric muscle contraction which occurs in postural tone (Hohtola 1981). Postural tone in mammals and birds is enabled by oxidative twitch-type muscle fibres, while in lower vertebrates it is maintained by slow and graded tonic fibres. The co-existence of sustained aerobic metabolism both in postural tone and shivering thermogenesis suggests that the origin of shivering arises from postural activity. A characteristic of shivering is an asynchronous firing pattern of the motor unit action potentials which prevents gross tremors and thereby possibly decreases convective heat loss (Hohtola and Stevens 1986). Tremors are not a prerequisite for thermogenesis although they emerge when the intensity of shivering increases in parallel with the augmented heat production.

Shivering can be quantified most accurately and easily by measuring the electrical events of a muscle (electromyogram, EMG). Electric currents (action potential, AP) in the muscle fibre are generated when the neurotransmitter acetylcholine is released from motoneuronal synapses. The AP moves along the muscle fibre at a speed of 2–5  $\text{m}\cdot\text{s}^{-1}$  and every part of the muscle fibre several centimeters long will meet the AP within a few milliseconds (Loeb & Gans 1986). The functional unit of the muscle, the motor unit

(MU), has a common  $\alpha$ -motoneuron which innervates all the muscle fibres of the MU simultaneously. The electrical signal emanating from the activation of the muscle fibres of a single MU is called a motor unit action potential, MUAP (DeLuca 1988). In a single MU, the MUAPs are generated repeatedly, the resulting sequence of MUAPs being termed a motor unit action potential train, MUAPT. The EMG, which is measurable with electrodes, is a result of the summation of MUAPTs in numerous spatially overlapping motor units.

West (1965) showed a linear relationship between the average peak-to-peak EMG and heat production in four species of birds. The use of various other EMG parameters in predicting heat production were systematically studied by Hohtola (1982) with pigeons. The most reliable parameter was obtained by rectifying and averaging the EMG signal, this mean rectified value is equivalent to the mean deviation or mean amplitude around the mean voltage. Another parameter, the root mean square voltage, equivalent to the standard deviation around the mean, was found to be almost as reliable. The correlation between the intensity of the EMG and heat production is not constant in a wide range of ambient temperatures (Hohtola 1982). At low temperatures, where metabolic rates are high, the correlation between the intensity of the EMG and heat production becomes weaker. Since the EMG electrode has a limited field in sensing myoelectric currents, the saturation of this field may be related to the decreasing correlation.

### ***2.2.2 Nonshivering thermogenesis***

Nonshivering thermogenesis (NST) comprises metabolic heat production by processes that do not involve skeletal muscle contractions (IUPS Thermal Commission 2001). NST is composed of both obligatory and thermoregulatory (facultative) components. Obligatory NST is independent of short-term changes in ambient temperature and it corresponds to basal metabolic rate. Thermoregulatory NST occurs below the thermoneutral zone in response to acute cold exposure. The term “nonshivering thermogenesis” is conventionally used to refer thermoregulatory NST and the same practice is adopted in this work.

A simple experiment which is used in mammals to reveal NST is the measurement of oxygen consumption and body temperature in the thermoneutral zone before and after noradrenaline injection. A parallel increase in oxygen consumption and body temperature in response to the injection indicates the existence of NST. In birds, this method cannot be successfully used since noradrenaline generally does not yield a calorogenic response (see Hissa 1988). Another way to reveal NST is to measure oxygen consumption and shivering intensity from muscles simultaneously and to compare the lower critical temperature and shivering threshold temperature (STT). Occurrence of STT lower than LCT is considered as evidence of NST.

In placental mammals, particularly in small-sized species and in neonatal and hibernating animals, the principal effector organ of NST is brown adipose tissue, BAT (Smith & Horwitz 1969, Janský 1973, Hayward & Lisson 1992). In cold-acclimated or cold-acclimatized individuals, NST in BAT replaces shivering thermogenesis and takes its role of “first line heat productive mechanism”. In mammals, BAT is located primarily in

the interscapular region overlying the cervical spinal cord and in smaller quantities in the thoracic, periaortic and perirenal positions (Smith 1964). Unlike white adipose tissue, BAT is characterized by a rich vascularization, which is responsible for its colour, a network of sympathetic fibers around every cell, multilocular adipocytes, and numerous mitochondria with dense cristae (Daniel & Derry 1969, Cannon & Nedergaard 1985). In response to acute cold exposure, the sympathetic nerve-endings of BAT release noradrenaline (NA). This NA binds to membrane  $\beta$ -adrenergic receptors, so stimulating lipolysis and the release of free fatty acids (FFA) (Nicholls & Locke 1984). FFAs released *in situ* are used as substrates for the respiratory chain and they apparently also activate thermogenesis in BAT. The mitochondrial uncoupling protein (UCP<sub>1</sub>, also called thermogenin) makes the mitochondrial inner membrane permeable to protons, so uncoupling the respiratory chain and oxidative phosphorylation. The activated uncoupling protein acts as a proton leak channel and when it short-circuits oxidative phosphorylation, the energy normally stored in the ATP is liberated as heat. Thermogenesis in BAT is also slightly stimulated via  $\alpha$ -adrenergic receptors (Mohell *et al.* 1987). In rodents, BAT thermogenesis contributes to the control of body weight, acting as the regulatory (facultative) part of diet-induced thermogenesis (Rothwell & Stock 1979, Himms-Hagen 1990). Similarly to acute cold exposure, overeating also activates BAT thermogenesis. The role of diet-induced thermogenesis in BAT is the conversion of excess energy into heat in order to prevent weight gain. BAT does not exist in marsupial and monotreme mammals (Hayward & Lisson 1992, Nicol *et al.* 1997, Rose *et al.* 1999) or in birds (Johnston 1971, Olson *et al.* 1988, Saarela *et al.* 1989, 1991).

Skeletal muscle has been proposed for the site of NST both in mammals and birds. However, the uncoupling of mitochondrial oxidative phosphorylation by UCP<sub>1</sub> has not been found outside of mammalian BAT. In birds, for example, UCP<sub>1</sub> is not even expressed in the skeletal muscles of those birds which have been suggested as candidates for possessing muscular NST (Denjean *et al.* 1999). Since 1997, new uncoupling protein homologs (UCP<sub>2</sub>, UCP<sub>3</sub>, UCP<sub>4</sub>, UCP<sub>5</sub>, avUCP, HmUCP) are found in various animal tissues including muscles and even in plants (Boss *et al.* 1998, Mao *et al.* 1999, Yu *et al.* 2000, Raimbault *et al.* 2001, Vianna *et al.* 2001). The two latter studies reported the discovery of avian UCPs that show 55% homology with UCP<sub>1</sub>. On the one hand, Raimbault *et al.* (2001) discovered avian-type UCP (avUCP) using cDNA libraries of chicken skeletal muscle. This avUCP is expressed exclusively in skeletal muscle. A high level of expression of avUCP mRNA was found in cold-acclimated and glucagon treated ducklings and in chickens with a high level of diet-induced thermogenesis. On the other hand, Vianna *et al.* (2001) reported the discovery of an uncoupling protein (HmUCP) from the swallow-tailed humminbird (*Eupetomena macroura*). The HmUCP mRNA is primarily expressed in skeletal muscle, but in addition in the heart and liver. Whether UCP<sub>1</sub> is the only true uncoupling protein is unsolved as yet (cf. Nedergaard *et al.* 1999). The uncoupling activity of other UCP homologs, including avUCP and HmUCP, have not yet been demonstrated. The recent finding that UCP<sub>1</sub>-ablated mice do not develop any NST but exhibit shivering when cold acclimated (Golozoubova *et al.* 2001) indicates that probably only UCP<sub>1</sub> can mediate adaptive NST in the cold.

The existence of NST in birds has been studied intensively during the last few decades. El-Halawani *et al.* (1970) studied the effects of cold acclimation on oxygen consumption and shivering in the gastrocnemius muscle of domestic chickens. Chickens



were not influenced by two-month cold acclimation but after 5 to 9 months shivering was greatly reduced while oxygen consumption was increased. This was regarded as evidence of NST. However, the responses of shivering intensity and oxygen consumption to acute cold-exposure were not studied and thus there was no evidence that *regulatory* NST really existed. Moreover, the ecological significance of NST achieved by such a long acclimation period can be questioned (Calder and King 1974). The most impressive array of evidence on muscular NST comes from cold-acclimated Muscovy ducklings (*Cairina moschata*) and king penguin chicks (*Aptenodytes patagonicus*) (for a review, see Duchamp *et al.* 1999). In these species, significant differences have been observed between the shivering threshold temperature and lower critical temperature (Barré *et al.* 1985, Barré *et al.* 1986a, Duchamp *et al.* 1989). In these studies, however, shivering was reported merely from gastrocnemius muscle, though unpublished recordings from pectoral muscle were briefly mentioned as showing a similar difference (Barré *et al.* 1985, Duchamp *et al.* 1989). Because cold acclimation may result in a shift of shivering to other muscles, the measurement of shivering merely from one muscle group is not extensive enough for revealing the existence of NST. In cold-acclimated Muscovy ducklings, Vittoria and Marsh (1996) also found that shivering in the gastrocnemius muscle was absent during cold exposure, but that in thigh muscles (*m. iliofibularis* and *flexor cruris*) muscle activity increased in parallel with the augmentation of the metabolic rate. Another possibility for the reason behind the disappearance of shivering, though only a hypothetical one since the matter has not been studied, is a shift of shivering to deeper regions of the same muscle.

It has been suggested that the endocrine control of avian muscular NST is based on the involvement of several hormones, including the glucagon, catecholamines and thyroid hormones (Duchamp *et al.* 1999). *In vivo* and *in vitro* experiments in some cold-acclimated bird species (muscovy ducklings, king penguin chicks, domestic chickens) indicate that these hormones are potential regulators for NST (e.g. Barré & Rouanet 1983, Barré *et al.* 1987, Eldershaw *et al.* 1997, Marmonier *et al.* 1997, Duchamp *et al.* 1999). The action of these hormones on the metabolism is generally dose dependent. It is also difficult to distinguish between the direct and indirect effects of these hormones on heat production. At the moment, the existence and mediation of avian NST has not yet been proved and the regulatory pathway has not yet been described. Two separate thermogenic processes have been proposed as accounting for avian muscle NST. The first one is based on the uncoupling of mitochondrial oxidation and phosphorylation (Barré *et al.* 1986b), and the second mechanism involves increased ATP-dependent sarcoplasmic reticulum  $\text{Ca}^{2+}$ -cycling (Dumonteil *et al.* 1994). Block (1994) suggested that the futile cycling of  $\text{Ca}^{2+}$  is a common feature in all kind of muscular thermogenesis. The only net effect of these futile cycles is the loss of ATP and release of heat either by alternating with passive flow of ions through membrane and active re-transport or by catalyzing chemical reactions between two substrates back and forth with different enzymes, e.g. between glucose and glucose-6-phosphate (Surholt & Newsholme 1983).

Both in birds and mammals, the resting muscle oxygen uptake (heat production) has been studied in perfused leg muscles. Muscle metabolism and performance are dependent on the regulation of the blood flow within the muscle. Vasoconstrictors, which increase the perfusion pressure in muscle, yield responses that can be divided into two types depending on their metabolic actions (Clark *et al.* 2000). In perfused rat hind muscle,

increased oxygen and nutrient uptake has been observed in response to the influence of noradrenaline, vasopressine and angiotensin II (Colquhoun *et al.* 1988, Tong *et al.* 1997, Newman and Clark 1998) and this is named type A response. Type B response results in decreased muscle oxygen consumption and nutrient efflux (Clark *et al.* 2000). It has been proposed that muscle has two distinct vascular routes, nutritive and non-nutritive, operating in parallel and regulated by vasoactive substances. The nutritive route is in close contact with muscle cells and the non-nutritive route functions as a vascular shunt leading the blood flow to the connective tissues and associated adipocytes (Clark *et al.* 2000). The nutritive/non-nutritive flow ratio has a great role in setting the basal metabolic rate. A high nutritive/non-nutritive flow ratio favours the acquisition of nutrients and hormones to the muscle cells and elevates the total metabolism in the muscle. However, when the non-nutritive flow is high, this favours the growth of fat tissue adjacent to the muscle. Although an increased nutritive flow *in vitro* results in increased muscular thermogenesis, this is unlikely to be thermoregulatory heat production but a part of obligatory heat production.

Specialized thermogenic tissues also exist in some fish species. Specialized heater cells warming the blood going to the brain and eye have been found in the modified eye muscles of the billfish, Xiphiidae and Istiophoridae (Carey 1982, Block 1987, Block 1994). These cells contain numerous mitochondria, hypertrophied T-tubule and sarcoplasmic reticulum membranes but lack organized myofibrillar structures and uncoupling protein. Heat production originates from intense  $\text{Ca}^{2+}$  cycling in the sarcoplasmic reticulum which is enabled by the rich content of  $\text{Ca}^{2+}$  ATPase in membranes. Heater cells have not been found in the tissues of birds or mammals.

### ***2.2.3 Postprandial excess heat production***

When a fasting animal begins to consume food, the metabolic rate quickly increases above the resting level. This postprandial excess heat production has been variously termed obligatory diet-induced thermogenesis (DIT), the thermic effect of food, specific dynamic action of feeding (SDA), the heat increment of feeding (HIF), or digestion-related thermogenesis (DRT). In a strict sense, many of these terms possess their own narrow specific meanings (see IUPS Thermal Commission 2001). In the literature, these terms are used in general as synonyms.

Postprandial heat production arises from complicated and combined metabolic reactions which are not clearly known. Heat production is dependent both on the amount of food digested and on the time elapsed since the meal. Augmented heat production has been explained as the obligatory utilization of ATP in the metabolic processing of ingested material (Himms-Hagen 1976). Postprandial heat production comprises several factors including: increased muscular activity; fermentation, hydrolysis and absorption in the intestine; neuronal and hormonal changes; increased active ion transport; increased protein turn-over in cells; and pharmacological effects of nutrients (Blaxter 1989). The magnitude of postprandial heat production is thought to be determined primarily by the rate of protein synthesis and turnover, thus reflecting the metabolic cost of growth (Jobling 1983, Carter & Brafield 1992, Janes & Chappell 1995). The magnitude of

postprandial heat production ranges from 30–31% of assimilated energy in protein to 13% in lipid and only 5–6% in carbohydrates (Harper 1971, Ricklefs 1974). Furthermore, postprandial heat production is believed to be mainly the result of the energy released from endogenous energy reserves since release of energy from ingested nutrients in the gut is slower (Schieltz & Murphy 1997). Vagal afferent signals obviously have a significant role in the onset of postprandial heat production (Székely 2000).

There are indications both in favour and against the idea of postprandial heat production substituting for regulatory thermogenesis in cold. The occurrence of substitution can be observed especially by comparing the metabolic rates of fed and starved animals at a wide range of ambient temperatures. The decrease in the ratio of the metabolic rate of fed animals to that of starved ones with decreasing ambient temperature indicates substitution. Bergman and Snapir (1965) compared the ratio in three breeds of domestic fowls and found a decline from 1.2 to 1.0 at ambient temperatures of 32°C and 16°C, respectively. Studies by Misson (1982) and Visser (1991), however, suggest that substitution does not occur in one or two-week-old domestic fowl chicks, respectively. In kestrels (*Falco tinnunculus*), about 50% of postprandial heat production substitutes for regulatory thermogenesis at temperatures below 10°C (Masman *et al.* 1989). Biebach (1984) found that substitution occurs in incubating starlings (*Sturnus vulgaris*) but Klaassen *et al.* (1989) did not find substitution in Arctic tern chicks (*Sterna paradisea*). More evidence supporting the role of postprandial heat production as a substitute for regulatory thermogenesis at least partially has been found in free-ranging verdins (*Auriparus flaviceps*) (Webster & Weathers 1990), in granivorous song birds (Meienberger & Daubenschmidt 1992), in blue grouses (*Dentragapus obscurus*) during winter (Pekins *et al.* 1992), in Adelie penguins (*Pygoscelis adeliae*) (Janes & Chappell 1995), in house wren chicks (*Troglodytes aedon*) (Chappell *et al.* 1997), in Brünnich's guillemots (*Uria lomvia*) (Hawkins *et al.* 1997) and in domestic pigeons during the night (Rashotte *et al.* 1997, Rashotte *et al.* 1999). There are also studies which support the idea of substitution in mammals, e.g. in golden hamsters (Simek 1975), in sea otters (*Enhydra lutris*) (Costa & Kooyman 1984) and in muskrats (*Ondatra zibethicus*) (MacArthur & Campbell 1994). However, the lack of substitution has been observed too, as in studies with short-tailed shrews (Platt 1974) and with star-nosed moles (*Condylura cristata*) (Campbell *et al.* 2000). As a result of all this evidence, it is justified to conclude that postprandial heat production is a true substitute for regulatory thermogenesis at least in some species and in some conditions.

Postprandial heat production is a part of the resting metabolism and is regulated more or less independently of thermoregulation (Schieltz & Murphy 1997). Some studies have also revealed that the active regulation of postprandial heat production for thermoregulatory purposes may occur during the night (Rijnsdorp *et al.* 1981, Rashotte *et al.* 1997). The storage of food in the crop for the night and the consumption of the food when necessary allows for postprandial heat production during the night in a regulated way, thus serving the needs of thermoregulation. The heat production arises from the processing of endogenous energy reserves in response to movements of bulk in the gut rather than from the processing of nutrients contained in the gut itself (Rashotte *et al.* 1997), a fact proved by the finding that even the feeding of non-digestible cellulose pellets results in increased heat production (Reinertsen & Bech 1994, Geran & Rashotte 1997).

### 2.2.4 Exercise thermogenesis

During shivering, mammals and birds apparently cannot utilize the full metabolic capacity of their muscles in thermogenesis since locomotion has been observed to have even more heat productive capacity (Marsh & Dawson 1989). Running and flying in birds can maximally yield metabolic rates from 10 to 12 times of the resting values, respectively, though more common values range between 5–10 times (Brackenbury 1984). Corresponding values for cold-induced thermogenesis are approximately 3–8 times the resting values (see Dawson & Whittow 2000).

Similar to postprandial heat production, exercise thermogenesis may be utilized for the substitution of regulatory thermogenesis in cold. As with postprandial thermogenesis, evidence is partly controversial. The extent of substitution varies among the species studied and with the ambient temperatures. Furthermore, all the information comes from adult animals. Signs in favour of substitution have been observed in budgerigars, whose flying oxygen consumption is constant between the ambient temperatures of 37–18°C (Tucker 1968) but whose resting oxygen consumption is doubled (Greenwald *et al.* 1967). In chaffinches (*Fringilla coelebs*), the differences between the oxygen consumption of voluntary activity and that of resting decreased between the temperatures of 32–5°C (Pohl 1969), indicating a partial substitution. In redpolls (*Carduelis flammea*), Pohl and West (1973) showed regulatory thermogenesis being replaced by exercise at very low temperatures (-30 to -45°C) during forced bipedal exercise. In higher temperatures (between -30 and 0°C) the costs of thermoregulation and exercise were additive. Further studies indicating substitution, partial or complete, have involved white-crowned sparrows (*Zonotrichia leucophrys gambelii*) (Ketterson & King 1977, Paladino & King 1984), Japanese quails (Nomoto *et al.* 1983a), free-ranging verdins (*Auriparus flaviceps*) (Webster & Weathers 1990, 2000), Gambel's quails (*Callipepla gambelii*) (Zerba & Walsberg 1992), free-ranging juncos (*Junco phaeonotus* and *Junco hyemalis*) (Weathers & Sullivan 1993), knots (*Calidris canutus*) (Bruinzeel & Piersma 1998), domestic pigeons (Hohtola *et al.* 1998), and Eastern house finches (*Carpodacus mexicanus*) (Zerba *et al.* 1999).

In two hummingbird species (*Amazilia cyanifrons* and *A. tzacatl*), Schuchmann (1979a) found the difference between the oxygen consumption during resting and hovering to be constant between the ambient temperatures 5–40°C indicating no substitution. Similarly, in another hummingbird, the booted racket-tail (*Ocreatus u. underwoodii*), substitution does not exist (Schuchmann 1979b). More studies indicating that the metabolic costs of thermoregulation and exercise are additive have involved white-throated sparrows (*Zonotrichia albicollis*) (Kontogiannis 1968) and dippers (*Cinclus cinclus*) (Bryant *et al.* 1985).

Exercise may result in an augmentation of heat loss due to increased forced convection. Goldstein (1983) observed the metabolic rate of Gambel's quail increasing linearly with increasing wind speed. A positive correlation between the metabolic rate and wind speed also occurs in verdins (Webster & Weathers 1988). When exercising and resting birds are compared in similar net convective conditions in cold, heat produced by exercise is completely used for substituting regulatory thermogenesis (Zerba & Walsberg

1992, Zerba *et al.* 1999). This may suggest that success in the prevention of excessive heat loss determines whether substitution can occur.

The existence of substitution suggests that exercise has an influence on shivering intensity. Exercise can affect shivering thermogenesis by inhibiting it either directly via neuronal interaction or indirectly via increased body temperature. Nomoto and Nomoto-Kozawa (1985) observed suppressed shivering due to exercise in domestic pigeons. Within one second after the onset of bipedal exercise on a treadmill, shivering in pectoral muscle decreased by 75%. Nomoto and Nomoto-Kozawa (1985) and Nomoto (1989) concluded that a direct neuronal inhibition is present. On the other hand, an exercising pigeon is capable of increasing shivering in the pectorals when the spinal cord is selectively cooled (Nomoto *et al.* 1983b, Nomoto & Nomoto-Kozawa 1985), which indicates that shivering can coincide with exercise. Hohtola *et al.* (1998) did not observe direct and abrupt inhibition of shivering in pectorals of pigeons during spontaneous walking or other types of voluntary movements (e.g. preening, feeding and pecking). Hohtola *et al.* (1998) suggested that startle reactions and direct neural inhibition may be involved in treadmill experiments where forced exercise is used. Hohtola *et al.* (1998) further concluded that voluntary movements may decrease the need for shivering indirectly by increasing internal heat production and body temperature. In humans, Hong and Nadel (1979) found graded inhibition of shivering during a pedaling exercise using a cycle ergometer, the inhibition increasing at any level of internal temperature when the intensity of exercise increased. The authors concluded that inhibition of a central origin exists and that exercise is accompanied with an integrated arousal response which has precedence over thermoregulatory activities. However, the study of Hong and Nadel (1979) also reports the simultaneous existence of exercise and shivering.

### **3 Outline of the thesis**

The aims of this study were to examine thermogenic mechanisms, their development and utilization in juvenile birds during the ontogeny of endothermy. The main focus of interest is shivering thermogenesis in muscle, when and where it develops and how its use is modified by the co-existence of the other forms of thermogenesis. The role of exercise, postprandial heat production and putative nonshivering thermogenesis is studied from this point of view. The perspective of the thesis ranges from the description of the ontogeny of thermogenesis and shivering to an investigation of the role of the other forms of thermogenesis. Paper I describes the endothermic development and attainment of homeothermy in the grey partridge. In Paper II, the development of shivering thermogenesis is studied in three precocial galliform species and in one altricial species. In Paper III, the existence of nonshivering thermogenesis after cold acclimation is investigated in Japanese quail chicks and domestic ducklings. In Paper IV, the participation of postprandial heat production in thermoregulation is examined in Japanese quail chicks. Paper V elucidates the role of exercise thermogenesis in thermoregulation of Japanese quail chicks. The specific subjects to be investigated were:

- 1) the efficiency of incipient shivering in heat production;
- 2) the utilization of nonshivering thermogenesis as a possible general phenomenon in cold-acclimated precocial chicks during cold spells;
- 3) the manner in which postprandial heat production influences shivering thermogenesis and the possible occurrence of substitution; and
- 4) exercise thermogenesis both as a potential benefit for young birds and as a possible influence on shivering.

## 4 Material and methods

Descriptions of materials and methods are presented in detail in the original papers. The following comprises a summary of the major experimental procedures and materials. However, the methods related to unpublished original data are presented in detail in this chapter.

### 4.1 Animals

Five species of birds were used in the experiments: grey partridge (*Perdix perdix*) (I, II), domestic fowl (*Gallus domesticus*) (II), Japanese quail (*Coturnix coturnix japonica*) (II, III, IV, V), domestic pigeon (*Columba livia*) (II) and Pekin duck (*Anas platyrhynchos*) (III). The age of the birds used varied from newly hatched to 39-day-old. Pigeons bred in outdoor aviaries and nestlings were fed and taken care of by their parents. The eggs of the other species used were incubated artificially and the hatched chicks were kept indoors (photoperiod 12L:12D) in plywood pens warmed by infrared lamps.

### 4.2 Temperature measurements

Ambient temperature ( $T_a$ ) (I–V), body temperature ( $T_b$ ) (I–V) and leg temperature ( $T_{leg}$ ) (III) were measured with copper-constantan thermoelements and recorded with a Yokogawa 3087 temperature recorder (I, II) or a Hewlett-Packard 34970A Data Acquisition/Switch unit connected to a PC (III, IV, V). Subcutaneous muscle surface temperatures from *m. pectoralis* were recorded in Japanese quail chicks with 0.7 mm copper-constantan thermoelements (TT-T-40, Omega, USA).

In domestic fowl chicks (Shaver), the development of body temperature and its diurnal rhythm was monitored from hatching for 43 days using 1.2 g XM-FH telemetric radio transmitters (Mini-Mitter Co., Sun River, USA). The transmitter contained an RC oscillator whose impedance and concomitant oscillation frequency was temperature dependent. The output frequencies of the transmitters were recorded using AM-receivers

(RA-1000-TH, Mini-Mitter Co.) connected to a PC. The transmitters, sealed in plastic capsules and coated with wax (Elwax) for protection against moisture, were calibrated in water baths at 31.7°C and 38.9°C.

Newly-hatched domestic fowl chicks were anesthetized with an intramuscular injection mixture of ketamine 25 mg·kg<sup>-1</sup> (Ketalar®, Parke-Davis S.A., Barcelona, Spain) and xylazine 2 mg·kg<sup>-1</sup> (Rompun®, Bayer AG, Leverkusen, Germany). The transmitters were implanted into the abdominal cavity through a small incision in the muscle layers of the abdomen. After implantation, chicks were placed to recover inside cages (60cm × 40 cm × 50 cm; length, width, height), each chick in a separate cage. Receivers were placed under the plastic floors of the cages. The cages were kept at a room temperature of 22°C. In one corner of each cage, a 150 W infrared lamp ensured a thermoneutral site inside the cage. After 12 h, three other chicks without transmitters were placed in each cage. The infrared lamps were gradually lifted as the chicks grew older. At the age of 21 d, two chicks without transmitters were removed from each cage.

### 4.3 Measurements of metabolic rate

Heat production was quantified with indirect calorimetry by measuring oxygen consumption either with a S-3A oxygen analyser (Applied Electrochemistry Inc.) (I–IV) or with a Servomex 1440C oxygen analyser (V) in a metabolic chamber. Oxygen consumption ( $\dot{V}O_2$ ) was calculated according to Hill (1972):

$$\dot{V}O_2 = \dot{V}(F_{in} - F_{out})(1 - F_{out})^{-1}m^{-1} \quad (4)$$

where  $\dot{V}$  is flow rate through the chamber,  $m$  is body mass,  $F_{in}$  is fractional oxygen content (0.20953) of the dry CO<sub>2</sub>-free inlet air, and  $F_{out}$  is oxygen content of the dry CO<sub>2</sub>-free outlet air. For calculation of the respiratory exchange ratio (RER), carbon dioxide production was also measured with a Servomex 1440C carbon dioxide analyser (IV). Oxygen consumption or carbon dioxide production was recorded either with a pen recorder (I, II) or with a Hewlett-Packard 34970A Data Acquisition/Switch unit connected to a PC (III, IV, V).

The metabolic rate was measured in either one or several constant ambient temperatures (I, IV, V) or during a slowly decreasing ambient temperature (II, III, IV). For the measurements, birds were resting in a non-postabsorptive state (I–V) or else metabolic rate was manipulated in part of the birds with either cold-acclimation (III), or fasting (IV) or forced bipedal exercise on a treadmill (V).

### 4.4 Ability to resist cooling

Grey partridge chicks aged 2–29 days (I) with dry or wetted plumage were exposed to an ambient temperature of 0°C for 20 minutes or until body temperature had decreased either to 25°C in chicks aged ≤ 11 days or to 30°C in older chicks.



## 4.5 Behavioural responses in temperature gradient

In grey partridge chicks aged 3–25 days, selection of preferred ambient temperature was studied in a temperature gradient created with infrared lamps (I). Movement activity in the thermal gradient was recorded with a video camera connected to a PC equipped with an image processing card and software. The activity counts in the four different parts of the gradient were used as an estimate of presence in different sections.

## 4.6 Measurements of shivering

Shivering EMGs were recorded from the pectoral (II–V), gastrocnemius (II–IV) and iliofibularis muscles (III). Measurements were performed using bipolar subcutaneous electrodes made of Teflon-coated multistranded steel wire. The EMG signals picked up with the electrodes were fed into differential amplifiers, displayed on an oscilloscope, converted either to mean rectified values ( $U_{mrv}$ ) (II) or to rectified mean square values ( $U_{rms}$ ) (III, IV, V) with analog integrators, and were recorded either with a pen recorder (II) or with a Hewlett-Packard 34970A Data Acquisition/Switch unit connected to a PC (III, IV, V). Samples of the unintegrated 1000-fold amplified raw EMGs were stored on C-cassette tapes using a stereo cassette deck (II, IV). Frequency spectra for tape-recorded EMG samples were calculated using the Fast Fourier Transform with a DATA 6000 signal analyser (Data Precision Inc.). In 21-day-old Japanese quails, intramuscular EMGs were recorded 5 mm deep from the thickest part of the breast muscle 5 mm laterally from the keel of the sternum with bipolar electrodes made of Teflon-coated multistranded steel wire (Medwire®, part 316SS7/44T, Mt. Vernon, USA).

## 4.7 Oxygen consumption of muscles *in vitro*

Muscle tissue samples were collected from the *musculus pectoralis*, *m. gastrocnemius* and *m. iliofibularis* (III). Respiration *in vitro* in muscle tissue homogenate was determined polarographically at a temperature of 25°C using a Clark-type oxygen electrode (Bachofer). The respiration was estimated as the activity of cytochrome *c* oxidase (COX) in tissue homogenates.

## 5 Results

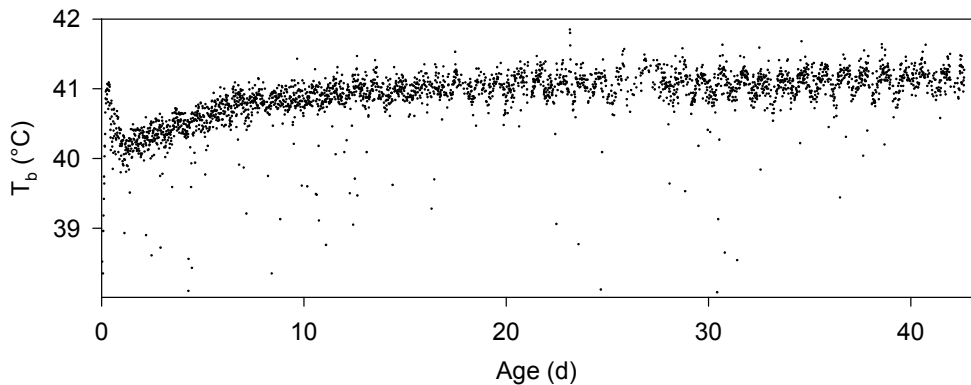
### 5.1 Ontogeny of endothermy

Cold tolerance improved with age and the increasing capacity to produce heat. Precocial birds obtained an ability to increase heat production in response to decreasing ambient temperature before or at the age of 1–2 days (I, II). The mass-specific heat production was age-dependent: a major increase in heat production occurred a few days after hatching, and thereafter the mass-specific heat production ( $\text{W}\cdot\text{kg}^{-1}$  or  $\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ) declined showing a biphasic pattern. In the grey partridge, the maximal mass-specific heat production was achieved before or at 5 days of age. In the Japanese quail, the corresponding age was 7 d. In the domestic fowl, the maximum occurred between days 1–2 and 7. The altricial species studied, the domestic pigeon, was able to increase heat production slightly in cold at 4 days of age but an intense thermogenesis did not occur until 6 days of age. During the 8 posthatching days observed, the mass-specific heat production increased smoothly. In all the species studied, both ambient temperature and age had a major effect on body temperature.

The wetting experiment in newly hatched grey partridge chicks (I) revealed that the ability to withstand cold ambient temperatures decreases dramatically if the insulation of plumage declines. The insulation of downy plumage was vulnerable to wetting, and at the age of three weeks its water impermeability was still improving although the insulation of dry plumage did not change appreciably. Due to the limited insulation of newly hatched chicks, the role of behavioural thermoregulation in resisting cooling was noticeable during the first two post-hatching weeks. In the experiment to study behavioural responses in the thermal gradient, chicks were found to be dependent on the external heat source. Moreover, the utilization of insulation in Japanese quail chicks was dependent on the nutritional state (IV). On the one hand, in chicks exposed to short-term fasting, the most crucial decline in conductance occurred before heat production started to increase during cold exposure. On the other hand, control quails increased insulation and metabolism simultaneously to defend their body temperature in cold. Furthermore,

behavioural thermoregulation in the form of huddling was used within 10 h from the beginning of the fasting.

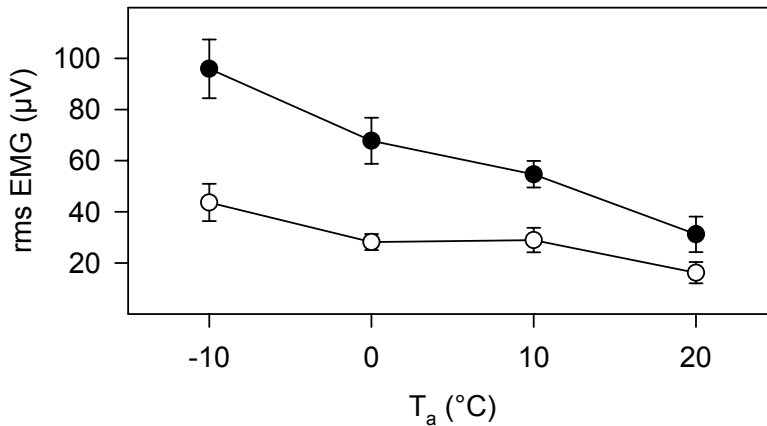
In all the species studied, body temperature measured from the cloaca increased gradually during the post-hatching development. Fig. 1 shows the development of body temperature in a domestic fowl chick between days 0–43. The major increase occurred during the first 10 days and thereafter body temperature increased only slightly but steadily. The diurnal rhythm in body temperature was only weakly visible soon after the hatching but the rhythm became more evident with age as the amplitude increased.



**Fig. 1. Development of body temperature recorded using telemetry in a domestic fowl chick (*Gallus domesticus*) between post-hatching days 0–43 at a room temperature of 22°C. The initial peak is probably due to a febrile response after the implantation of the transmitter (Marjoniemi and Hohtola, unpublished).**

## 5.2 Development of shivering thermogenesis

In precocial birds, first signs of shivering thermogenesis appeared in leg muscles, and in altricial pigeons in breast muscles (II). A clear increase in shivering amplitude during cold exposure occurred in breast muscles of pigeons, domestic fowl, quail and partridge at 2, 7, 7 and 10 d of age, respectively. In the gastrocnemius, the equivalent ages were 4, 1–2, 1 and 1 d. In the gastrocnemius of two-day-old pigeons, the amplitude of the EMG increased due to movement activity. The earliest shivering response observed in the pigeons and partridge did not result in significant heat production. The amplitude of shivering was dependent on the adult size being higher in small species. The amplitude of shivering varied within different locations of breast muscles of Japanese quail. Fig. 2 shows the amplitudes of integrated EMGs measured from surface and intramuscular locations at four different ambient temperatures. The amplitude of the EMG increased with decreasing ambient temperature in both locations, but the surface EMGs were more intense than the intramuscular ones.

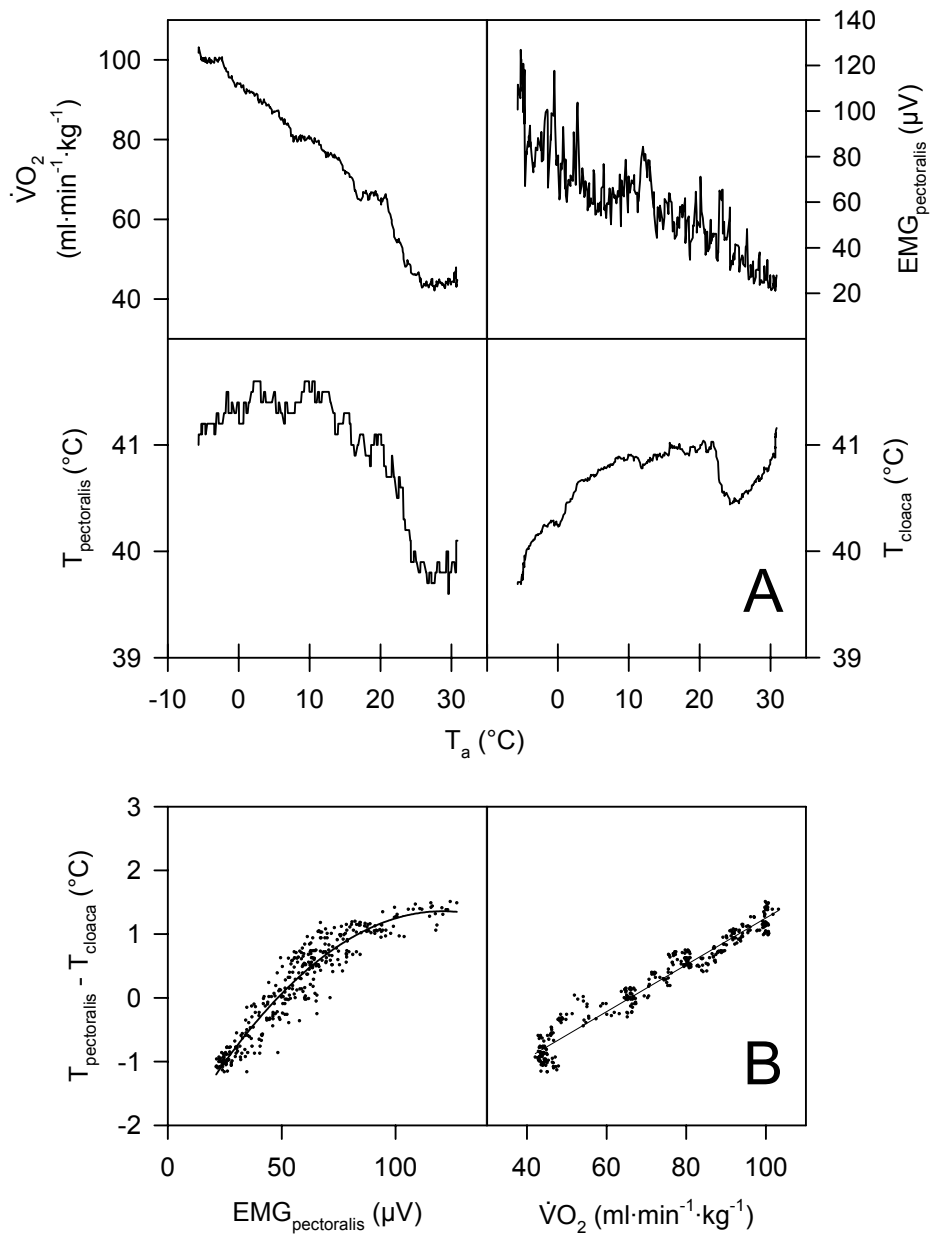


**Fig. 2.** The intensity of shivering EMGs in surface (solid circles) and intramuscular (open circles) locations of pectoral muscles of 21-d-old Japanese quail chicks at four different ambient temperatures.  $N = 4-5$  at each point. Vertical bars indicate SE. (Marjonemi, unpublished).

The shivering threshold temperature was found to be dependent on the muscle studied, on the age of the bird and on cold-acclimation (II, III) but not on the nutritional state of a chick (IV). In galliform chicks, except for the Japanese quail, shivering in the gastrocnemius started at a higher temperature than in pectoralis. During postnatal development the order changed towards a higher onset temperature for the pectoralis. In Japanese quail chicks, the shivering threshold temperatures did not differ between the pectoralis and the *m. gastrocnemius*. In the pigeon, shivering (or muscle activity in gastrocnemius) began at 2 d of age at the same temperature in the pectoralis and the gastrocnemius and thereafter shivering was always triggered at a higher temperature in the pectoralis.

The galliform chicks shivered in bursts and only in the fowl, from the age of 14 d onwards, was regular bursting or “true bursting” visible. In the pigeons, a bursting-type of shivering was the first form of shivering observed, but continuous shivering also developed parallel with the increased heat productive capacity. The shivering pattern was not influenced by the nutritional state of the bird (IV).

It was found that the size of the bird had an effect on the shivering frequencies. The median frequency of shivering had a tendency to increase with growth and maturation. Similarly, the frequency content of the EMG was dependent on the adult size, being higher in small species.



**Fig. 3. A:** Oxygen consumption, root mean square EMG of the pectoralis, surface temperature of the pectoral muscle and temperature of the cloaca during steadily decreasing ambient temperature ( $0.33^{\circ}\text{C}\cdot\text{min}^{-1}$ ) from  $32$  to  $-5^{\circ}\text{C}$  in a 21-day-old Japanese quail chick. **B:** Temperature difference between the pectoral muscle and the cloaca first versus rms voltage of the EMG and second versus the oxygen consumption (Marjoniemi, unpublished).

Slight 2–3°C hypothermia was observed to have no appreciable effect on the frequency content of shivering (II) while fasting clearly decreased shivering frequencies (IV). Fig. 3A shows the effect of shivering thermogenesis on the surface temperature of pectoral muscle and  $T_{\text{cloaca}}$  during a steadily decreasing ambient temperature in a 21-day-old Japanese quail chick. The temperature gradient between the muscle and the cloaca increased with increased shivering thermogenesis. The correlation of the temperature difference between the muscle and the cloaca to the rms voltage of the EMG was curvilinear and to the oxygen consumption linear (Fig. 3B).

### 5.3 Cold acclimation and nonshivering thermogenesis (III)

Three-week cold acclimation resulted in less changes in ducklings than in quails. In ducklings, no clear changes in the metabolism occurred *in vivo* during cold exposure, nor in measurements of cytochrome *c* oxidase activity in muscles *in vitro*. The most prominent induced changes were increased body weight, increased leg temperature in cold and a decrease in relative weights of liver and leg muscles. In the quail, cold acclimation induced changes in the metabolic rate (higher oxygen consumption in cold, a shift in the LCT from 23.8°C to 21.1°C, a lower shivering threshold temperature in *m. pectoralis* and higher respiration *in vitro*) and morphometric changes including an increase in body mass, relative weights of heart and intestine, and a decline in the water percentage of the *m. gastrocnemius*.

Cold-acclimated Japanese quail chicks and Pekin ducklings lacked clearly distinguishable NST. The amplitude of the shivering EMGs paralleled the increasing oxygen consumption in at least one of the muscles studied. In ducklings, the amplitudes of shivering were low (<30µV) both in the control and cold acclimated groups. In both species, the highest shivering amplitudes were recorded from cold-acclimated birds.

## 5.4 Modulation of shivering by the other forms of thermogenesis

The amplitudes of shivering EMGs measured during cold exposure were found to be dependent on the co-existence of postprandial thermogenesis (IV) or exercise thermogenesis (V).

### 5.4.1 Postprandial thermogenesis (IV)

After 29–35 h of fasting, the metabolic rate in the thermoneutral zone decreased 39%. As the ambient temperature decreased, so the difference in the metabolic rate between the control and the fasting group declined, the difference at 12°C being less than half of that observed at thermoneutrality. Furthermore, the body temperature decreased approximately 2°C due to fasting.

Fasted chicks increased their shivering thermogenesis to compensate for the decrease in postprandial thermogenesis. The amplitude of the shivering EMG increased in the pectoralis but the change was not significant in the *m. gastrocnemius*. The  $\dot{V} O_2$ -to-EMG ratio decreased due to fasting, also indicating compensation for the decreased postprandial heat production with shivering.

#### 5.4.2 Exercise thermogenesis (V)

After commencement of the bipedal exercise, the amplitude of shivering in the *m. pectoralis* decreased significantly within 20 s. Exercise suppressed shivering completely at the ambient temperature of 25°C, and partially with a quantity of 20μV at 15°C and 0°C. This indicates that exercise substitutes for shivering just as the increase in the  $\dot{V} O_2$ -to-EMG ratio during exercise does. The magnitude of change in the  $\dot{V} O_2$ -to-EMG ratio indicates that substitution is largest at 25°C and smallest at 0°C. The physical strain of exercise, measured as oxygen consumption, was dependent on the ambient temperature. Between 15°C and 0°C it increased from 72.3 to 143.7 ml·min<sup>-1</sup>·kg<sup>-1</sup>. Due to shivering suppression and increased forced convection, hypothermia developed the faster the colder the ambient temperature was: at a rate of 1.4, 3.3 and 5.3 °C·h<sup>-1</sup> at ambient temperatures of 25, 15, and 0°C, respectively.

## 6 Discussion

### 6.1 Ontogeny of endothermy

In the precocial chicks in the present study, the development of homeothermy was characterised by the early attainment of shivering thermogenesis in leg muscles and the use of behavioural thermoregulation (I, II). Chicks of the three galliform species studied, were endothermic at the age of 1–2 days or earlier, as soon as they were able to increase heat production in response to a decrease in ambient temperature. This timing of endothermy does not differ from that observed in earlier studies in Galliformes (e.g. Aulie & Moen 1975, Matthew 1983, Gdowska *et al.* 1993, Dietz & Kampen 1994). The metabolic rate was age-dependent, and the biphasic development of mass-specific resting metabolic rate, observed in precocial species (e.g. Visser 1998), was clearly visible in the grey partridges and the Japanese quails (I, II), the maximum occurring approximately at days 5 and 2–7, respectively. In Japanese quail, the earlier study by Freeman (1967) dated the maximum mass-specific RMR to the age of 5 days. In the domestic fowl, the maximum can be dated as occurring between days 1–2 and 7. The mass-specific metabolic rates ( $W \cdot kg^{-1}$ ) in chicks of two domestic fowl strains (Pilch and Isabrown/Warren) calculated from the data of Visser (1991), show that the maximum occurs approximately at 6 days of age, which is again in line with this present study. After the breakpoint, the decrease in the mass-specific metabolic rate most likely results from the decrease in mass of the metabolic active tissue/BW ratio (Dietz *et al.* 1995, Weathers & Siegel 1995) and from the growth rate (Klaassen & Bech 1992). The resting metabolism showed a positive correlation with the growth rate, possibly partially due to the high proportion of postprandial heat production during the highest growth phase.

The altricial species in the present study, the domestic pigeon, was endothermic at 6 d of age when a thermogenic response to the decline in ambient temperature was already apparent. The regulatory thermogenesis was enabled by shivering principally in the breast muscles. The appearance of endothermy is consistent with the development of brain temperature regulation in which the most rapid increase occurs during the first 5–6 post-hatching days (Arad 1989). In line with this Arad's work as well, is the study by



Ginglinger and Kayser (1929), where the pigeon nestling's ability to maintain a body temperature at mild cold ( $\sim 30^{\circ}\text{C}$ ) was observed to improve markedly between the post-hatching days 3–6. In addition, Koskimies and Lahti (1964) found that the cooling rate in the pigeon also decreases between days 0–2. However, since the pigeon lacks regulatory thermogenesis at that age, as was observed in this present study, this fall most probably is attributable to an increase of body mass and a decrease of conductance. The metabolic scope in the above three studies increased after the age of 6 days but in the present study the increase in mass-specific resting metabolic rate with age was not clearly visible. The data of the pioneer studies by Ginglinger and Kayser (1929) and Riddle *et al.* (1932) indicate that the metabolic rate reaches its maximum at 9–11 days of age and thereafter decreases. Thus it seems obvious that the development of the resting metabolic rate in the pigeon shows a similar biphasic pattern as in precocial species and is similar to that in another large-sized altricial species, in white-necked ravens (Mishaga & Whitford 1983). This development in the pigeon differs from small-sized altricials, where the mass-specific RMR generally increases from a low hatchling level more or less linearly to the high adult level (e.g. Dawson & Evans 1960, Shichun *et al.* 1979, Clark 1982, Olson 1992).

The ability to withstand cold ambient temperatures decreases significantly if insulation is lost. Wet plumage in small chicks leads to an increase in the cooling rate even though heat production is increased (Hissa *et al.* 1983). In precocial chicks, the ability to withstand cool ambient temperatures is attained (Aulie and Moen 1975) and subsequently locomotory activity is increased (Boggs *et al.* 1977) just when the hatching down dries. Even in such a good thermoregulator as the eider duckling, which is capable of increasing regulatory heat production as a late embryo, wet hatchling down offers very poor insulation (Steen & Gabrielsen 1988). A wet eider hatchling is unable to compensate adequately in cold for the increased heat loss but soon becomes exhausted. Steen *et al.* (1989) observed that only the drying of the down in eider chicks enables thermoregulation. In aquatic precocial chicks, hatching down, once dried, is no longer easily wetted. In contrast to that, the plumage of the grey partridge chicks of the present study remained vulnerable to wetting for weeks (I). At the age of three weeks, the water impermeability of the plumage was still improving although the insulation of the dry plumage was not appreciably getting any better. This indicates that especially during rainy and cool days, the chicks' ability to thermoregulate is poor in the wild in comparison with the laboratory conditions, and the chicks are absolutely dependent on their parents for warming.

Due to the limited insulation, the role of behavioural thermoregulation in resisting cooling was enhanced during the first post-hatching weeks (I). Chicks were found to be dependent on the external heat source, as in experiment to study behavioural responses in thermal gradient. With the attainment of thermal independence, movement activity also increased (I) as in chicks of the willow ptarmigan (Boggs *et al.* 1977, Pedersen & Steen 1979). The utilisation of insulation was also found to be dependent on the nutritional state, as was seen in the Japanese quail chicks (IV). In chicks exposed to short-term fasting, the most crucial decline in conductance occurred during cold exposure after which heat production started to increase, while in control quails increased insulation and metabolism simultaneously to defend their body temperature in cold. This is a novel finding, which suggests that in a normal nutritional state, chicks do not have to utilise

their full insulative capacity. It is also possible that postprandial heat production increases circulation in surface tissue layers and thus exposes chicks to greater heat loss (Misson 1982). The decreased conductance due to fasting has been observed previously, e.g. in domestic fowl chicks (Visser 1991). The decreased conductance in each individual chick and the further decrease due to mutual behavioural thermoregulation in the form of huddling, as seen in fasted Japanese quails (IV), result in energy sparing which obviously has a great value for survival during food deficiency.

In all the species studied in the present study, both ambient temperature and age had a major effect on body temperature (I, II, Fig. 1). The body temperature measured from the colon increased during the post-hatching development. In Galliformes, the most rapid change was observed simultaneously with the achievement of maximum mass-specific metabolic rate. In the pigeon, the development of body temperature in thermoneutrality made a quantum leap between post-hatching days 2–4. The study by Arad (1989) revealed that both in the development of brain and body temperature, the major change occurs within the first 5–6 post-hatching days. In domestic fowl chicks in the present study, the major increase occurred during the first 10 days and thereafter body temperature increased only slightly but steadily (II). This is consistent with the results obtained with bantam hen chicks published by Myhre (1978). Although a diurnal rhythm in body temperature was poorly visible in newly-hatched domestic fowl chicks (Fig. 1), it clearly exists in their movement activity (Höchel *et al.* 1999) and heart rate (Moriya *et al.* 1999). In muscovy ducklings (*Cairina moschata*), a diurnal rhythmicity in  $T_b$  is already apparent in one-day-old chicks (Nichelmann *et al.* 1999). Because internal rhythms usually develop with increasing amplitude, a small initial amplitude in  $T_b$  may not be apparent.

## 6.2 Development of shivering thermogenesis

This study is both one of the few systematic studies of the development of shivering thermogenesis in young birds and also the largest of them. The earlier studies have revealed that the first signs of shivering can be detected in hatchlings or even in late embryos in precocial birds, whereas in altricial nestlings shivering appears during later post-hatching development, (e.g. Whittow & Tazawa 1991). However, incipient shivering is difficult to observe without electromyography. Aulie and Moen (1975) did not observe visible shivering tremors in chicks of willow ptarmigan (*Lagopus lagopus*) before the age of two days, but this did not mean that younger chicks lack shivering thermogenesis since they were capable of increasing heat production slightly when exposed to cold. Obviously, they only lacked visible tremors. Subsequent EMG recordings from the chicks of willow ptarmigan revealed that newly-hatched chicks also produce heat by means of shivering (Aulie 1976). EMG recordings were used in the present study, and in all the species of studied this work, even the earliest measurements revealed myoelectrical activity (II). However, the beginning of shivering was apparent only when the amplitude of the EMG increased in response to cold exposure.

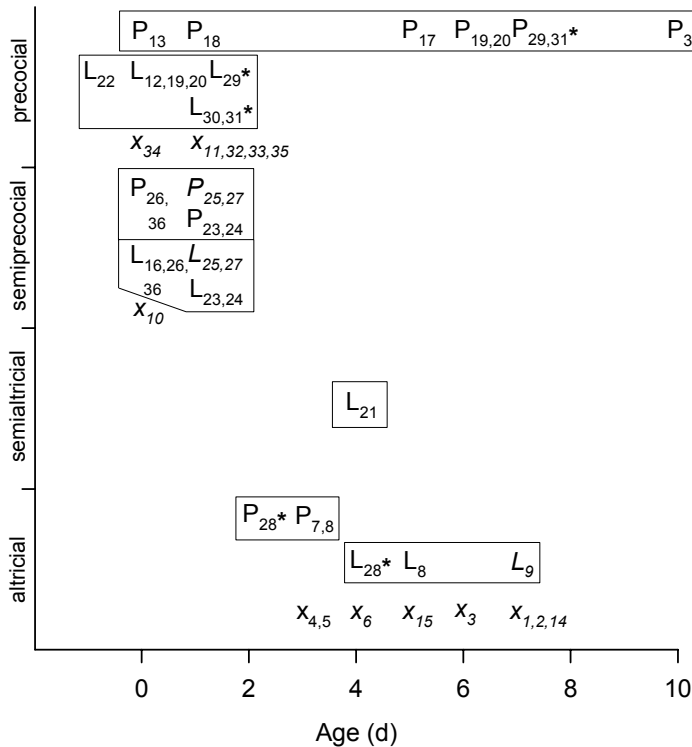


Fig. 4. Development of shivering thermogenesis in chicks of different development modes. Chicks are classified according to Starck and Ricklefs (1998b). Data is based on the direct observations of the age at which shivering is first observed. P = pectoralis; L = leg muscle; x = whole body; italics = visual or acoustic observations or palpation; normal font = measurements with vibrometry or electromyography; \* = data from the present study. References: 1) *Passer montanus* (Shichun *et al.* 1979), 2) *Iridoprocne bicolor* (Marsh 1980), 3) *Sturnus vulgaris* (Clark 1982), 4) *Troglodytes aedon* (Odum 1942), 5) *Parus atricapillus* (Odum 1942), 6) *Zonotrichus leucophrys* (Morton & Carey 1971), 7) *Delichon urbica* (Steen *et al.* 1989), 8) *Agelaius phoeniceus* (Olson 1994), 9) *Sula bassanus* (Montevicchi & Vaughan 1989), 10) *Anous stolidus pileatus* (Mathiu *et al.* 1991), 11) *Somateria mollissima*, (Myhre & Steen 1979), 12) *Somateria mollissima* (Steen & Gabrielsen 1988), 13) *Fulica americana* (Sutter & MacArthur 1992), 14) *Pelecanus erythrorhynchus* (Evans 1994), 15) *Gymnorhinus cyanocephalus* (Clark & Balda 1981), 16) *Larus delawarensis* (Dawson *et al.* 1976), 17) *Tetrao urogallus* (Hissa *et al.* 1983), 18) *Lagopus lagopus* (Aulie 1976), 19) *Meleagris gallopavo* (Dietz *et al.* 1997), 20) *Numida meleagris* (Dietz *et al.* 1997), 21) *Bubulcus ibis* (Hudson *et al.* 1974), 22) *Gallus domesticus* (Peters *et al.* 1961), 23) *Podiceps cristatus* (Keskpaik *et al.* 1968), 24) *Podiceps auritus* (Keskpaik *et al.* 1968), 25) *Larus occidentalis* (Dawson & Bennet 1981), 26) *Larus occidentalis* (Eppley 1987), 27) *Puffinus pacificus* (Mathiu *et al.* 1992), 28) *Columba livia* (Marjoniemi & Hohtola 1999), 29) *Gallus domesticus* (Marjoniemi & Hohtola 1999), 30) *Perdix perdix* (Marjoniemi & Hohtola 1999), 31) *Coturnix c. japonica* (Marjoniemi & Hohtola 1999), 32) *Tringa totanus* (Myhre & Steen 1979), 33) *Gallinago gallinago* (Myhre & Steen 1979), 34) *Gallus domesticus* (Randall 1943), 35) *Lagopus lagopus* (Myhre *et al.* 1975), 36) *Larus ridibundus* (Keskpaik & Davydov 1966).

All three precocial galliform species of the present study utilized mainly leg muscles in shivering thermogenesis during the first post-hatching week (II). This kind of utilization is obvious, because in newly-hatched precocials most of the muscle mass is in the legs, and leg muscles are also more mature in comparison to the pectorals (Aulie 1976, Ricklefs 1979). From the second week onwards, the pectorals were also recruited for shivering (II). Especially in domestic fowl, the shivering in the pectorals remained at a low level even at three weeks of age. In this species, the importance of leg muscles in heat production is obviously greater even in adults due to the very low proportion of aerobic muscle fibres in the pectorals observed by histochemical analyses (Barnard *et al.* 1982, Smith *et al.* 1993). In the present study, the amplitude of the shivering in the pectoral muscles was apparently dependent on the adult size being larger in small species (II), thus reflecting the negative correlation between the metabolic rate and the body mass. The adult body masses for both sexes of the domestic chicken (leghorn), grey partridge and Japanese quail are approximately 670–1020g, 380–450g and 100–150 g, respectively (Ichilcik & Austin 1978, Dunning 1992). In the present study with these three galliforms species, the shivering intensity level in the breast muscles was also observed to show a positive correlation with the adult bird's flight endurance, which depends on the level of oxidative metabolism of breast muscles.

In the altricial domestic pigeon, shivering in pectorals was present at two days of age and the pectorals had a predominant role in heat production (II). Breast muscles are also the principal site of thermogenesis from the beginning in Passeriformes (Olson 1994). In the young pigeons, there was also an evident cold-induced increase in the shivering amplitude in the leg muscles (II), which thus seem to have an assisting role in heat production during severe cold. This is an interesting observation, since generally, the role of the pectorals has been emphasized in the heat production of the altricial birds in the cost of the leg muscles. Despite the fact that EMG signs of shivering begin early in pigeons, intense thermogenesis appeared clearly later than in Galliformes (II). The appearance of shivering both in the precocials and altricial species of the present work are consistent with many earlier fragmental observations and few systematic measurements of shivering in young birds, which are now first time comprehensively reviewed (Fig. 4). Several conclusions of the ontogeny of shivering can be done from the figure. 1) Both in precocials and semiprecocials, shivering appears at a late embryo stage or soon after hatching. 2) In most precocials, the beginning of shivering in leg muscles clearly precedes the onset in the pectorals. 3) In semiprecocials, shivering in breast muscles coincides with the onset in legs, or only a few hours later. 4) In semialtricial and altricial nestlings, the beginning of shivering clearly occurs later than in precocial and semiprecocial chicks. 5) Moreover, in semialtricial and altricial development modes, the onset of shivering in the pectorals precedes the beginning of shivering in leg muscles.

In order to compare the shivering intensities of different species and of different individuals within a species, the standardization of the attachment site of the recording electrode is essential. For example, in EMG measurements with bipolar wire electrodes, the recording site has been found to result in more variation than the electrode material or its dimensions (Gans & Gorniak 1980). Similarly in the breast muscle of Japanese quail chicks in the present study, the amplitude of the shivering at the surface and intramuscular locations varied (Fig. 2): the amplitudes of the EMG increased with decreasing ambient temperature in both locations, but the surface EMGs were

consistently more intense than the intramuscular ones. Moreover, in the gastrocnemius muscle, the subcutaneous electrodes on the surface of the muscle yielded more intense EMG amplitudes when placed near the distal end of the muscle than when they were placed on the middle of the muscle (personal observation). The reason for the variation of the amplitude may be due to fact that the proportion of different types of muscle fibres varies within muscle (e.g. Rosser & George 1986) as well as the distribution of muscle fibres in motor units (the fibres of some motor units may be clumped and in others distributed), so resulting in different EMG amplitudes in different locations. Moreover, the damage in muscle tissue due to implantation of the electrode inside muscle, may affect the recorded EMG intensity (c.f. Fig. 2). Therefore comparisons of shivering intensities between individuals should be made due caution and an awareness of these and other potential error sources.

Measurements in 21-day-old Japanese quails verified that shivering truly produced an increase in muscle temperature (Fig. 3A). The correlation of the temperature difference  $T_{\text{muscle}} - T_{\text{cloaca}}$  to rms voltage of the EMG was curvilinear and to oxygen consumption linear (Fig. 3B), just as also Hohtola (1982) observed in adult pigeons. Thus the present study confirms that direct muscle temperature recordings quantify shivering thermogenesis even more reliably than EMG recordings. In adult birds, the amplitude of EMG usually correlates well with the heat production or oxygen consumption (Hohtola 1982). The present study clearly showed that both in altricial and precocial hatchlings an early cold-induced increase in shivering does not necessarily cause a significant increase in heat production (II). For example, in one-day-old partridges and 2 to 4-day-old domestic pigeons, the EMG intensity increased in cold without a simultaneous measurable augmentation in heat production. Thus, even before muscles are mature enough for thermogenesis, their motoneuronal control and myoelectrical control of shivering are functional. In precocial birds, motoneuronal maturation seems to occur during the embryo stage. For example, in Japanese quail chicks, the motor end plates are stained when using the acetylcholine esterase staining method (see Karnovsky & Roots 1964) already immediately hatching both in the pectoral and leg muscles, thus indicating the mature synaptic junctions (Marjoniemi & Hohtola, unpublished). However, not only motoneurons, but also muscles have to reach a certain minimal level of maturation before thermogenesis is enabled. A water fraction of  $\leq 0.85$  in skeletal muscles seems to be the bottom limit for heat production in these muscles in many species (Ricklefs & Webb 1985, Dietz *et al.* 1997, Visser 1998). In four-week-old Pekin ducklings and three-week-old Japanese quails, all muscles both in cold-acclimated and warm-acclimated individuals were clearly below this fraction (III). Furthermore, all muscles also showed increased shivering activity in cold which was accompanied by increased heat production.

### 6.3 Cold acclimation and nonshivering thermogenesis

The existence of NST in young birds has been studied almost merely in Muscovy ducklings and king penguin chicks. Over ten years ago, Connolly *et al.* (1989) called for rigorous studies to confirm the existence of avian NST in other bird species. However, before the present work, no new species have been used. The putative development of

NST as a result of cold acclimation includes the assumption that NST gradually replaces shivering. In the studies performed with Muscovy ducklings and king penguin chicks, the absence of shivering thermogenesis was reported merely for the gastrocnemius muscle. Nevertheless, as the study by Aulie and Tøien (1988) in domestic chickens revealed, different muscles can have different threshold temperatures for shivering; in the leg muscles (*musculus iliotibialis*) of the chicken, shivering was observed to appear at 32°C but in breast muscles only at 20°C. This finding was confirmed by Carey *et al.*'s (1989) observation in winter acclimatized adult house finches (*Carpodacus mexicanus*), that the thermal thresholds in leg muscles for the onset of shivering were substantially below the thresholds for the onset in the pectoralis; in pectoralis shivering appeared approximately at the ambient temperature of 20°C, while in the gastrocnemius, tibialis and peroneus muscles the corresponding temperatures were -5, -11, and -14°C, respectively. The conclusion one has to draw is that the possibility for different threshold temperatures in different muscles should always be taken into account and all shivering should be excluded before the lack of shivering can reliably be interpreted as evidence of NST. For example, Vittoria and Marsh (1996) showed with more extensive EMG measurements that in cold-acclimated Muscovy ducklings, shivering is present in two thigh muscles (*musculus iliofibularis* and *m. flexor cruris lateralis*) even when it is absent in gastrocnemius. This finding suggests that shivering is probably the major source of regulatory heat production in cold-acclimated Muscovy ducklings too.

In the present work, oxygen consumption increased concomitantly with the EMG activity both in the warm and the cold-acclimated birds in at least one of the muscles studied (III). Thus all the heat production can be explained by shivering thermogenesis and no evidence for the existence of NST was found. The intensity of the EMG did not decrease due to cold acclimation but in contrast, it increased. One could claim that the chicks were only partially cold acclimated or that the chicks had grown past or had not yet reached the stage where the capacity of NST is most evident. However, the biological significance of NST occurring in a very short age-period and yet requiring a long exposure to cold is difficult to conceive. Nevertheless, it is possible that with a lower acclimation temperature NST could have developed. However, the intensity of cold-exposure used in cold acclimation should be "ecologically sensible" and similar to that in the wild. For most newly-hatched chicks, continuous three-week cold acclimation at 5°C is far removed from their natural conditions. Moreover, a severe hypothermia caused by a single period of severe cold exposure may increase uncoupled respiration (Skulachev & Maslov 1960). By exposing sheared domestic pigeons to an ambient temperature of -15°C and by letting birds' body temperatures fall to 5–10°C, these authors were able to increase mitochondrial uncoupled oxidation artificially. However, it is questionable if NST thus obtained has any biological significance and if increased uncoupled oxidation is just a result of damage in mitochondrial membranes.

In adult winter-acclimatized pheasants and grey partridges, Hohtola *et al.* (1989) found no clear cold-induced shivering in breast or leg muscles even at -30°C, although oxygen consumption was increased. In Hohtola *et al.*'s study, because the temperature of the major muscle groups was lower than the colonic temperature and because the thermal gradient between the colon and the muscles even increased in cold, they concluded that the major muscle groups did not participate in cold-induced thermogenesis by shivering or any other heat productive mechanism. This finding suggests that in adult birds, a high

degree of localisation of shivering may exist or alternatively a non-muscular regulatory source of heat may exist. In the chicks of the present study, shivering increased as also the oxygen consumption (II–V). Furthermore, the experiments in which the muscle temperatures were measured showed an increase in the electrical activity of the muscles with a simultaneous increase in the muscle-body temperature gradient (Fig. 3). Therefore, it is justified to conclude that shivering was truly the mechanism, which produced the thermoregulatory heat.

The studies by Saarela and Heldmaier (1987) and Saarela *et al.* (1995) with adult Japanese quails and European finches (*Carduelis* sp.), respectively, showed that in extreme cold (from -40 to -60°C) the intensity of shivering levelled off or even began to decrease while oxygen consumption was still increasing. The authors raised the possibility that increased  $\dot{V}O_2$ -to-EMG ratio indicates the existence of NST acting as a secondary source of heat and supporting shivering thermogenesis in severe cold. A similar change in the  $\dot{V}O_2$ -to-EMG ratio was observed in the cold-acclimated Japanese quail chicks of the present study too (III). However, Hohtola (1982) found in pigeons that the correlation between the mean rectified voltage of the EMG and the metabolic rate decreased with decreasing ambient temperature. This decrease may reflect the saturation of the limited field sensed by the electrode. Recruitment of motor units farther from the recording site in the same muscle or different muscles is thus undetectable. Theoretically, when the intensity of shivering increases, negative and positive currents of the myoelectric signal are more likely to contact measurement electrode at the same time and thus cancel each others electrically. Any prediction of total muscular heat production based on recordings of the EMG from a single muscle has to be made with caution. As Hohtola *et al.* (1998) emphasised, the conclusion on the presence of NST should not be drawn only on the basis of the changes in the  $\dot{V}O_2$ -to-EMG ratio before more direct explanations can be ruled out. In any case, it remains clear that shivering was the first line defence mechanism against cold in the species of the present work.

#### **6.4 Adjustment of shivering thermogenesis by postprandial heat production and exercise**

Thermal by-products of feeding and locomotion may be of benefit in energy saving especially in juvenile birds. The present study clearly showed that the intensity of shivering is dependent on the co-existence of postprandial heat production or exercise thermogenesis (IV, V). Shivering as a form of regulatory heat production is a flexible mode of thermogenesis and its magnitude can be adjusted according to the magnitude of obligatory thermogenesis. This adjustment works towards energy saving and the avoidance of the summation of different modes of heat production. Moreover, in mammals possessing BAT, nonshivering thermogenesis adjusts the use of shivering indirectly via body temperature. It has been found that if NST alone is insufficient to maintain a constant temperature in the upper spinal cord, then shivering is initiated (Heldmaier *et al.* 1990). This finding suggests that the use of NST in BAT adjusts the use of shivering as well. The indications in favour of the existence of NST in birds also show shivering being flexibly modulated by putative avian NST, use of which precedes the use

of shivering. If no adjustment occurs, the nervous system controlling shivering would not respond to the increased heat production and to the changed thermal balance of the body.

The potential for the greatest thermal benefit from postprandial heat production exists in small-sized endothermic animals that consume relatively large high-protein diets and inhabit thermally stressful microhabitats (Campbell *et al.* 2000). Young precocial chicks fulfil these criteria by feeding mostly on insects and other invertebrates and by being frequently exposed to temperatures below the thermoneutral zone (e.g. Chappell 1980). The present study clearly showed that fasted Japanese quail chicks used shivering to compensate for the decrease in postprandial heat production (IV). In other words, the decreased shivering in the *ad libitum* fed chicks means that postprandial heat production has the capacity to substitute for shivering thermogenesis. In a review on the energetics of postnatal growth, Weathers (1996) concluded that postprandial heat production has no value for growing chicks because feeding stimulates peripheral circulation and thus facilitates heat loss. However, this worthlessness may be the case in thermoneutrality only where postprandial heat production is equivalent to excess heat which must be dissipated in order to maintain thermal balance and to avoid an increase in body temperature. In contrast to thermoneutrality, in cold, where additional heat production is needed, postprandial heat production may be gradually harnessed to thermoregulation until its whole capacity is used and in addition regulatory thermogenesis is also needed. The results of the present study indicate that also during postprandial heat production, shivering is adjusted indirectly via changed body temperature. The successful use of postprandial heat production in thermoregulation possibly necessitates an ability to adjust thermal conductance. In the Japanese quails in the present study, the dissipation of postprandial heat was seen in the differences in the thermal conductance of the control and fasting groups (IV). Because the magnitude of postprandial thermogenesis cannot be actively regulated, its proportion used for thermoregulatory purposes is regulated by the use of insulation. This hypothesis is supported by the fact that in thermoneutrality, the thermal conductance was higher in fed chicks but in cold there were no differences between the fed and fasted chicks. Thus it is possible that the successful use of postprandial heat production is dependent on the insulative capacity available and in birds with poor insulation, as in newly hatched altricials, postprandial heat is lost to the surroundings.

Although breast muscles do not participate in bipedal exercise in any way, bipedal exercise interferes with the shivering in these muscles (V). In pigeons, the inhibition of shivering is initiated by afferent input from leg muscles and sole skin (Nomoto 1989); from femoral muscles, the inhibitory neural circuit mediating this inhibitory response via afferents includes at least one interneuron in the spinal cord. In the present study, similarly to exercising pigeons in cold (Nomoto & Nomoto-Kozawa 1985), shivering increased during exercise in response to decreased body temperature in the Japanese quail chicks. It seems obvious that during exercise, shivering is adjusted first via inhibitory nervous circuits and later augmented indirectly via the activity of peripheral thermoreceptors in response to a decrease in body temperature. Body temperature may also increase when the exercise is intense and only some of the excess heat is dissipated to the surroundings (e.g. Brackenbury *et al.* 1981, Nomoto *et al.* 1983a, Nomoto *et al.* 1983c). It is obvious that in exercising birds, increased body temperature results in a decrease in shivering, just as in resting birds. However, Nomoto and Nomoto-Kozawa



(1985) showed with exercising domestic pigeons that when the spinal cord is cooled with thermodes, intense shivering is initiated in the pectorals, but after termination of the cooling the shivering intensity decreases.

In the present study, due to suppression of shivering and to increased forced convection during exercise, hypothermia developed faster the colder the ambient temperature was (V). Although exercise interacted with regulatory thermogenesis, partially inhibiting it, the benefit of exercise, if any, was restricted to temperatures slightly below thermoneutrality. By optimizing the duration of exercise and the speed of locomotion to the prevailing ambient temperature, a better benefit may be possible. However, this hypothesis has not been studied. Moreover, extremely few experiments concerning the substitution of facultative heat production by exercise in juvenile birds can be found in literature. The data from the studies which do exist, indicate that exercise has only a little benefit for thermoregulation as the present study also indicates. For example, Modrey and Nichelmann (1992) observed that voluntarily exercising turkey chicks aged 1–10 days prefer 1.5–2.5°C lower  $T_a$  than resting birds. This preference may indicate a partial substitution in temperatures only slightly below thermoneutrality. In free-living arctic shorebirds chicks, Chappel (1980) found no significant benefit from exercise heat at ambient temperatures 3–7°C.

The benefit of exercise thermogenesis can be divided into energetic and thermal advantages. The energetic advantage results from the substitution of shivering with exercise heat production. The thermal advantage depends on the fate of body temperature as a result of exercise. However, energetic advantage may accompany a thermal disadvantage, as observed in exercising adult rats (Mäkinen *et al.* 1996). In the Japanese quails chicks of the present study, exercise was not utilized in thermoregulation in cold either in an energetically or a thermally favourable way. In some adult birds (see section 2.2.4), the energetic advantage and the thermal advantage coexists even though the birds are small sized. The co-existence of these advantages may suggest that the potential for substitution is always present and that success in prevention of heat loss during exercise determines whether substitution is complete, partial or non-existent.

## 7 Conclusions

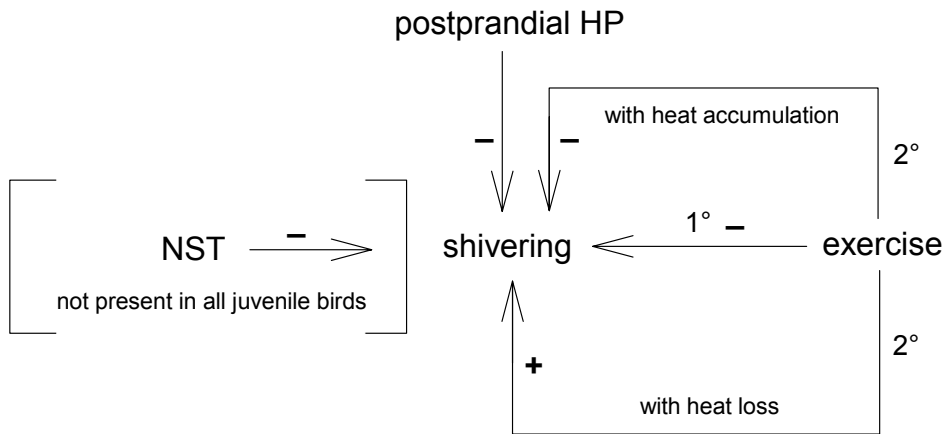
The development of shivering thermogenesis is the determinant for the beginning of endothermy in juvenile birds. The heat productive capacity of shivering develops gradually. Depending on the species, incipient shivering may have only a small capacity in sustaining body temperature in cold.

The occurrence of shivering has to be ruled out with careful and extensive measurements of the EMG before the existence of avian NST can be proved. It seems obvious that the occurrence of NST is not a general phenomenon in juvenile birds. Until further evidence can be provided, the existence of NST in young birds remains doubtful. Consequently, shivering thermogenesis is probably the only mode of regulatory heat production in juvenile chicks.

At least in some juvenile birds, postprandial heat production can be used successfully to replace shivering thermogenesis at least partially in cold, as was shown in the present study with Japanese quail chicks.

Exercise, although possessing heat production potential, is a poor source of compensatory heat production for shivering in young birds since it results in excessive heat loss due to weak insulation.

Interactions between shivering thermogenesis and other forms of thermogenesis are summarised in Fig. 5. Shivering is a flexible mode of thermogenesis and its magnitude can be adjusted according to the magnitude of obligatory thermogenesis. The adjustment works towards energy saving by avoidance of the summation of different modes of heat production. The prerequisite for successful adjustment of shivering is adequate insulation, whose role in preventing excessive heat loss is pronounced during exercise. Thus the energetics of posthatching thermoregulation includes the potential for optimization of energy use in order to avoid dissipation of waste energy as heat.



**Fig. 5. Observed and suggested interactions between shivering thermogenesis and other forms of thermogenesis in juvenile birds. + = augmentation, - = inhibition, 1° = direct neuronal action, 2° = indirect action via changed body temperature.**

## 8 References

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