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COMMENTARY

The evolution of endothermy is explained by thyroid hormone-mediated responses to cold in early vertebrates

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ABSTRACT

The evolution of endothermy is one of the most intriguing and consistently debated topics in vertebrate biology, but the proximate mechanisms that mediated its evolution are unknown. Here, we suggest that the function of thyroid hormone in regulating physiological processes in response to cold is key to understanding the evolution of endothermy. We argue that the capacity of early chordates to produce thyroid hormone internally was the first step in this evolutionary process. Selection could then act on the capacity of thyroid hormone to regulate metabolism, muscle force production and cardiac performance to maintain their function against the negative thermodynamic effects of decreasing temperature. Thyroid-mediated cold acclimation would have been the principal selective advantage. The actions of thyroid hormone during cold acclimation in zebrafish are very similar to its role during endothermic thermogenesis. The thyroid-mediated increases in metabolism and locomotor performance in ectotherms eventually resulted in sufficient heat production to affect body temperature. From this point onwards, increased body temperature per se could be of selective advantage and reinforce thyroid-induced increases in physiological rates. Selection for increased body temperature would promote those mechanisms that maximise heat production, such as increased Na^+/K^+-ATPase activity, futile cycling by SERCA, and mitochondrial uncoupling, all of which are regulated by thyroid hormone. The specific end point of this broader evolutionary process would be endothermic thermoregulation. However, considering the evolution of endothermy in isolation is misleading because the selective advantages that drove the evolutionary process were independent from endothermy. In other words, without the selective advantages of thyroid-mediated cold acclimation in fish, there would be no endotherms.

KEY WORDS: Thermoregulation, Locomotion, Metabolism, Heat production, Aerobic capacity

Introduction

The advantages that have led to the evolution of an endothermic physiology are still under debate. A widely accepted model is that directional selection for incremental increases in aerobic capacity and sustained activity led to the evolution of endothermy (the 'aerobic capacity' model) (Bennett and Ruben, 1979; Nespolo et al., 2011). The greater scope for physical activity means that endotherms tire less quickly and are therefore better able to forage, escape predators and migrate (Clarke and Pörtner, 2010). Increased physical activity requires a greater capacity for energy (ATP) production, and oxidative metabolic capacities of endotherms are consequently an order of magnitude greater than those of ectotherms (Bennett and Ruben, 1979). In contrast, the 'parental care' model suggests that increased non-shivering thermogenesis permitted parents to control incubation temperatures and thereby provided the selective advantage leading to endothermy (Farmer, 2000). An alternative model posits that selection for greater capacities of the visceral organs permitted greater energy assimilation, which would have supported increased locomotor capacity and thereby parental care (Koteja, 2000). However, these models have some weaknesses. For example, the aerobic capacity model has been criticised because it assumes a mechanistic link between resting metabolic rate and maximal aerobic capacity, which is not necessarily the case (Farmer, 2000). However, the parental care model does not account for the increased energetic and behavioural (e.g. foraging) costs of endothermy, and the potential costs of increased incubation temperatures may outweigh the benefits (Angilletta and Sears, 2003). Additionally, whether increases in body temperature per se represented a selective advantage underlying the evolution of endothermy is debatable, because metabolic rates would have needed to change substantially to cause even a small increase in body temperature (Bennett and Ruben, 1979; Bennett et al., 2000). It is more likely that other evolutionary forces selected the prerequisite physiology in the first place. Ultimately, however, the increased magnitude and stability of body temperature resulting from metabolic processes would have become advantageous because the physiological performance of early endotherms would increasingly be uncoupled from environmental variation (Nespolo et al., 2011).

Regardless of which model is the most appropriate – and it may well be a combination of all in different contexts – an understanding of the proximate mechanism that could have mediated the physiological changes leading to endothermy is lacking. At the core of the transition from ectothermy to endothermy is enhanced energy metabolism, cardiovascular function to provide the extra oxygen required and muscle function to sustain increased physical activity (Bennett and Ruben, 1979; Walter and Seebacher, 2009; Clarke and Pörtner, 2010; Nespolo et al., 2011). Heat production is facilitated because the metabolic system of endotherms is much less efficient than that of ectotherms. The membranes of endotherms are more leaky to ions such as sodium so that endotherms require relatively higher activities of the molecular pumps (e.g. Na^+/K^+-ATPase) that restore ion balance across membranes (Wu et al., 2004; Walter and Seebacher, 2009). The higher activities of ion pumps require greater amounts of ATP produced in mitochondria, the powerhouses of the cell. This higher ATP demand is compounded by futile cycling of ion pumps, particularly the sarco-endoplasmic reticulum calcium ATPase 1 (SERCA1). SERCA 1 transfers calcium ions from the muscle cell to an internal storage compartment (the sarcoplasmic reticulum) to facilitate muscle relaxation (Berchtold et al., 2000). Futile cycling by SERCA 1 means that the protein undergoes the ATP-consuming conformational changes without, however, transporting calcium; instead, the energy gained from converting

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The Journal of Experimental Biology (2014) doi:10.1242/jeb.088880

Glossary

Acclimation
Reversible physiological response within the lifetime of a non-embryonic organism that compensates for the effect of an environmental change. Thermal acclimation refers to compensation for a change in temperature, and cold acclimation denotes a compensatory physiological response that counteracts the negative thermodynamic effect of a decrease in temperature.

ATPases
Enzymes that catalyse a reaction which uses the energy released from converting ATP to the lower-energy ADP molecule. ATPases often are involved in the transport of ions. For example, Na^+/K^+-ATPase transports ions across cell membranes, and the sarco(endo)plasmic reticulum calcium ATPase (SERCA) transports calcium into the intracellular sarcoplasmic or endoplasmic reticulum.

Calcium cycling in muscle
Neural signals lead to muscle contraction by stimulating dihydropyridine and ryanodine receptors in muscle cells, which causes calcium release from the sarcoplasmic reticulum in the muscle cell. The calcium binds to troponin inside the muscle cell, which results in an interaction between actin and myosin and, hence, muscle contraction. Muscle relaxation is facilitated by re-sequestration of calcium back into the sarcoplasmic reticulum by SERCA.

Ectotherm
An organism with metabolic rates and consequent heat production that are too low to affect body temperature.

Endotherm
An organism that produces sufficient metabolic heat at rest to affect its body temperature, with the result that body temperature is often higher than environmental temperature. The resting metabolic rate of endotherms is 5–10 times higher than that of ectotherms.

Futile cycling
Futile cycling occurs when an enzyme such as an ATPase undergoes conformational changes that consume ATP but without actually achieving the catalytic step. For example, SERCA1 undergoes futile cycling by using ATP and undergoing conformational changes without, however, transporting calcium into the sarcoplasmic reticulum.

Mitochondrial electron transport
The energy contained in food is converted into chemical energy stored in ATP. Hydrolysis of ATP to the lower energy ADP releases the energy that drives most cellular processes. Mitochondria produce most of the ATP in the cell by transporting electrons along a series of carrier proteins situated in their inner membrane. Electron transport results in the pumping of protons (H^+) across the membrane to establish a gradient. The release of this proton gradient fuels ATP production by a mitochondrial enzyme called ATP synthase. However, a relatively large proportion of the proton gradient may be lost as heat without ATP production as a result of protons leaking passively through the membrane. Endotherms have maximised this proton leak to produce heat.

PGC-1α
The peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is a protein that acts as a coactivator of transcription factors. It is an important molecular control mechanism of metabolism.

Thyroid hormone
The thyroid gland produces the iodinated (i.e. adding iodine) amino acid hormones thyroxine (T_4) and triiodothyronine (T_3). T_4 is relatively inactive, and it is converted into its active forms T_3 and T_2 by deiodinase enzymes in cells. Thyroid hormones have a broad range of function, but are best known for their effects on development and metabolism.

Thyroid receptors
Thyroid receptors are nuclear proteins that when bound by thyroid hormone act as a transcription factor to regulate the expression of target genes.

Thyroid response elements
Thyroid response elements are binding sites on the promotor regions of particular genes. Thyroid response elements are bound by thyroid receptors when these are in complex with thyroid hormone. The binding of thyroid receptors to thyroid response elements regulates the expression of the gene associated with the promotor region.

Transcription factor
A protein that binds to DNA to regulate the transcription (expression) of genes.

ATP to ADP is released as heat (Arruda et al., 2008). As a result, mitochondria in endothermic cells work at a much higher rate to supply the increased ATP demand.

A further inefficient step occurs in the membranes of the mitochondria themselves. To produce the energy necessary to drive the production of ATP from the lower energy ADP molecule, a number of proteins in the inner mitochondrial membrane transport protons to the intermembrane space. The resulting electrochemical gradient is dissipated through an enzyme (ATP synthase) that harnesses the energy released to produce ATP. However, the electrochemical gradient may also be dissipated by protons leaking back through the mitochondrial membrane at sites other than the ATP synthase. As a result, the electrochemical energy stored in the proton gradient is released as heat rather than being used for ATP production. Mitochondria of endotherms are much more leaky to protons and also have specialised proteins [uncoupling proteins (UCPs) and adenine nucleotide translocase (ANT)] (Brand, 2005; Walter and Seebacher, 2009) that facilitate proton leak. Hence, proton leak and futile cycling lead to increased flux through mitochondria and to increased heat production. Ultimately, the metabolic heat produced by these processes while the animal is at rest becomes sufficient for endotherms to maintain relatively high and stable body temperatures.

Increased sustained physical activity, in contrast, requires increased maximal mitochondrial (aerobic) capacity to supply working muscle with ATP, as outlined in the aerobic capacity model (Bennett and Ruben, 1979). Mitochondrial density and function are determined by proteins (transcription factors) that regulate gene expression programs (Egan and Zierath, 2013; Moyes, 2003), such as peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) and its targets (Lin, 2009). Endotherms have greater cellular densities of mitochondria with greater bioenergetic capacities than ectotherms (Hulbert and Else, 1981). Additionally, increased sustained physical activity also relies on muscle function and cardiovascular performance (Clarke and Pörtner, 2010).

Endotherms tend to have higher proportions of oxidative muscle fibre types that are characterised by greater oxidative metabolic capacities. Additionally, muscle power output is largely determined by myosin heavy chain composition (Johnston and Temple, 2002) and the efficacy of calcium cycling within the muscle cell. Calcium cycling refers to the dynamics of calcium release from the sarcoplasmic reticulum by ryanodine receptors and it re-sequestration by SERCA, which determines muscle force production and fatigue resistance (Berchtold et al., 2000).

Muscle performance, cardiovascular function and metabolic capacity are therefore essential components underlying the transition from ectothermy to endothermy. All three physiological systems may have co-evolved to support the high activity levels of endotherms. More parsimoniously, however, all three systems may be controlled by a single regulator, the evolution of which provides the key to understanding the evolution of endothermic physiology. Here, we propose that thyroid hormone represents this regulator.

Thyroid hormone is a universal regulator of growth and development in vertebrates, and additionally regulates thermogenesis in birds and mammals. The classic model for thyroid hormone action is through interactions with thyroid receptors (a type of nuclear receptor) in peripheral tissues (Hulbert, 2000). Thyroid receptors regulate gene expression by binding thyroid response elements in the promoters of target genes. Depending upon their conformational state and the type of thyroid hormone elements they bind (positive or negative), thyroid receptors either promote or repress gene transcription (Aranda and Pascual, 2001; Pandya et al., 2010). Recently it was shown that thyroid hormone also acts through non-genomic (non-transcriptional) pathways, although the specific mechanisms that underlie these effects remain obscure (Goglia, 2005). Thus, in addition to its control over gene expression, thyroid hormone can also regulate protein function directly. In birds and mammals, thyroid hormone regulates the metabolic, muscular and cardiac mechanisms that form the core of endothermy (Hulbert, 2000). Importantly, however, thyroid hormone already regulates these levels of physiology in ectothermic vertebrates, specifically in response to cold. Its trajectory throughout animal evolution can therefore explain how intermediate states were selectively advantageous in response to cold.

The internalisation of thyroid hormone production is the first evolutionary step towards more effective metabolic regulation

Many invertebrates cannot synthesise thyroid hormone internally, and must acquire it from dietary sources (Eales, 1997; Flatt et al., 2006; Davey, 2007). Hence, concentrations of thyroid hormone in these organisms are intrinsically tied to food availability and quality. Not surprisingly, therefore, thyroid hormone has evolved a role in signalling nutritional status in some of these groups (Eales, 1997; Heyland and Hodin, 2004; Heyland et al., 2004; Miller and Heyland, 2010). For example, in feeding echinoderm larvae, thyroid hormone regulates the allocation of energy to larval feeding structures in nutrient-poor conditions, thereby facilitating nutrient uptake. In nutrient-rich environments, however, thyroid hormone directs the allocation of energy to metamorphosis or juvenile structures to promote development (Heyland and Moroz, 2005). In the Pacific sand dollar, *Dendraster excentricus*, thyroid hormone derived from ingested unicellular marine algae accumulates in the pelagic larvae until a critical concentration threshold signals metamorphic competence (Heyland and Hodin, 2004); this process also mediates the transition from a pelagic to a benthic lifestyle. Developmental processes are intrinsically linked to energy metabolism, and thyroid hormone stimulates metabolism in several invertebrate groups (Davey, 2007; Flatt et al., 2006). It is therefore possible that thyroid hormone regulates growth and development in echinoderms and other thyroid hormone-sensitive invertebrates at least in part by regulating metabolism. However, the precarious supply of thyroid hormone from dietary sources would preclude it from assuming the essential functions it has in vertebrates. For example, the regulation of mammalian thermogenesis by thyroid hormone could not continually maintain body temperature if its availability were tied to the availability of particular food sources.

In contrast to most invertebrates, all chordates and some derived echinoderms and molluscs have evolved the capacity for internal thyroid hormone synthesis using exogenous sources of iodine (Heyland and Moroz, 2005; Heyland et al., 2006; Holzer and Laudet, 2013). This process occurs in the endostyle of non-vertebrate chordates, which is homologous to the thyroid gland in vertebrates (Eales, 1997). Interestingly, the primary role of the endostyle is to gather food particles during filter feeding, a feature that further reflects the evolutionary link between thyroid hormone and diet. Internal thyroid hormone production would have been advantageous at this stage because species that synthesise thyroid hormone internally also evolved non-feeding larval forms, which reach metamorphic competence more quickly than larvae from species that obtain thyroid hormone from food (Wray and Bely, 1994). Without the need for feeding structures and behaviour, non-feeding larvae allocate their energy reserves to increased rates of development instead. Selection would favour increased rates of development and the decreased risks inherent during planktonic feeding, and thereby internal thyroid hormone production (Heyland et al., 2006; Wray and Bely, 1994).

The evolution of the endostyle or thyroid gland in early chordates and vertebrates would have permitted an increased degree of regulatory autonomy. Thyroid hormone concentrations would no longer be limited by diet. Because its concentrations could be regulated internally, it would be free to evolve signalling roles for environmental parameters that do not necessarily coincide with food availability. In many classic examples of vertebrate metamorphosis, for instance, thyroid hormone triggers metamorphosis in response to specific environmental cues (Crockford, 2009; Laudet, 2011). Importantly, it regulates the changes in physiology that allow animals to undergo major shifts in response to environmental conditions during development, such as the shift from freshwater to marine environments (Crockford, 2009; Laudet, 2011). In many species of fish, thyroid hormone signals the transition from pelagic to benthic environments, and in amphibians it mediates the move from aquatic to terrestrial or amphibious environments (Crockford, 2009; Laudet, 2011). These roles of thyroid hormone in metamorphosis are similar to its role in feeding echinoderm larvae discussed above, with the fundamental difference that here thyroid hormone signals metamorphosis in response to cues unrelated to diet. Thus, thyroid hormone has evolved to regulate animal physiology in response to a range of environmental signals. Thyroid hormone can also regulate reversible changes in phenotype in mature vertebrates as a response to less permanent transitions in the environment, such as seasonal thermal variation. Specifically, thyroid hormone mediates thermogenesis to maintain a constant body temperature in birds and mammals (Hulbert and Else, 1981; Walter and Seebacher, 2009; Cannon and Nedergaard, 2010), and cold acclimation to maintain locomotor performance during cold exposure in ectothermic zebrafish (Little and Seebacher, 2013; Little et al., 2013).

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Thyroid hormone regulates thermal responses in an ectothermic vertebrate

The role of thyroid hormone in regulating thermal responses is best known from studies in mammals and birds (Hulbert, 2000). However, we have recently shown that it also regulates thermal acclimation in an ectothermic vertebrate, the zebrafish (*Danio rerio*) (Little and Seebacher, 2013; Little et al., 2013). Thyroid hormone acts to maintain high levels of locomotor performance, muscle function, metabolic capacity and heart rate in response to cold. During cold acclimation, thyroid hormone increases the mRNA concentration and activity of metabolic enzymes, as well as the mRNA concentration of PGC-1α, which controls their transcription. Thyroid hormone also upregulates the mRNA concentration and activity of SERCA in skeletal and cardiac muscle during cold acclimation in zebrafish. These effects on SERCA coincide with faster tail beat frequencies and higher maximum heart rate in cold-acclimated fish (Little and Seebacher, 2013; Little et al., 2013; Little and Seebacher, 2014). Interestingly, thyroid hormone also stimulates Na^+/K^+-ATPase activity in fish (Madsen and Korsgaard, 1989), which is a typical component of the cold acclimation response (Schwarzbaum et al., 1992). Centrally, thyroid hormone increases sympathetic outflow to the heart during rest and exercise, thereby increasing heart rate in cold fish (Little and Seebacher, 2014). These changes in metabolism, and cardiac and muscle function coincide with improved sustained swimming performance at low temperatures. The net result of these actions is that thyroid hormone buffers locomotion in fish from the thermodynamic effects of low temperature. It is likely, therefore, that thyroid hormone regulatory activity was under strong positive selection because it would improve fitness in variable environments (Husak et al., 2006; Le Galliard et al., 2004). Even incremental increases in thyroid hormone-mediated locomotor performance and physical activity under cold conditions would represent clear selective advantages for accessing food and energy assimilation, parental care and territorial gains. Additionally, ectotherms have restricted activity times that are closely tied to the operative thermal environment (Seebacher and Grigg, 2001). An extension of activity times, either daily or seasonally, would be of particular selective advantage before the evolution of endothermy. Hence, an individual with extended activity times resulting from improved performance at low temperatures would have had an obvious advantage.

Thyroid hormone affects the same physiological pathways during cold acclimation in ectotherms as during thermogenesis in endotherms

The pathways regulated by thyroid hormone for thermogenesis during cold exposure in endotherms are homologous to those that it stimulates during acclimation in zebrafish (Fig. 1). During cold exposure, endotherms increase their metabolic rates and thereby heat production for thermoregulation. Thyroid hormone mediates these responses to cold in skeletal muscle and in brown adipose tissue (Cannon and Nedergaard, 2004) locally via thyroid receptors. Thyroid receptors induce expression of metabolically important genes and thereby modulate mitochondrial capacity and metabolic flux (López et al., 2010). Thyroid receptors interact with the metabolic co-activator PGC-1α and its target transcription factors to

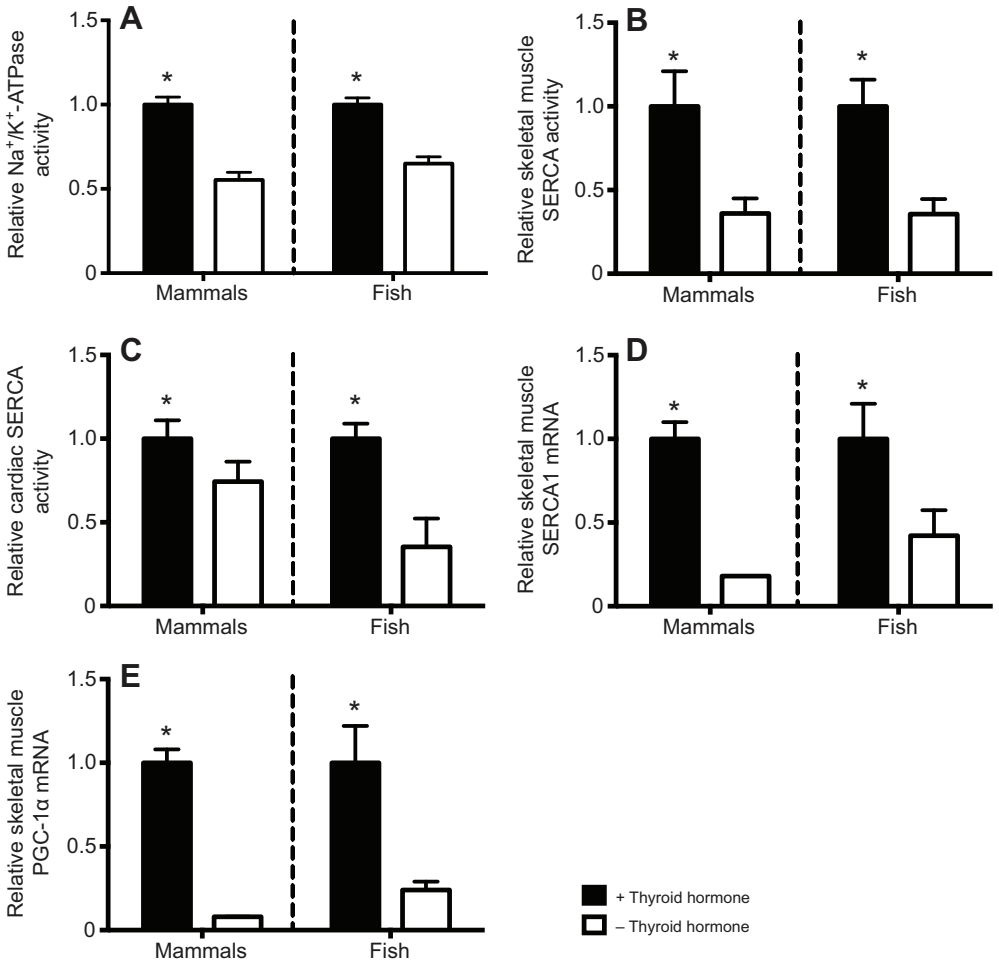

Fig. 1. Mammals and fish show similar physiological responses to thyroid hormone. In both groups, pharmacologically manipulated thyroid levels cause changes in relative Na^+/K^+-ATPase activity (A), skeletal muscle SERCA activity (B), cardiac SERCA activity (C), skeletal muscle SERCA1 mRNA levels (D) and PGC-1α mRNA levels (E). Note that '+ thyroid hormone' and '– thyroid hormone' are used to represent either hyperthyroid and control treatments, respectively, or control and hypothyroid treatments, respectively, depending upon the study referenced. Asterisks represent statistical significance at $P<0.005$; data were extracted from the following studies: A (Ismail-Beigi and Edelman, 1971; Madsen and Korsgaard, 1989); B,D (Simonides et al., 2001; Little and Seebacher, 2013); C (Kiss et al., 1994; Little and Seebacher, 2014); and E (Weitzel et al., 2003; Little et al., 2013).

alter gene expression, including that of PGC-1α itself (Knutti and Kralli, 2001; Yuan et al., 2013). As a result, in response to cold there is an increase in the expression of mitochondrial proteins involved in energy metabolism.

In endotherms, thyroid hormone also stimulates the activity of Na^+/K^+-ATPase and SERCA, thereby increasing ATP use (Silva, 1995). Intriguingly, as well as increasing SERCA activity in ectotherms and thereby enhancing muscle performance, thyroid hormone particularly enhances SERCA1 isoform expression in zebrafish (Little and Seebacher, 2013). SERCA1 is the only isoform that undergoes futile cycling in addition to pumping calcium into the sarcoplasmic reticulum to facilitate muscle relaxation. The heat released by SERCA1 futile cycling is a major contributor to thermogenesis in mammals and in billfish heater organs (Arruda et al., 2008; Block, 1994). Thyroid hormone also induces mitochondrial uncoupling by promoting the expression of uncoupling proteins in mammals, which facilitate proton leak through the mitochondrial membrane and thereby heat production (van den Berg et al., 2011). Similarly, in birds, uncoupling protein expression is decreased with hypothyroidism, although uncoupling proteins are more likely to be associated with reactive oxygen species defence rather than heat production (Rey et al., 2010; Walter and Seebacher, 2009).

Additionally, in mammals, thyroid hormone interacts with the hypothalamus to modulate sympathetic activity and thereby β-adrenergic receptor-induced heat production in brown adipose tissue and skeletal muscle, as well as heart rate and cardiac output (Cannon and Nedergaard, 2004; Carr and Kranias, 2002). These effects of thyroid hormone on sympathetic tone of the heart in mammals are mirrored in zebrafish, where increased sympathetic output results in improved cardiac performance under cold conditions.

Thyroid hormone as the proximate mechanism underlying the evolution of cold responses leading to endothermy

We suggest that endothermy is a specific outcome of a broader evolutionary process that was driven by compensatory responses to low temperature (Fig. 2). Discussion of the evolution of endothermy can be misleading, therefore, because it considers the specific outcome rather than the progressive selective advantages gained in the process. Hence, the major selective advantages of endothermy proposed by the various models outlined in the Introduction were all important at different points in the evolutionary process and are not mutually exclusive.

The internalisation of thyroid hormone production in early chordates was the first step in the evolutionary process to buffer animals from cold, because it permitted consistent physiological regulation in response to environmental variability. From that point onwards, the regulatory capacity of thyroid hormone could have been selected for to maintain metabolism, and muscle and cardiac function against the negative thermodynamic effect of decreasing temperatures. The selective advantage would have been cold acclimation and the benefits it confers in decoupling physiology and performance from thermal variability. In particular, consistent and increased metabolic flux at low temperatures along with increased cardiac output would have improved locomotor performance and physical activity (St-Pierre and Charest, 1998; Johnston and Temple, 2002). This is of course at the core of the aerobic capacity model. However, there is no need to invoke endothermy at this point because the selective advantages gained by incremental compensation for cold would have been sufficient by themselves in driving the evolutionary process. The regulation of cold acclimation by thyroid hormone in zebrafish (Little et al., 2013) is an example of the selective advantages gained at this stage of the evolutionary process. Cold acclimation is common in many ectotherms and it is not necessarily associated with thermoregulation, although the two can be linked (Guderley, 2004; Glanville and Seebacher, 2006; Seebacher, 2005).

Beyond cold acclimation, there are distinct selective advantages from improved locomotor performance (Le Galliard et al., 2004; Husak et al., 2006; Irschick et al., 2008). Selection for increased locomotor activity and the underlying increases in metabolic capacity and cardiac function would have eventually led to sufficient heat retention in tissues to influence body temperature. This would have been achieved at a lower metabolic rate in terrestrial organisms than in aquatic organisms because of the greater heat loss in water. Additionally, larger animals lose heat at a lower rate so that body size would have been an important correlate in metabolic heat retention. Not surprisingly, metabolic heat production influences body temperature only in the largest and most active fish that regularly encounter cold temperatures, such as lamnid sharks, tuna and billfish (Dickson and Graham, 2004). In terrestrial animals, however, heat retention would have also contributed to body temperature in smaller and less active animals. Once the point has been reached in the evolutionary process where sufficient metabolic heat is generated to affect body temperature, increased body

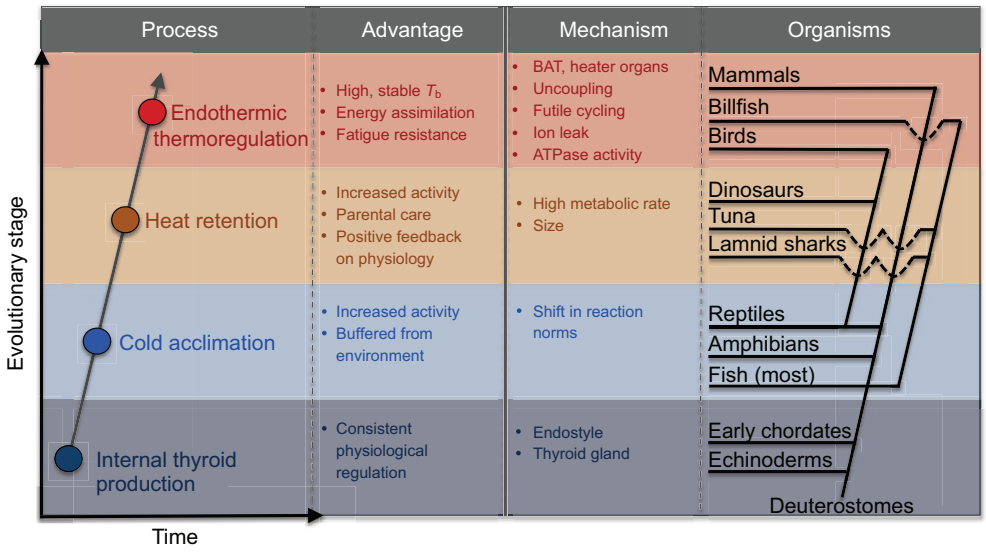

Fig. 2. Summary of the thyroid-mediated evolutionary process that led to endothermy. The evolutionary trajectory of thyroid hormone function that ultimately led to the evolution of endothermy (left panel). Over evolutionary time, various selective advantages (second panel from left) led to the internalisation of thyroid hormone production, cold acclimation, heat retention and endothermic thermoregulation (left panel). Different thyroid-controlled mechanisms (second panel from right) underlie the evolutionary responses in different organisms (right panel). The phylogenetic relationships in the right panel are approximate only and the organisms included are not comprehensive. T_b, body temperature.

temperature itself becomes a selective advantage for incubating offspring and parental care, for example (Farmer, 2000). Increased body temperatures would also provide a positive feedback on physiological rates, thereby accelerating increases in the magnitude of muscle function and fatigue resistance, metabolism itself, and the capacity of visceral organs to assimilate energy, for example (Koteja, 2000). It is important to stress that the evolutionary process that led to increases in these physiological capacities started with thyroid hormone-induced cold acclimation; increased body temperatures ultimately reinforced this process, but did not cause it. Selection for increased body temperatures would favour processes that maximise heat production, such as mitochondrial uncoupling, futile cycling by SERCA and high Na^+/K^+-ATPase activity, and eventually brown adipose tissue in mammals and heater organs in billfish. The mechanisms that underlie these heat-producing processes are all regulated by thyroid hormone, and are the hallmarks of endothermic thermoregulation, which is the last step in the evolutionary process (Fig. 2). Along the evolutionary trajectory, however, the evolution of endothermic thermoregulation cannot be considered in isolation. In other words, without the selective advantages of thyroid-regulated cold acclimation in fish there would be no endotherms.

Competing interests

The authors declare no competing financial interests.

Author contributions

Both authors conceived the ideas and wrote the manuscript.

Funding

This work was funded by an Australian Research Council Discovery Grant to F.S.

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