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Adipose tissue development during early life: novel insights into energy balance from small and large mammals

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Since the rediscovery of brown adipose tissue (BAT) in adult human subjects in 2007, there has been a dramatic resurgence in research interest in its role in heat production and energy balance. This has coincided with a reassessment of the origins of BAT and the suggestion that brown preadipocytes could share a common lineage with skeletal myoblasts. In precocial newborns, such as sheep, the onset of non-shivering thermogenesis through activation of the BAT-specific uncoupling protein 1 (UCP1) is essential for effective adaptation to the cold exposure of the extra-uterine environment. This is mediated by a combination of endocrine adaptations which accompany normal parturition at birth and further endocrine stimulation from the mother’s milk. Three distinct adipose depots have been identified in all species studied to date. These contain either primarily white, primarily brown or a mix of brown and white adipocytes. The latter tissue type is present, at least, in the fetus and, thereafter, appears to take on the characteristics of white adipose tissue during postnatal development. It is becoming apparent that a range of organ-specific mechanisms can promote UCP1 expression. They include the liver, heart and skeletal muscle, and involve unique endocrine systems that are stimulated by cold exposure and/or exercise. These multiple pathways that promote BAT function vary with age and between species that may determine the potential to be manipulated in early life. Such interventions could modify, or reverse, the normal ontogenic pathway by which BAT disappears after birth, thereby facilitating BAT thermogenesis through the life cycle.

Adipose tissue: Development: Feeding: Prolactin: Thermoregulation

Following the ‘rediscovery’ of brown adipose tissue (BAT) in 2007⁽¹⁾ there has been a substantial amount of innovative research into the assessment of both its function and regulation⁽²⁾. This work has largely been undertaken in human adults with little consideration of its developmental aspects⁽³⁾. The majority of animal studies have utilised rodent models as these have the advantages of offering gene deletion approaches, have a short duration and can be constrained within a closely controlled and confined environment⁽⁴⁾. However, as will be considered in more detail

later, there are fundamental differences in early life development between small (i.e. rodents) and large (i.e. sheep) mammals, which could have a pronounced impact on our understanding of BAT function throughout the life cycle.

In adult human subjects, BAT is primarily sited in the supraclavicular regions where it is present across a wide range of ages^(5–7). This is not unexpected given that this is one of the main sites of BAT originally identified⁽⁸⁾ and more recently confirmed⁽⁹⁾ in infants. The initiation of non-shivering thermogenesis in both human subjects and

Abbreviations: BAT, brown adipose tissue; PRLR, prolactin receptor; UCP1, uncoupling protein.

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large mammals, such as sheep, is one critical factor in determining metabolic adaptation to the cold challenge of the extra-uterine environment⁽¹⁰⁾.

The substantial potential of BAT to contribute to energy balance is not limited to the period immediately after birth because its maximal rate of heat production through the life cycle is in the order of 300 W/kg compared with 1 W/kg in all other tissues^(11,12). In the 1980s it was calculated that in adult human subjects, only 40–50 g of BAT would be required for it to be able to contribute 20% of daily energy expenditure^(13,14), an estimation that has now been confirmed⁽¹⁵⁾. Surprisingly, despite the important role of the early life nutritional environment on adipose tissue development, especially in large mammals such as sheep, there has been only a limited amount of research into its impact on BAT⁽³⁾. However, in rodents, at least, there have now been three specific sub-types of adipose tissue identified:

- (1) ‘Classical’ BAT located within the interscapular region that possesses large amounts of the BAT-specific uncoupling protein 1 (UCP1)⁽¹⁶⁾ and arises from a *myf5*, muscle-like cell lineage⁽¹⁷⁾.
- (2) White adipose tissue located in a large number of different locations throughout the body.
- (3) Brown adipose in white tissue depots that are not derived from a *myf5*-positive lineage⁽¹⁸⁾.

The extent to which comparable classifications can be made for adipose tissue development in large mammals remains to be established but, as this review will go on to highlight, it is likely that a different relationship could be present. Extensive studies are now required to explore the complexity of the multiple pathways involved in BAT regulation, and the differential effects of short- and long-term challenges. These will need to be undertaken across a range of different species in order to realise transferable interventions designed to promote BAT function and, thus, prevent excess body fat accumulation. At present, however, the majority of rodent studies have investigated the effects of a ‘high-fat’ dietary challenge⁽⁴⁾ conducted in a thermoneutral environment resulting in variable outcomes within BAT⁽¹⁹⁾.

Metabolic adaptation to the extra-uterine environment

BAT is maximally recruited at birth when the rapid appearance of UCP1 initiates non-shivering thermogenesis⁽²⁰⁾. It coincides with maximal heat production which is rarely matched again at any other stage of life. This process is highly dependent on the degree of maturation at birth and, in altricial species such as the rat, this maturation is delayed until around 7 d after birth when the hypothalamic–pituitary axis becomes fully functional⁽²¹⁾. If this maturation process is inhibited, hypothermia and death rapidly result, although in some genetic manipulations of imprinted genes, there are still a small number of survivors⁽²²⁾. Interestingly, surviving offspring that were generated to lack the imprinted delta-like homologue 1/preadipocyte factor, and iodothyronine deiodinase type 3, fail to adapt effectively to the change in diet at weaning and were subsequently unable to maintain UCP1 longer

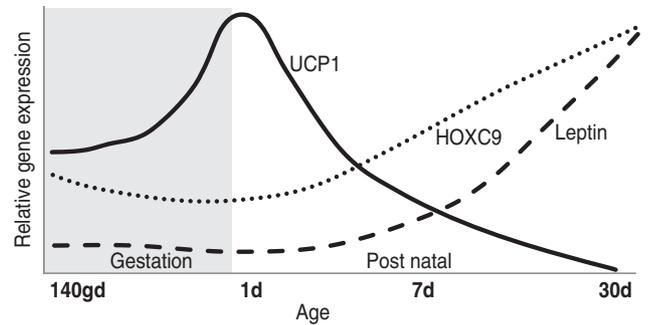


Fig. 1. Summary of the changes in gene expression for markers of brown (i.e. uncoupling protein 1 (UCP1))⁽²⁸⁾, white (i.e. leptin)⁽²⁹⁾ and brown adipocytes in white (i.e. homeobox C9 (HOXC9))⁽³⁰⁾ in perirenal–abdominal adipose tissue between fetal and postnatal life (term = 145 d gestation (gd)) in the sheep.

than 11 d after birth. The extent to which a comparable developmental window is apparent in precocial mammals remains to be demonstrated.

Differences in BAT development are mediated, in part, by changes in thyroid function that has been established in both small⁽²³⁾ and large⁽²⁴⁾ mammals to be critical for the onset of BAT thermogenesis. Thus, the latter is influenced by the ability of triiodothyronine to serve as a bipotential mediator of mitochondrial biogenesis⁽²⁵⁾. In addition to the effects of triiodothyronine, the rapid increase in UCP1 around the time of birth in large mammals (as summarised in Fig. 1) is also dependent on the rapid appearance of a range of other endocrine stimulatory factors including cortisol, prolactin, leptin and catecholamines⁽²⁶⁾. Their secretion in the fetus/newborn is dependent on the stress of the normal birth process which results in intense activation of the central nervous system⁽²⁰⁾. The magnitude of response is, however, dependent on exposure of the offspring to the cool temperature challenge of the extra-uterine environment⁽¹⁰⁾. To date, the effect of this stimulus has only been studied in the perirenal–abdominal depot of the sheep. This depot, which represents about 80% of all adipose tissue in the newborn sheep, is characterised by the rapid loss of its UCP1 expression⁽²⁷⁾ and its subsequent apparent morphological conversion to white adipose tissue, coincident with a greatly increased leptin synthetic capacity (Fig. 1).

In rodents, it has been suggested that the conversion of *myf5* myogenic progenitors to brown adipocytes is regulated by bone morphogenetic protein 7 acting through PRDM16 (PRD1-BF1-RIZ1 homologous domain containing 16)^(17,31). The optimum marker for brown adipose in white adipocytes, however, appears to be homeobox C9⁽³⁰⁾ and exhibits a similar ontogeny to leptin (Fig. 1). The extent to which brown adipose in white characteristics can be manipulated in early life remains to be established; although, in the adult mouse gene expression for homeobox C9 is unaffected by cold exposure⁽³⁰⁾.

Milk composition and brown adipose tissue thermogenesis

Feeding also appears to be vital in switching on BAT function in rodents⁽³²⁾ in which fat composition of milk

Table 1. Comparison of the milk composition between the rat, sheep and human subjects

Macronutrient (g/100 ml)	Stage of lactation	Species		
		Rat	Sheep	Human
Fat	Early	18.0 ⁽³³⁾	9.5 ⁽³⁹⁾	2.3 ⁽⁴⁰⁾
	Late	10.0 ⁽³³⁾	6.0 ⁽³⁴⁾	3.8 ⁽⁴¹⁾
Protein	Early	6.5 ⁽³³⁾	10.8 ⁽³⁹⁾	5.8 ⁽³⁵⁾
	Late	8.3 ⁽³³⁾	5.4 ⁽³⁴⁾	1.9 ⁽³⁵⁾
Lactose	Early	2.7 ⁽³³⁾	3.0 ⁽³⁹⁾	2.0 ⁽³⁵⁾
	Late	3.7 ⁽³³⁾	5.1 ⁽³⁴⁾	5.0 ⁽³⁵⁾

peaks at birth and then declines up to 1 week after birth⁽³³⁾. Then even after this stage of lactation, the fat content remains around three times higher than that of either sheep⁽³⁴⁾ or human subjects⁽³⁵⁾. Protein concentrations are also elevated in rodents but, while these remain unchanged through lactation in the rat⁽³³⁾ they fall dramatically over the first 4 d of lactation in human breast milk⁽³⁵⁾. This primarily reflects the very different composition of colostrum in human (and sheep) milk⁽³⁶⁾ compared with that of rodents and which is necessary for providing immune protection immediately after birth. Human milk is also characterised as having a high cortisol content⁽³⁷⁾ although the extent to which this could further promote UCP1 synthesis in the newborn, as demonstrated in the fetus⁽³⁸⁾, has yet to be investigated. Therefore, milk composition between small and large mammals (including human subjects) is very different (as summarised in Table 1). The impact of alterations in milk composition resulting from pre or postnatal changes in maternal diet within species has been largely overlooked.

Human milk also has a high prolactin⁽⁴²⁾ content which is implicated in promoting BAT development and ensuring BAT thermogenesis is maintained through postnatal development⁽⁴³⁾. This could result in enhanced BAT activity during later life, preventing excess adipose tissue deposition around central organs and increasing BAT sensitivity to thermogenic challenges. At the same time, diurnal variation in prolactin uptake within the human breast⁽⁴²⁾ may further regulate BAT function in the infant. Prolactin is known to have a rapid thermogenic effect in neonatal sheep⁽⁴⁴⁾, at least when administered intravenously, but whether this response is present when prolactin is ingested enterally or is modulated between BAT depots has yet to be examined. It appears that not only is prolactin an important potential regulator of UCP1 in the perinatal period⁽⁷⁾, newly identified depots of BAT in the sternal and clavicular regions of juvenile sheep⁽³⁾ may be highly sensitive to prolactin as gene expression for UCP1 across these depots is highly positively correlated with that for the long form of the prolactin receptor (PRLR) (r^2 0.95; $P < 0.01$).

There is also increasing evidence of differences in feeding behaviour⁽⁴⁵⁾, preferences⁽⁴⁶⁾ and growth trajectory⁽⁴⁷⁾ between breast and bottle-fed infants. It is, therefore, possible that the absence of BAT activating endocrine factors within formula milk differentially resets energy regulation between breast- and formula-fed infants.

One factor that has restricted our ability to assess BAT thermogenesis in larger cohorts of human subjects has been the limitations of methods available to assess BAT activity: positron emission tomography with F-18 fluorodeoxyglucose; single photon emission tomography scanning with tracers such as I-123-meta-iodobenzylguanidine or Tc-99m-tetrofosmin; and tissue biopsy^(48–50). These invasive techniques have substantial disadvantages relating to the administration of comparatively large amounts of radiopharmaceuticals to healthy individuals, the use of single substrates to identify uptake into BAT, together with the static information they reveal and their relatively high costs. We have now developed the use of thermal imaging as a rapid, safe, and acceptable technique for use in human subjects, confirming previous findings in both rodents⁽⁵¹⁾ and human subjects⁽⁵²⁾ of the rapidity with which BAT thermogenesis is initiated and showing that there is a pronounced reduction in activity of BAT with age⁽⁵³⁾.

Using thermal imaging⁽⁵³⁾, we are now investigating the potential thermogenic effects of individual food ingredients. This has demonstrated a significant thermogenic effect of drinking milk in young children (Fig. 2), which results in up to 0.7°C rise in BAT temperature, thus indicating a role in dietary-induced thermogenesis. It is likely that the macronutrient composition determines the magnitude of response⁽⁵⁴⁾ that has been shown in adults to be promoted by protein and reduced by fat⁽⁵⁵⁾.

The prolactin receptor and brown adipose tissue development

PRLR are one potential mediator by which the maternal nutritional environment can alter adipose tissue composition. PRLR are highly expressed in fetal adipose tissue⁽⁵⁶⁾ where they are more responsive to external challenges than in the fetal liver⁽⁵⁷⁾, for example. The increased amount of fetal BAT deposited when maternal food intake is raised is accompanied by an increased PRLR abundance⁽⁵⁸⁾, whereas sub-optimal maternal food intake has the opposite effect⁽⁵⁶⁾. In adults, it is known that the plasma concentration of prolactin is inversely related to the abundance of its receptor⁽⁵⁹⁾ and could be important in explaining seasonal differences in BAT function in adult human subjects⁽⁶⁰⁾. To date, studies on the direct effects of prolactin on fetal adipose tissue have not been undertaken. This results, in part, from the potential confounding influence of placental lactogen on adipose tissue development as, potentially, it can also bind to the PRLR. The relative binding of placental lactogen, as compared to prolactin, to the PRLR varies markedly between tissues and a 100–1000 times greater molar concentration of placental lactogen is required to achieve the same binding as prolactin in both the liver and ovary⁽⁶¹⁾. In contrast, in fibroblasts, placental lactogen binding to the PRLR is 250-fold greater than that of prolactin⁽⁶²⁾. To date, comparable studies have not been undertaken in adipocytes; although it is notable that placental lactogen only has a very limited lipolytic effect^(63,64) suggesting a low affinity of placental lactogen for the PRLR in adipose tissue. Moreover, in the newborn sheep, direct stimulation of the PRLR

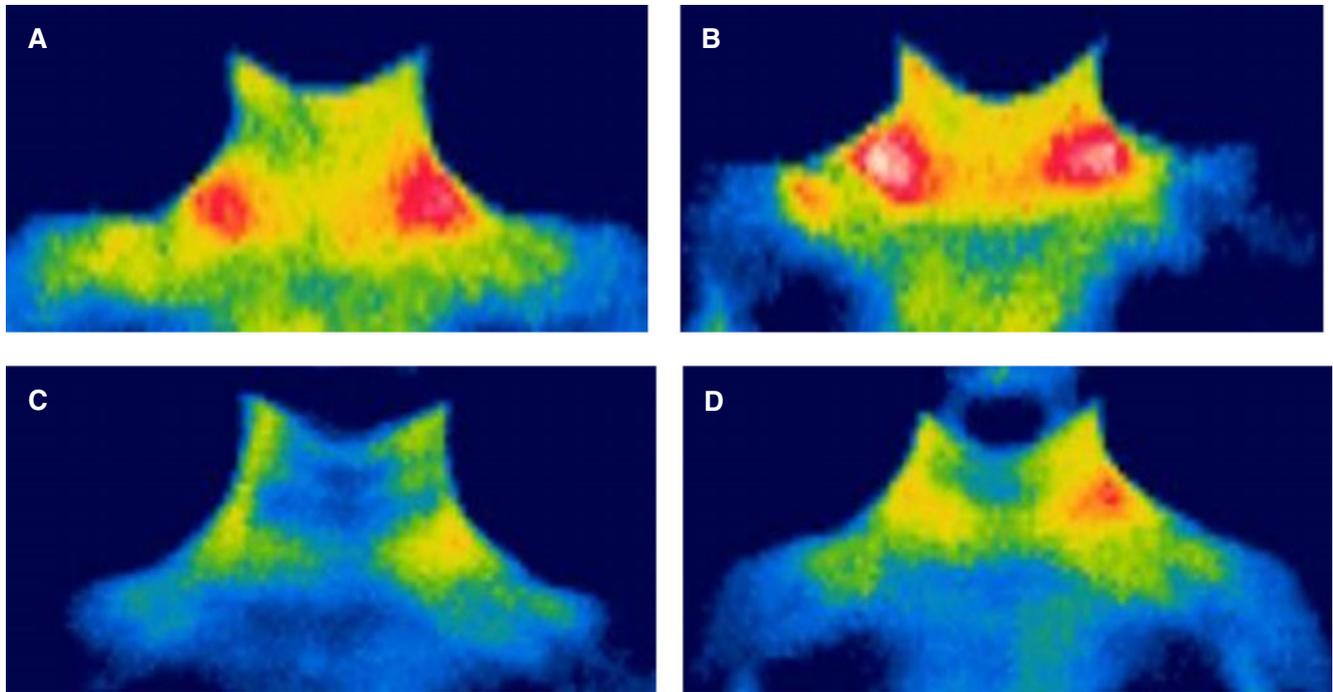


Fig. 2. (Colour online) Comparison of the change in temperature of brown adipose tissue located within the supraclavicular region of an 8-year-old child in the fed state before (A) and after (B) placing one hand in cold water for 5 min (A, B) or before (C) and after (D) drinking 100 ml warm (about 70°C) semi-skimmed milk (adapted from Symonds *et al.*⁽⁵³⁾). Each challenge was accompanied with a mean increase in temperature of the supraclavicular region of about 0.7°C.

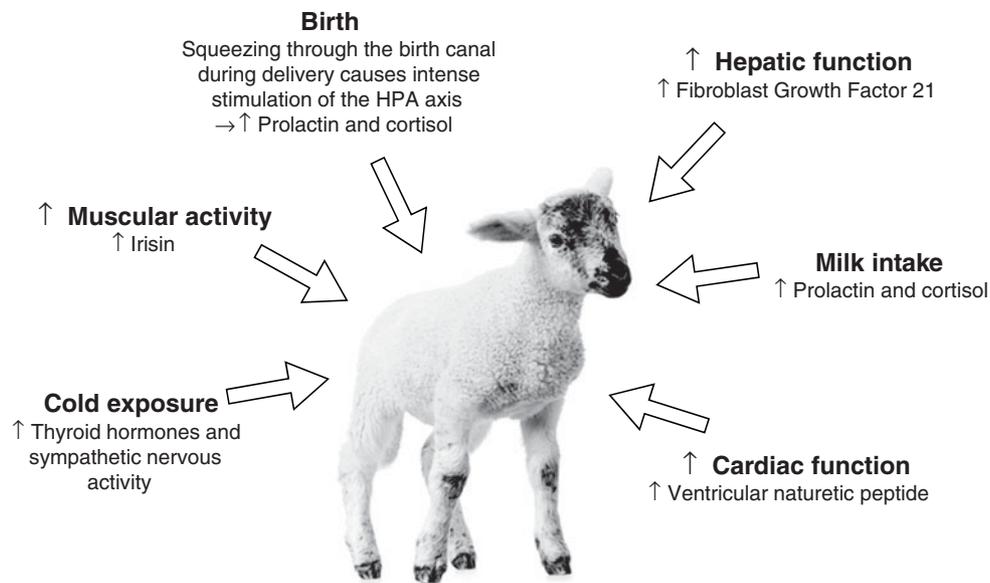


Fig. 3. Summary of the primary factors that can activate non-shivering thermogenesis in brown adipose tissue of the neonatal sheep. HPA, hypothalamic–pituitary–adrenal; ↑, increase.

promotes BAT thermogenesis⁽⁴⁴⁾. These results have been confirmed in a mouse PRLR knockout model which demonstrated that BAT growth and development were severely impaired, as was the expression of a number of brown adipogenic genes including UCP1⁽⁶⁵⁾.

Given the important role prolactin has in establishing and maintaining pregnancy, it is perhaps surprising that its exact role in fetal development is still uncertain⁽⁶⁶⁾. In sheep, although maternal plasma prolactin is decreased 100-fold by short day length and offspring circulating

prolactin and progesterone concentrations also decrease, there are few obvious consequences for pregnancy, birth weight or postnatal growth⁽⁶⁷⁾.

PRLR are also expressed in white adipose tissue and have been implicated in the regulation of adult fat mass. Two recent genome-wide association studies have identified a variant, rs4712652, adjacent to the prolactin gene that is associated with excess body weight^(68,69). Furthermore, as may be expected for a gene that is closely involved in reproductive function, this relationship is gender specific with the risk A allele of rs4712652 strongly correlated with BMI and fat mass in males but not females⁽⁶⁹⁾. Previous clinical studies have demonstrated that correction of hyperprolactinaemia in human subjects improves body weight; although it has a much greater effect on reducing body weight in males compared to females. However, obese females with a normal prolactin profile, have a short-term body weight reduction, associated with a lowering in prolactin following administration of the dopamine D2 receptor agonist bromocriptine⁽⁷⁰⁾. Clearly, further studies are needed to explore this relationship further especially from a developmental perspective.

External factors stimulating brown adipose tissue thermogenesis

There is now increasing evidence in rodents that a number of other tissues and endocrine factors can promote BAT function in rodents. One important example is the liver as, in rats, the postnatal maturation of BAT has been shown to relate to the onset of feeding and initiation of hepatic function, mediated by the release of fibroblast growth factor 21, which can also promote BAT function⁽⁷¹⁾. This factor has also been shown to have a physiological role in adipose tissue of adult mice in which gene expression within adipose tissue is raised by chronic cold exposure, whereas it is depressed in the liver⁽⁷²⁾. Fibroblast growth factor 21 also appears to promote the 'browning' of some white adipose tissue depots where it acts in an autocrine/paracrine manner to enhance the abundance of the nuclear receptor coactivator PPAR α 1 α . It may, however, have only a modest role in energy balance as deletion of the fibroblast growth factor 21 gene does not impair BAT gene expression and the normal diurnal variation in body temperature is maintained with cold exposure. This is accompanied by a small (but non-significant) reduction in temperature which may be linked to increased recruitment of shivering thermogenesis. However, as fibroblast growth factor 21 can have widespread metabolic effects and results in skeletal fragility⁽⁷³⁾, it is unlikely to have immediate therapeutic potential.

An important link between muscle and BAT development has been highlighted from studies focused on the heart⁽⁷⁴⁾ and skeletal muscle⁽⁷⁵⁾. The heart, acting through cardiac natriuretic peptides, can regulate BAT thermogenesis in adult mice⁽⁷⁶⁾. This response appears to be mediated by ventricular, or cardiac B-type, natriuretic peptide, which promotes UCP1 expression; although this effect is potentially greater in inguinal white fat, compared

to interscapular BAT. Given the relatively high expression of UCP1 in both human⁽⁷⁷⁾, ovine⁽³⁾ and mouse⁽³⁰⁾ epicardial fat, it will be interesting to see whether this, and other depots, respond in a similar manner⁽⁷⁸⁾.

Skeletal muscle also has a potential role in regulating BAT function. Exercise in adult mice and human subjects results in increased secretion of a new hormone, irisin, which promotes energy expenditure in mice⁽⁷⁵⁾. Irisin is a membrane protein that is cleaved from FNDC5 (fibronectin type III domain containing 5) and promotes the expression of UCP1 in white adipose tissue, but not BAT. The magnitude of response differs across white fat depots in mice and is greatest within inguinal fat. This study also demonstrated some fascinating differences in the gene response of muscle to exercise between mice and human subjects with leucine-rich α -2-glycoprotein, a novel member of GTPase-activating proteins⁽⁷⁹⁾, showing the greatest response in human subjects, but being unresponsive to exercise in mice⁽⁷⁵⁾. Intriguingly, leucine-rich α -2-glycoprotein has previously been linked to cell growth and cancer^(80,81) and animal studies have shown cancer *per se* can promote BAT function⁽⁸²⁾.

Birth could be considered to represent a period of rapid muscular activity and is associated with a dramatic rise in muscle oxygenation⁽⁸³⁾. In precocial mammals such as sheep, birth is also accompanied by an increase in voluntary muscular activity, and with the onset of shivering thermogenesis which is, in turn, dependent both on BAT function⁽⁸⁴⁾ and on the magnitude of thermal challenge⁽⁸⁵⁾. The process of adaptation at birth may, therefore, provide further insights into the cross-talk between different muscle and fat depots, together with their ability of other organs to promote BAT function in early life as summarised in Fig. 3.

In conclusion, multiple pathways can be recruited to promote BAT function. These may also promote the 'browning' of white adipose tissue and, thus, reverse the normal ontogenic pathway by which BAT disappears from some depots after birth. An increased understanding of these interactions and especially the role of feeding in further activating BAT in the postnatal period could result in novel interventions aimed at enhancing BAT thermogenesis throughout the life cycle.

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