REVIEW

Brown adipose tissue development and function and its impact on reproduction

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Abstract

Although brown adipose tissue (BAT) is one of the smallest organs in the body, it has the potential to have a substantial impact on both heat production as well as fat and carbohydrate metabolism. This is most apparent at birth, which is characterised with the rapid appearance and activation of the BAT specific mitochondrial uncoupling protein (UCP)1 in many large mammals. The amount of brown fat then gradually declines with age, an adaptation that can be modulated by the thermal environment. Given the increased incidence of maternal obesity and its potential transmission to the mother's offspring, increasing BAT activity in the mother could be one mechanism to prevent this cycle. To date, however, all rodent studies investigating maternal obesity have been conducted at standard laboratory temperature (21°C), which represents an appreciable cold challenge. This could also explain why offspring weight is rarely increased, suggesting that future studies would benefit from being conducted at thermoneutrality (~28°C). It is also becoming apparent that each fat depot has a unique transcriptome and show different developmental pattern, which is not readily apparent macroscopically. These differences could contribute to the retention of UCP1 within the supraclavicular fat depot, the most active depot in adult humans, increasing heat production following a meal. Despite the rapid increase in publications on BAT over the past decade, the extent to which modifications in diet and/or environment can be utilised to promote its activity in the mother and/or her offspring remains to be established.

Key Words

- ▶ adipose tissue
- ▶ diabetes
- ▶ metabolism
- ▶ obesity
- ▶ pregnancy

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Introduction

Although brown adipose tissue (BAT) may be one of the smallest fat depots in the adult, it has the potential to have a substantial influence on energy balance, as well as glucose and lipid metabolism (Cannon & Nedergaard 2012b). BAT could be considered as the body's natural radiator, which can be rapidly stimulated during thermal

or dietary challenges (Symonds *et al.* 2015). Brown fat is able to generate large amounts of heat due to the presence of a unique uncoupling protein (UCP)1 on the inner mitochondrial membrane (Cannon & Nedergaard 2004). When stimulated, UCP1 enables the free flow of protons across the mitochondria, by-passing the usual

production of ATP, which occurs in the mitochondria of all other organs (Cannon & Nedergaard 2004). At maximal stimulation, brown fat has the capacity to generate up to 300 times more heat per unit mass than any other organ in the body (Symonds *et al.* 2015) and, as such, can account for up to 10% of total daily heat production (Klingenspor & Fromme 2012). In large mammalian species, including humans, brown fat is first activated around the time of birth following cold exposure to the extra-uterine environment and intense endocrine stimulation (Symonds 2013, Symonds *et al.* 2015). The activity of brown fat then gradually declines, with the possible exception of during puberty (Gilsanz *et al.* 2012) when there may be an increase (Symonds *et al.* 2016). Furthermore, many of the more recent findings on brown fat are in accord with those demonstrated nearly 50 years ago following the first comprehensive studies on the distribution and function of brown fat in neonatal (Hull & Segall 1966) and adult humans (Heaton & Nicholls 1977) but which were not always published at the time (Aldiss *et al.* 2017).

Since the rediscovery of brown fat in adult humans in 2009, there has been a steep increase in publications on the subject (Fig. 1). This has been accompanied with the finding that there are three types of fat depots: namely brown, white and beige adipose tissue (Cypess *et al.* 2014). The latter has the largest potential as a therapeutic target in the prevention of obesity and/or diabetes. This is because, from an adult perspective, beige (or recruitable) fat can be present in many white depots as clusters of pre-adipocytes that on differentiation to the mature adipocyte possess UCP1 (Nedergaard & Cannon 2013). Although the relative abundance of UCP1 in beige fat is only c.10% of that found in 'classic' brown fat, that it resides in white fat of much greater abundance confers potential for sustained functional significance (Cannon & Nedergaard 2012a). Complementary lineage-tracing studies in mice have also suggested that beige and white adipocytes share a

common developmental lineage, whilst brown adipocytes may originate from the same embryonic precursor as skeletal muscle (Harms & Seale 2013). To date, the primary stimulus of the beiging process appears to be a reduction in ambient temperature, acting through an increase in the activity of the sympathetic nervous system (Cannon & Nedergaard 2012b). However, as it becomes clear that the development of each fat depot is unique, a substantial amount of research is still required.

It is now recognised that many of the experimental protocols adopted do not replicate the human environment (Maloney *et al.* 2014), in which cold exposure is the primary stimulus for UCP1 (Chondronikola *et al.* 2014). This is especially the case for many studies in rodents where exposure to the standard housing temperature used of 20–21°C represents an appreciable cool challenge (Fischer *et al.* 2018). Furthermore, rodent models of obesity in which healthy animals are 'simply' switched to a high-energy diet (usually in the form of extra fat) and rapidly become obese is not the typical pathway to obesity in humans (Symonds *et al.* 2011). In humans, excess adiposity is the consequence of a much smaller change in energy balance over a prolonged period of time and is typically accompanied by a range of behavioural adaptations such as reduced activity and more frequent snacking (Weinsier *et al.* 1998, Diaz-Zavala *et al.* 2017). In this context, it is much more likely that enhanced brown or beige fat will provide an additional target to promote weight loss or indeed the maintenance of a healthy weight.

Adipose tissue development and the thermal environment

The development and maturation of adipose tissue varies substantially between large and small mammals. The latter are born with an immature hypothalamic–pituitary axis after a short gestation with very little capacity for independent thermogenesis at birth (Symonds *et al.* 2007) and the abundance of UCP1 increases postnatally in parallel with brain maturation (Symonds & Budge 2009). This contrasts with large mammals, such as humans and sheep, in which parturition and birth are accompanied with intense endocrine stimulation of BAT fully developed at birth and an exponential rise in UCP1 (Symonds 2013, Symonds *et al.* 2015). The magnitude of response is dependent on the thermal environment, the route of delivery (Symonds *et al.* 1995) and can also be modulated by the mother. Prolonged maternal cold

Figure 1

Summary of the number of publications relating to brown fat over the past 50 years.

exposure, for example, promotes overall foetal growth and fat deposition in the foetus and enhances the newborn's capacity to thermoregulate after birth (Symonds *et al.* 1992). Metabolic adaptation around the time of birth is accompanied by rapid mobilisation of lipid and glycogen reserves to meet such a pronounced increase in metabolic rate (Mellor & Cockburn 1986) that is seldom reached at any other time of life (Symonds *et al.* 1989). At the same time, the increase in blood oxygenation following the onset of breathing and exposure to the extra-uterine environment is paralleled by a rise in circulating glucose and free fatty acids (Symonds *et al.* 2015). Concomitantly, plasma short-chain fatty acids, such as acetate, decline (Symonds *et al.* 2015), although the direct impact this has on the subsequent postnatal disappearance of brown fat is unknown.

The impact of temperature on adipose tissue function persists after birth when rearing in a warm temperature accelerates the loss of UCP1 and promotes lipid deposition (Symonds *et al.* 1996). Maternal diet can also modulate the amount of brown fat in the newborn, with an increase in food intake promoting the relative abundance of UCP1 (Budge *et al.* 2000), although the postnatal consequences remain to be explored. The thermal environment also has a modulatory role in determining the metabolic outcomes (Maloney *et al.* 2014): an effect seen in adults as well as during early life. For rodents, both during development and in adulthood, it would appear necessary that they are housed at thermoneutrality (i.e. ~28°C) when the aim is to mimic any effects of deviation from thermal neutrality in humans. As such, standard housing conditions (i.e. c.21°C) adopted in all developmental rodent studies represent an appreciable cool stimulus, which would stimulate thermogenesis in brown fat (Maloney *et al.* 2014). Rearing in a cool temperature also impacts on a wide range of other physiological functions including cardiovascular control and the capacity to replicate metabolic-related diseases in mice as these appear to be housing temperature dependent (e.g. Giles *et al.* 2017). Indeed, epidemiological evidence from the United States suggests that rising environmental temperatures could contribute to enhanced risk of diabetes (Blauw *et al.* 2017), whilst a Canadian study indicated a similar relationship with the onset of gestational diabetes (Booth *et al.* 2017).

To date, the influence of the thermal environment has been ignored in studies examining the impact of the developmental origins of adult disease. This should be rectified and it must be noted that the thermoneutral zone in newborn and young animals is actually closer to their body temperature (Cannon & Nedergaard 2011).

Recent studies have suggested that low-birth-weight offspring have raised UCP1 compared to normal-sized litter mates (Dumortier *et al.* 2017). Growth-restricted offspring would be expected to be much more susceptible to cold stress due to their lower body weight, greater surface area to body weight ratio and reduced substrate availability, providing additive stress to increase non-shivering thermogenesis – an effect less likely to be seen if animals were housed at thermoneutrality. Furthermore, the modest perturbations in glucose homeostasis typically seen in offspring born to dams fed low-protein diet (Dumortier *et al.* 2017) may not be present if they were maintained in a thermoneutral environment with its inherently reduced thermogenic stimulus and brown fat activity. This also means that the pre-diabetes phenotype that occurs with ageing in this model would be expected to develop much earlier and provides an example of sub-optimal experimental protocols adopted in these types of studies.

In addition to the confounding effects of the environmental temperature adopted in studies of metabolic activity and brown fat function, common errors include the potentially confounding effects of gender and foetal number and the potential inflation of any effects when the much larger number of offspring are considered the unit of assessment for maternal interventions in place of the mother (Symonds *et al.* 2009). Taken together, it is not surprising that comparatively little progress has been made into elucidating the primary mechanisms by which maternal obesity may programme the offspring, let alone designing, and testing, credible interventions that could impact on obese pregnant women. Such methodologies may also explain why a majority of rodent models of maternal obesity either have no impact on birth weight (Symonds *et al.* 2013a) or increase the incidence of intra-uterine growth retardation (Panchenko *et al.* 2016).

Brown fat thermogenesis and the control of body temperature

In accord with anatomical studies conducted in newborn infants nearly 50 years ago (Aherne & Hull 1966), the main depot of brown fat identified from positron emission tomography-CT (PET-CT) studies in adult humans resides within the supraclavicular depot (Au-Yong *et al.* 2009, van Marken Lichtenbelt *et al.* 2009). Due to the relatively close location of this depot to the skin, it has proven feasible to measure changes in its activity using thermal imaging (Law *et al.* 2017). Such studies have shown that

Figure 2

Infrared thermographs of (A) a lean and (B) an obese child showing increased supraclavicular hotspot temperature overlying BAT in the lean individual (35.4°C) compared with obese (34.5°C). Images after analysis to locate the hottest 10% pixels show (C) a centralised hotspot (red) within the supraclavicular region of interest (blue line) in the lean individual and (D) a more dispersed heat signature in the obese. BAT, brown adipose tissue.

the temperature of the supraclavicular region is at least 0.5°C higher than the surrounding area (Fig. 2), whilst the relative difference between this area and adjacent regions increase further when brown fat is activated. There is also a close relationship between body temperature and brown fat function during development so that, in the sheep, for example, body temperature increases immediately after birth to ~1°C higher (Fig. 3A) than would be expected in the adult and then gradually declines as brown fat is lost (Symonds *et al.* 1989). The relationship between body temperature and brown fat function in adult humans indicates that the reduced activity of BAT seen with obesity is accompanied by a lower body temperature (Grimaldi *et al.* 2015). This is particularly noticeable during the day (Fig. 3B), after feeding and is indirect evidence of defective dietary-induced thermogenesis (Symonds 2013), although further studies are required to confirm these findings.

Maternal obesity and BAT as a potential therapeutic target

In the United Kingdom for example, overweight or obesity affects almost half the women of child-bearing age (16–44 years) (Health and Social Care Information Centre 2016), and represents an important health issue for women and their children. Inadequate diet is clearly a factor and could relate to the much greater purchase of processed foods compared with the rest of Europe (Monteiro *et al.* 2018). Maternal obesity during pregnancy can directly influence infant size and metabolic characteristics from conception onward, is one of the main predicting factors

of later offspring obesity (Dabelea & Crume 2011) and can have trans-generational effects. With increased BMI and reduced insulin sensitivity, an enhanced trans-placental gradient exposes the foetus to more nutrients, especially glucose (Catalano *et al.* 2009). The consequent increase in

Figure 3

Summary of changes in body temperature over the life cycle and the effect of obesity. (A) Shows the rise in temperature after birth in young sheep when maintained at thermoneutrality (red line) or in the cold (blue line). (B) Shows difference in diurnal temperatures between lean and obese adult humans.

foetal insulin secretion (Pedersen 1954) stimulates foetal growth and energy storage, increasing fat mass, and birth weight (Modi *et al.* 2011). Longer term adverse effects on the offspring can include the maintenance of a heavier body weight and greater adiposity, thereby increasing the risk of developing heart disease and diabetes in adulthood (Dabelea & Crume 2011, Reynolds *et al.* 2013, Gaillard 2015).

Obesity during pregnancy is particularly resistant to existing lines of treatment, with weight rebound common, suggesting that prevention or amelioration of the development of obesity in women prior to pregnancy is critical (Symonds *et al.* 2013b). Reducing hyperglycaemia in obese mothers throughout conception, pregnancy and lactation could interrupt early-life obesity in offspring, reducing their long-term risk of metabolic disease. Consequently, a primary goal in achieving such outcomes could be to target brown fat (Symonds *et al.* 2017). This may be achieved by treating obese females with metformin, a biguanide drug commonly used in diabetes treatment. It primarily decreases circulating glucose by inhibiting hepatic gluconeogenesis, which is insufficiently suppressed in insulin resistance, and by increasing glucose transporter (GLUT)4-dependent glucose uptake into muscle and white fat (Grisouard *et al.* 2010, Turban *et al.* 2012). Metformin lowers body weight, plasma glucose, glycosylated haemoglobin, triglycerides and non-esterified fatty acids, in humans (Diabetes Prevention Program Research Group 2002) and mice (Geerling *et al.* 2014).

Metformin has also been shown to have a direct effect on thermogenic tissues. In cell culture, brown adipocytes show a dose-dependent response (1–100 µmol/L) to metformin, increasing phosphorylation of hormone-sensitive lipase, AMP-activated kinase and acetyl-coA carboxylase, and lipolysis, indicating lipid consumption (Geerling *et al.* 2014). In ApoE3-transgenic mice with diet-induced obesity, metformin administration induces selective uptake of very-low-density lipoproteins triglycerides into brown fat, induces expression of thermogenic genes including peroxisome proliferator-activated receptor gamma, coactivator 1α (PGC1α) and hormone-sensitive lipase and lowers lipid content in brown fat (Geerling *et al.* 2014). These effects are consistent with enhanced thermogenic activity without the need for cold stimulation.

Oral treatment with metformin is routinely used to reduce hyperglycaemia in pregnancy (Hughes & Rowan 2006) with few maternal side effects (Domecq *et al.* 2013)

and no reported adverse effects on foetal development (Cassina *et al.* 2014). Metformin is transported across the placenta (Vanky *et al.* 2005, Eyal *et al.* 2010) and is present at low concentrations in breast milk (Hale *et al.* 2002, Eyal *et al.* 2010). In rodents, maternal treatment with metformin during pregnancy has no reported effect on offspring health when weaned to a low-fat diet but offspring are protected from excess body weight gain and the adverse metabolic effects of subsequent exposure to a high-fat diet (Salomäki *et al.* 2014). In a clinical intervention study, giving metformin to obese pregnant women had no apparent effect on their weight gain, glucose tolerance or the weight and adiposity of their newborn infants (Chiswick *et al.* 2015). However, whether the absence of any immediate outcomes in the newborn could mask protective effects at a later time point for these children has not been studied.

Both pre (Salomäki *et al.* 2014) and early postnatal (Liang *et al.* 2016) exposure to metformin has been linked to sustained effects on offspring thermogenic activity through brown and beige fat. When obese pregnant mice were treated with metformin, both their male and female offspring gained less weight and fat when subsequently exposed to a high-fat diet in adulthood (Salomäki *et al.* 2014). The offspring were also protected from the diet-induced onset of glucose intolerance. White adipocyte size was reduced, suggesting an improved secretory profile (Skurk *et al.* 2007), and UCP1 gene expression was raised (Salomäki *et al.* 2014). Metformin treatment of the offspring through lactation further modulated brown fat function in pups whose mothers were fed a high-fat diet post-partum (Liang *et al.* 2016). In this mouse study (Liang *et al.* 2016), the lipid content of milk was raised, as was fat mass. Critically, although metformin had no effect on body weight, it did restore UCP1 abundance and function in vitro (Liang *et al.* 2016). However, only modest effects on rectal temperature were seen, maybe due to the standard (cool) housing temperature adopted and offspring were only examined up to one month of age. Taken together, these studies demonstrate the potential of metformin to protect offspring of mothers fed a high fat diet from adverse effects. They were, however, limited to the effects of short-term metformin administration or acute exposure to an obesogenic diet in pregnancy (in the case of the clinical study (Chiswick *et al.* 2015) omitting conception and early pregnancy), or to lactation, and measured only short-term outcomes. Therefore, it would appear necessary to apply this intervention in a manner more relevant to the clinical situation.

Depot-specific developmental profiles and their contribution to heat production in adulthood

The most widely studied brown fat depot in rodents is the interscapular one, although in humans, the primary depot resides in the neck i.e. within the supraclavicular region (Cypess *et al.* 2013). A comparable depot has now been identified in mice, which shows a number of similarities to interscapular fat from as early as 18.5 days post conception (Mo *et al.* 2017). This includes some molecular and morphological (e.g. mitochondrial) characteristics, although its postnatal growth is constrained. Furthermore, gene expression of specific putative beige or white markers, were very different in the supraclavicular region compared with interscapular, inguinal and epididymal (i.e. white fat) depots. For example, *HoxC8* and *Zic1* were absent from the supraclavicular depot, which also showed a separate principal component analysis (Mo *et al.* 2017). In sheep, the equivalent depot is found in the sternal region and has a very different postnatal ontogeny to the more widely studied perirenal-abdominal depot (Henry *et al.* 2017). The sternal depot, unlike other fat depots in the sheep, retains UCP1 into adulthood, where it exhibits a pronounced thermogenic response to feeding (Henry *et al.* 2017). In this regard, it is comparable to the supraclavicular depot in humans, which increases in temperature after a meal (Scotney *et al.* 2017). The unique gene profile of supraclavicular fat shown when candidate genes were measured in both mice (Mo *et al.* 2017) and sheep (Henry *et al.* 2017) has recently been extended to the five major fat depots in young sheep when analysed using microarrays (Fainberg *et al.* 2018).

Undertaking microarray analysis at two important time points in early postnatal life of sheep, coincident with the transition of fat from a depot in which brown characteristics dominate (i.e. 7 days of age) to when brown fat is scarce (i.e. 28 days of age) (Symonds *et al.* 2015), has illustrated the unique nature of each depot (Fainberg *et al.* 2018). Machine learning algorithms, in conjunction with weighted gene co-expression network analysis, demonstrated that the five depots examined (i.e. sternal, perirenal, pericardial, subcutaneous, omental) could be segregated into defined sets of modules containing co-expressed genes, indicative of separate functions. The developmental changes markedly differed between depots despite them showing a similar macroscopic morphology (Fainberg *et al.* 2018). This means that, at 28 days when, in sheep, fat is considered primarily white (Symonds *et al.* 2015), each adipose depot kept a distinct gene expression

profile (Fainberg *et al.* 2018). Consequently, although adipose tissue has been considered a metabolic organ with important functions beyond lipid storage, this varies between depots, especially during development. Adipose tissue, therefore, has a range of functions depending on location (Macotela *et al.* 2012). This concept was formulated by using a computer-assisted supervised learning algorithm, to demonstrate that, during postnatal development, each fat depot contains a transcriptome which forms dynamic networks with unique sets of genes (Barabasi & Oltvai 2004). Over the first month of life, in sheep, these gene networks are reorganised by accommodating novel members and/or losing some of their original components (Fainberg *et al.* 2018). Dynamic changes in gene regulation with age are rarely examined but do enable the identification of important regulatory relationships. These will have crucial depot-specific roles to enable differentiation and the adaptation necessary to modulate metabolic homeostasis (Macotela *et al.* 2012). Despite recent efforts to elucidate the cellular and transcriptome composition of different fat depots (Lidell *et al.* 2013, Rockstroh *et al.* 2015), the influence of genetic, endocrine or environmental factors on fat development remains largely unknown. It is further likely that these differences are mediated in part by sympathetic innervation that have recently been shown to include dense arborizations within adipose tissue that regulate the beiging process, at least in adult mice (Jiang *et al.* 2017).

Epicardial adipose tissue

One of the more widely studied depots resides within the epicardial region (Aldiss *et al.* 2016) which retains UCP1 into adulthood (Sacks *et al.* 2013) and, as such, is likely to have a role in heat generation and protecting the heart against the cold (Sacks & Symonds 2013). Epicardial adipose tissue may also have additional roles within the heart apart from thermogenesis, such as the regulation of vascular tone and the modulation of inflammation (Antonopoulos & Antoniadis 2017). One of the reasons this depot has been widely studied is its accessibility at the time of surgery and the ease with which small amounts can be removed for subsequent analysis. This has enabled a more detailed ontogeny during early life and demonstrated how utilising contemporary systems biology approaches, such as through construction of gene networks, can elucidate the transcriptional function (Ojha *et al.* 2016). Computational methodology, through the development of open source packages (e.g. WGCNA,

DAVID and Cytoscape), provides a complementary method to understand adipose tissue biology. Such an approach enables the subdivision of genes into regulatory pathways on the basis of their relative expression. By segregating genes into functional groups, changes in the topology of intra-modular networks enabled the identification of biologically important genes within these pathways. For example in the neonatal (i.e. aged between 1 and 22 days) network, high connectivity of hub genes that regulate cellular activities, particularly those associated with cell differentiation and mitochondrial function, were identified (Ojha *et al.* 2016). It also emphasises the intricate co-ordination of multiple processes that control thermogenesis in epicardial adipose tissue of neonates, as well as the manner in which these gene-to-gene interactions shift towards lipogenesis with age (Fig. 3). The same study indicated significant correlations, both positive and negative, between age and negative Z scores for growth (Ojha *et al.* 2016), which could link adipose tissue dysfunction to environmental factors which promote cardiovascular diseases (Ojha *et al.* 2013). Indeed, the concept of specialisation of epicardial fat genes into functional groups, is based not only on maturation through age but also on location in different anatomic sites within the epicardial fat layer per se, namely pericoronary, periatial and periventricular (Gaborit *et al.* 2015).

Ultimately, the transcriptome in epicardial adipose tissue in early life appears to be sensitive to a longer term reduction of cardiac performance which is not dissimilar to that found in adults with advanced coronary artery disease (McAninch *et al.* 2015).

The structure and architecture of adipose tissue thus differs between the neonate, infant and child with pronounced regulatory effects on UCP1 (Fig. 4). In particular, epicardial adipose tissue retains discrete islands of UCP1 positive cells persisting beyond the neonatal period (Ojha *et al.* 2016). These thermogenic cells exhibit

developmental adaptations in their transcriptional architecture, which could modulate cellular function, and this appears to continue throughout life (Chechi *et al.* 2017). Although epicardial fat has limited flexibility and responsiveness in the newborn period, with age and maturation it becomes more responsive to environmental stimuli (Ojha *et al.* 2016). These unique, developmentally-regulated gene interactions and metabolic-related pathways are potential targets for intervention strategies designed to promote BAT function, and potentially reduce the risk of later heart disease.

In conclusion, the study of brown fat has undergone a marked renaissance over the past 10 years, although a majority of studies have adopted cold adapted mice. To make substantial progress over the next decade, focus will need to be on human-relevant studies, together with those which explore the extent to which changes in early life can enhance brown fat function throughout life.

Declaration of interest

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Figure 4

Summary of the changes in epicardial adipose tissue composition through the life cycle.

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