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REVIEW

Renaissance of brown adipose tissue research: integrating the old and new

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The recent demonstration of active brown adipose tissue (BAT) in adult humans, along with the discovery of vast cellular and metabolic plasticity of adipocyte phenotypes, has given new hope of targeting adipose tissue for therapeutic benefit. Application of principles learned from the first wave of obesity-related BAT research, conducted 30 years earlier, suggests that the activity and/or mass of brown fat will need to be greatly expanded for it to significantly contribute to total energy expenditure. Although the thermogenic capacity of human brown fat is very modest, its presence often correlates with improved metabolic status, suggesting possible beneficial endocrine functions. Recent advances in our understanding of the nature of progenitors and the transcriptional programs that guide phenotypic diversity have demonstrated the possibility of expanding the population of brown adipocytes in rodent models. Expanded populations of brown and beige adipocytes will require tight control of their metabolic activity, which might be achieved by selective neural activation, tissue-selective signaling or direct activation of lipolysis, which supplies the central fuel of thermogenesis.

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The past 4 years has seen an explosion of interest in brown adipose tissue (BAT) that has been fueled by the unequivocal identification of active BAT in adult humans, and by the growing appreciation of the intrinsic metabolic and cellular plasticity of mammalian adipocyte phenotypes. Whether and how this new appreciation might translate into new therapeutics for the treatment of obesity-related disorders is uncertain at present. Nevertheless, there is no doubt that these recent findings have ushered in a renaissance of interest into mechanisms by which various adipose tissue depots might contribute to energy expenditure. The recent interest in BAT as a therapeutic target was preceded by a 'first wave' of research that examined, and largely abandoned, the adipocyte/thermogenesis approach. Although this first wave of research lacked the molecular and genetic tools of today and was based on a simplistic view of adipocyte complexity, this experience nevertheless provides important parameters that may help define and inform current approaches.

THE CENTRAL ROLE OF BAT IN NONSHIVERING THERMOGENESIS, AND THE MYTH OF DIET-INDUCED THERMOGENESIS

The first wave of interest in BAT as a potential target for obesity-related disease was triggered by three key discoveries in the late 1970s and early 1980s. At that time, the concept of luxuskonsumption, that the efficiency of metabolism diminished under conditions of overnutrition, had been discussed for decades. Aside from the fact that the phenomenon only had anecdotal support, it certainly lacked a physiological mechanism and tissue target. Three papers were subsequently published that crystallized the concept of diet-induced thermogenesis into testable physiological reality. The first paper was the clear demonstration by Foster and Frydman that BAT is the dominant site of cold-induced nonshivering thermogenesis in rats.[1,2] It was well known that cold adaptation increased the thermogenic responsiveness of rats to injection of norepinephrine (NE), and the work of Foster and Frydman demonstrated, using measurement of blood flow and oxygen extraction, that BAT is the dominant (but not exclusive) site of NE-induced thermogenesis in rats. It should also be noted that the relative contribution of BAT was significantly less in warm-adapted animals, and that the response of more selective activators of BAT (that is, β3 receptor agonists) was less than NE.[3] Nonetheless, experiments in the intervening years have clearly established that BAT is critical for nonshivering thermogenesis in rodents, and that in the absence of functional brown fat alternative mechanisms that are less effective must be engaged.[4,5]

In addition, in the late 1970s, Young and Landsberg[6–8] published a series of papers demonstrating that nutritional interventions, such as sucrose feeding, increase the activity of the sympathetic nervous system (SNS) to several key targets, including BAT. As it was well established that SNS activity activates BAT and leads to its expansion during cold stress,[9–11] a potential link between diet, overnutrition and BAT thermogenesis could be drawn.

Perhaps, the influential paper for founding the first wave of BAT research was published in late 1979 in *Nature* by Rothwell and Stock.[12] In that paper, rats were fed a complex 'cafeteria' diet that promoted overconsumption of energy, but did not result in the expected retention of energy in the form of carcass fat. According to the authors, the 'missing' energy implied a thermogenic process that was triggered by diet, perhaps in a manner similar to that observed in pigs fed a low-protein diet.[13] These investigators went on to show that cafeteria-fed rats had larger brown fat depots and were significantly more responsive to the thermogenic effects of NE injection. As BAT is the dominant site of energy expenditure in cold-adapted rats, and sympathetic activity is the main driver of cold-induced BAT expansion, the clear inference was that BAT is a major site of diet-induced thermogenesis. If so, then one might propose that defects in diet-induced BAT thermogenesis might contribute to obesity.

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In the next 15 years, scores of studies were produced that addressed the phenomenon of diet-induced thermogenesis and the potential role of BAT. The general outcome of this work was inconclusive, at best. The concept of diet-induced thermogenesis in rodents remains highly controversial: in fact, the same cafeteria feeding protocol used by Rothwell and Stock[12] as evidence of BAT involvement failed to increase BAT thermogenesis when directly measured by blood flow studies.[14] Despite much effort, there is no conclusive demonstration that overnutrition increases responsiveness of humans to catecholamines or increases metabolic rate in a manner that is not predicted by increased body mass.[15–17]

It is important to note that the lack of evidence for clear involvement of BAT in diet-induced thermogenesis[4] is not to deny the importance of BAT-mediated thermoregulatory thermogenesis in energy balance, particularly in small rodents. For example, the difference in energy accumulation in pair-fed ob/ob and wild-type mice markedly declines as housing temperature increases, owing mostly to greater energy retention by wild-type mice.[18] Conversely, cold adaptation greatly increases insulin sensitivity and glucose transport in BAT.[19,20] Thus, adrenergic activation of BAT clearly provides a sink for excess calories and a means of avoiding systemic lipotoxicity during overnutrition.[21,22]

THE HOPE OF B3 ADRENERGIC RECEPTORS (ADRB3)

The major physiological regulator of brown fat thermogenesis is activation by the SNS. Work performed on rodents established that the responsiveness of animals to the thermogenic effects of NE correlated so well with the degree of cold adaptation that the two phenomena became nearly synonymous.[23] NE is known to increase the metabolic rate in humans, and work in the 1960s established that the thermogenic effects of NE in man depend on lipolysis and fatty acid oxidation.[24,25] Experiments in rodents have clearly established that mobilized free fatty acids are central to BAT thermogenesis: first, free fatty acids are direct allosteric activators of uncoupling protein-1 and are sufficient to trigger thermogenesis in the absence of protein kinase A activation.[26,27] Second, free fatty acids are the fuel of BAT thermogenesis, and defects in fatty acid mobilization,[28] activation,[29] transport[30] or oxidation[31] profoundly disrupt cold-induced thermogenesis. Thus, lipolysis is both necessary and sufficient to induce thermogenesis, and it may be the final common pathway by which catecholamines elevate metabolic rate.

Perhaps, the clearest demonstration of the role of adipocyte lipolysis came from the discovery, in 1984 by Arch *et al.*[32,33] at Beecham Pharmaceuticals, of novel compounds that activated adipocyte lipolysis selectively and thereby triggered robust thermogenesis. These compounds were highly effective in increasing thermogenesis, and when given chronically they reproduced the beneficial effects of cold adaptation on insulin sensitivity in rodent models. Years later, the molecular reality of the 'atypical beta receptor' was confirmed with the molecular cloning of the β3-adrenergic receptor,[34–36] which provided new tools for improving compound efficacy and selectivity, and manipulating the system genetically. Selective β3 agonists have been extremely important tools for probing adipose tissue function *in vivo*. These agents activate classic BAT, recruit inducible 'beige' adipocytes in subcutaneous white adipose tissue (WAT) depots, and promote proliferation and differentiation of brown adipocytes in abdominal WAT.[37–40] However, ADRB3 agonists have not been proven to be effective for obesity-related disorders in humans (although one, mirabegron,[41] is an approved treatment for urinary incontinence). Why? One reason almost certainly relates to species differences in the expression of ADRB3. In humans, ADRB3 is expressed in BAT, but not in WAT,[36,42,43] and experiments from the Lowell laboratory demonstrated that ADRB3 must be expressed in both WAT and BAT to achieve full thermogenic response.[44] Indeed, the thermogenic response of BAT alone is only 20–25% of that achieved in wild-type mice. These results indicate that adrenergically mediated thermogenic responses require activation of both BAT and WAT, and the latter may be involved in uncoupling protein-1-independent thermogenesis.[37,45]

NEW ERA: HUMANS HAVE COLD-ACTIVATABLE BAT, AND ADIPOSE TISSUE EXHIBITS TREMENDOUS CELLULAR AND METABOLIC PLASTICITY

As mentioned in the introduction, the renaissance of interest in BAT derives from the synergy of two lines of research. The first line of research is the clear demonstration that many, and perhaps most, adult individuals have depots of BAT that can be activated by mild cold stress.[46–49] The second line of research is the recognition of the tremendous heterogeneity of adipocytes throughout the body with regard to cellular origins and metabolic plasticity.[50–52] This new research raises the hope that one might target the intrinsic cellular and metabolic plasticity of adipocytes and adipocyte progenitors for therapeutic benefit. These are clearly early days, and it may be useful to reflect on how conclusions from the first wave of BAT research might be integrated to advance the exciting new era.

Positron emission tomography (PET) imaging studies conclusively demonstrate that mild cold stress produces an impressive increase in uptake of ^{18}F fluorodeoxyglucose. One key question is as follows: what does ^{18}F fluorodeoxyglucose uptake mean with respect to energy expenditure? On one hand, glucose uptake is thought to supply $<10\%$ of calories burned in BAT, as fatty acids are the main source of fuel for BAT thermogenesis. Thus, elevated glucose uptake, although small (<20 kcal), could be the 'tip of the iceberg' with respect to energy expenditure. To address this question, we applied the logic used by Foster and Frydman[2] and used PET to directly image blood flow and oxygen extraction during cold-induced BAT activation.[53,54] The results of these experiments demonstrate that despite high ^{18}F fluorodeoxyglucose uptake, the increases in oxidative metabolism, as measured by 15O PET, were <10 kcal per day. As oxygen delivery sets the upper limit on oxygen consumption, those studies that have measured blood flow agree that the metabolic rate of cold-activated supraclavicular BAT is <20 kcal per 100 g per day.[53–56] To put this in perspective, BAT of cold-adapted rats can achieve blood flow of 1000 ml per min per 100 g versus 15–20 ml per min in warm-adapted humans. The clear conclusion is that the abundance and/or magnitude of thermogenic activation of BAT would need to be increased by 20- to 50-fold to achieve an effect similar to brief exercise.

PET imaging experiments are not without limitations. PET imaging averages over several cubic millimeters of tissue, and thus microscopic patches of brown adipocytes may not register above the background of typical white adipocytes. In this regard, we and others have noted that individuals with PET-defined BAT exhibit an increase in metabolic rate in response to cold, whereas those without brown fat do not. This observation suggests three possibilities: first, thermogenesis may be because of widely distributed brown adipocytes in white fat whose activity is difficult to image by PET. In this regard, we noted that the activity of white fat was also correlated with cold-induced energy expenditure.[53,57] The second possibility is that BAT engages downstream thermogenic mechanisms, perhaps by the release of hormones such as FGF21.[58,59] Last, BAT thermogenesis in humans may simply be a small component of a larger metabolic response that is driven by a common factor, such as the SNS. Regardless, identification of the sites of cold-induced energy expenditure and the signaling mechanisms involved are important unresolved questions.

The amount and activity of brown fat varies widely among individuals, and an important task of future research is to identify the signaling and transcriptional networks that guide commitment and differentiation of progenitors into active brown adipocytes. In this regard, recent lineage-tracing studies have

highlighted the developmental and anatomic heterogeneity among adipocytes that can express uncoupling protein-1.[40,52,60] As detailed in the following papers, work by Kajimura has demonstrated the importance of PRDM16 in specifying the brown fat lineage, including the central role of EMH1.[61–63] Work from the Tseng laboratory was among the first to establish the cellular complexity of cells capable of uncoupling protein-1 expression,[51] including the critical role of BMP7 signaling in classic and inducible brown adipogenesis.[64–66] Each of these discoveries has provided new insights and tools for assessing brown fat function. Our own lineage-tracing experiments have identified progenitors in abdominal white fat that are capable of brown or white adipogenesis, depending on activating signals.[39,40] The cells are a subset of stromal vascular cells that express PDGFRA, Scal and CD34, and are highly committed to the adipocyte lineage.[67] Interestingly, these cells appear to be the main source of new adipocytes that are produced in response to cell death in adult adipose tissues.[39] Given the large heterogeneity among adipocyte depots in rodents, at present it is unclear how our understanding of a specific marker in mice will translate to humans. As reported in this compilation, work from the Enerback laboratory suggests similar heterogeneity of classical BAT, and beige adipose tissue is likely to be found in humans.[68,69]

Although making more BAT may be necessary for achieving therapeutic effect, precise means of regulating the thermogenic and endocrine functions of brown adipocytes will need to be discovered. The SNS is well established as the dominant regulator of classic brown fat function. Activation of the SNS is stimulus dependent, and cold stress triggers a pattern of activation that is distinct from other physiological stimuli.[70] The work of Bartness and colleagues[71,72] has provided a deeper understanding of the circuits involved in the regulation of BAT and WAT, which may lead to a more effective means of activation of energy expenditure, without unintended effects on the cardiovascular system. Alternative means of increasing the thermogenic potential of BAT include retinoic acid, TGR5 agonist, thyroid hormone and irisin (reviewed in Villarroya and Vidal-Puig[73]). Recent results suggest that transient receptor potential channels may provide a novel means of indirect and direct activation of BAT-dependent thermogenesis.[74,75]

As mentioned above, there are compelling data indicating that BAT thermogenesis relies on lipolysis, and that both brown and white adipocytes contribute to the total thermogenic response of lipolytic agents. In view of the failure of ADRB3 agonists to work as thermogenic agonists in humans, other means of activating lipolysis need to be exploited. In this regard, the recent appreciation that natriuretic peptide signaling leads to activation of classic and inducible brown fat by the Collins laboratory offer a novel strategy for inducing energy expenditure without the deleterious effects of systemic sympathomimetics.[76,77] In addition, recent advances in our understanding of lipolytic mechanisms that are downstream of protein kinase A may offer new targets for fat-specific activation of lipolysis and catabolic remodeling of both BAT and WATs.[78,79]

CONFLICT OF INTEREST

The author declares no conflict of interest.

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DISCLAIMER

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