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REVIEW

Neural control of white, beige and brown adipocytes

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Reports of brown-like adipocytes in traditionally white adipose tissue (WAT) depots occurred ~30 years ago, but interest in white adipocyte 'browning' only has gained attention more recently. We integrate some of what is known about the sympathetic nervous system (SNS) innervation of WAT and brown adipose tissue (BAT) with the few studies focusing on the sympathetic innervation of the so-called 'brite' or 'beige' adipocytes that appear when WAT sympathetic drive increases (for example, cold exposure and food deprivation). Only one brain site, the dorsomedial hypothalamic nucleus (DMH), selectively browns some (inguinal WAT (IWAT) and dorsomedial subcutaneous WAT), but not all WAT depots and only when DMH neuropeptide Y gene expression is knocked down, a browning effect is mediated by WAT SNS innervation. Other studies show that WAT sympathetic fiber density is correlated with the number of brown-like adipocytes (multilocular lipid droplets, uncoupling protein-1 immunoreactivity) at both warm and cold ambient temperatures. WAT and BAT have sensory innervation, the latter important for acute BAT cold-induced temperature increases, therefore suggesting the possible importance of sensory neural feedback from brite/beige cells for heat production. Only one report shows browned WAT capable of producing heat *in vivo*. Collectively, increases in WAT sympathetic drive and the phenotype of these stimulated adipocytes seems critical for the production of new and/or transdifferentiation of white to brite/beige adipocytes. Selective harnessing of WAT SNS drive to produce browning or selective browning independent of the SNS to counter increases in adiposity by increasing expenditure appears to be extremely challenging.

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INTRODUCTION

This review will focus on a topic that the authors are repeatedly queried about—what is the sympathetic nervous system (SNS) innervation of brite/beige adipocytes? That is, is there 'special' sympathetic innervation of the subpopulations of brown adipocyte-like fat cells harbored within what are traditionally considered to be the province of white adipocytes only? Unfortunately, little is actually known about this topic, but because the central and peripheral neuroanatomy of the SNS innervation of white adipose tissue (WAT) and brown adipose tissue (BAT) is known, we believe some of this general knowledge is highly relevant to answer this question. In this review, we also will give a brief historical perspective of the seminal studies of what we now term brite or beige adipocytes, the initial findings of which are as much as 30 years old.

MATERIALS AND METHODS

Search strategy and outcome

PubMed was searched through all years using the search sequences 'beige adipocytes AND sympathetic nervous system AND innervation' (0 hits), 'brite adipocytes AND sympathetic nervous system AND innervation' (0 hits), 'beige adipocytes AND sympathetic nervous system' (1 hit,[1] a review) and 'brite adipocytes AND sympathetic nervous system' (1 hit,[2] a review). Harms and Seale[1] have a section on the topic of this review discussing the conclusion that SNS drive browns white adipocytes, but do not discuss the neuroanatomical evidence and related data on this issue, whereas Virtanen *et al.*[2] very briefly discuss and support the conclusion again without the neuroanatomical evidence and related issues. Therefore, these two articles are included in the review, but the rest of the literature is present by the discretion of the authors.

A brief historical perspective on brown adipocytes in humans and brite/beige adipocytes in non-human animals

There is an interesting parallel between the clamor caused by the 'discovery' of BAT in normal adult humans appearing in several articles in 2009[refs 3–7] and the 'discovery' of brite/beige cells in recent more years. In terms of the former, the presence of BAT in normal adult humans should not have been a shock, as Nedergaard *et al.*[8] presented evidence as early as 1996 from clinical studies of humans undergoing fluoro-deoxy-glucose positron emission tomography for the detection of cancerous tumors.[9,10] This rediscovery/discovery of BAT in normal humans indicated an additional potential mechanism to increase thermogenesis beyond locomotor and general metabolism contributions to heat production. Similarly, the 'discovery' of brownish or 'brite/beige' adipocytes by a variety of investigators (for review see: Harms and Seale[1]) at about the same time as the brown adipocyte 'rediscovery' in normal humans discussed above, suggested yet another potential mechanism to increase heat production. Together or singly the possibility of combating increasing or existing obesity with increasing energy expenditure by brown or brite/beige adipocytes has generated some hope for this side of the energy balance equation to counter increasing adiposity, because the ability of pharmacological and/or behavioral approaches to decrease food intake in the long term has failed to a large degree, but with some success seen with various extreme surgical manipulations of the gastrointestinal system.

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To our knowledge, the first use of the term 'brite' for these brownish adipocytes that exhibit, at the minimum, uncoupling protein-1 (UCP-1) that is normally associated with 'true brown adipocytes' and/or multilocular lipid droplets (by contrast with unilocular lipid droplets for traditional white adipocytes) was coined by the Nedergaard/Cannon laboratory in late 2009 online, but in print 2010.[11] The first use of the term 'beige', to our knowledge, was by the Seale laboratory[12] in a commentary about a paper by Vegiopoulos *et al.*[13] The first reports of the presence of 'brown adipocytes' among white adipocytes in traditionally white adipocyte depots to our knowledge, however, occurred 26 years earlier in the pioneering studies of Young *et al.*[14] They reported brown adipocytes in the parametrial WAT pad of BALB/c mice acclimated to the cold, as identified morphologically by light and electron microscopy, and as possessing increases in a marker of brown adipocytes, mitochondrial UCP-1 content,[14] the finding of which the field and we turned a blind eye toward because such a presence was almost heretical, given the Zeitgeist of the time that WAT and BAT were separate tissues with separate functions. Loncar *et al.*[15] soon after reported that young (10–13 weeks old) domestic cats exposed to − 30 °C for 1 h twice a day during a week displayed brown-like adipocyte changes, most notably increases in mitochondria volume and surface density of mitochondrial cristae in several WAT depots not seen in the room temperature controls and more typical of brown adipocytes, not white ones. That was an effect in cats, not a typical laboratory animal, but Loncar in 1991[(ref. 16)] demonstrated what he termed 'convertible adipose tissue in mice' that were cold acclimated for 2 weeks. These common laboratory animals had increases in IWAT UCP-1 messenger RNA (mRNA) that were reversible with several weeks of warm exposure. Still, this finding did not gain wide acceptance or interest compared with that occurring now for brite/beige cells. At about the same time (1992), however, in a particularly thorough study, Cousin *et al.*[17] in laboratory rats found both mRNA and protein for UCP-1 in several WAT depots (mesenteric, epididymal, retroperitoneal and inguinal) that was especially striking in periovarian WAT (which can possess some *bona fide* brown adipocytes; for example, see Giordano *et al.*[18]). As with BAT, cold exposure or β-adrenoceptor agonist treatment exacerbated the UCP-1 in these WAT depots. The UCP-1-bearing periovarian adipocytes exhibited other characteristics of the brown adipocytes, such as increases in mitochondrial crista density and multilocular lipid droplets.[17] They concluded that WAT and BAT are really a continuum,[17] a concept made popular and additionally strongly supported by converging histological and cellular measures more recently by Cinti[19] and termed by him—the 'adipose organ' to remove the distinction between WAT and BAT. Thus, for both of these similar concepts, WAT changes its morphology and function as energy demands are changed. Thus, with increases in energy demand (for example, cold exposure), whitish adipocytes brown, whereas in times of decreases in energy demands, the brownish tissues whiten.[17,19,20] At about the same time (1994), Jean Himms-Hagen *et al.*[21] demonstrated that chronic administration of the selective β_3-adrenoceptor agonist, CL-316 243, triggered the multilocular phenotype in adipocytes normally thought of, at least at that time, as exclusively WAT pads containing only unilocular white adipocytes, including the mesenteric WAT, IWAT, epididymal WAT (EWAT) and retroperitoneal WAT[21] depots. Finally, although well after these seminal studies reporting brite/beige adipocytes in traditionally WAT depots 2002[(ref 22)], but before the most recent flurry of brite/beige cell 'rediscovery', we found that Siberian hamsters (*Phodopus sungorus*) transferred from long 'summer-like' photoperiods (light:dark 16:8 h) to short 'winter-like' photoperiods (light:dark 8:16 h) in the laboratory with typical vivarium ambient temperature (~22–23 °C) had increased WAT β_3-adrenoceptor and UCP-1 gene expressions in retroperitoneal WAT (the only WAT pad tested for these responses[22]). These data showed for the first time, to our knowledge, that a naturally occurring reversal of an obese state (~50% body fat to ~20% body fat[23]) that induces increases in the sympathetic drive to WAT[24] also triggers browning, doing so without decreases in ambient temperature.[22]

Collectively, the finding of brownish (brite/beige) adipocytes in WAT depots that were thought to be the province of only white adipocytes, has a 30-year history, but despite this, only recently has this been recognized as a common phenomenon across many mammalian species including humans. The history of white adipocyte browning has suffered the same fate as that of BAT in normal human adults—the original findings did not nicely fit into the often dogmatic conceptions of the times and, thus, were largely ignored leading to a 'rediscovery' of both phenomena. Finally, as can be seen above from the origins of the study of brite/beige adipocytes, the common thread among the disparate studies of several species is stimulation of the WAT depot and its adipocytes by the SNS or more specifically, stimulation of β-adrenoceptors possessed by these cells.

A common factor in WAT browning

In the seminal studies of browning of WAT depots traditionally thought of as 'pure' white adipocytes discussed above, and in the current literature with one exception[25] (addressed below), there are increases in β_3-adrenoceptor stimulation occurring either naturally by the norepinephrine (NE) released by sympathetic nerve terminals in WAT or by β_3-adrenoceptor agonist stimulation. This stimulation is the principal initiator of lipolysis in white and brown adipocytes in mammals (for a review, see Bartness *et al.*[26,27]), including of course humans, with two notable exceptions in humans (atrial natriuretic peptide; for review see Lafontan *et al.*[28] and perhaps adrenal medullary epinephrine with exercise[29]). The link between increases in lipolysis and browning has not been established, but we believe it is more than coincidental that there are 'pockets' of cells undergoing lipolysis (that is, lipolysis does not occur uniformly within a WAT depot (nor across WAT depots, see below)) and at least the initial browning of white adipocytes also occurs in similar pockets (for example, see Vitali *et al.*[30] and Barbatelli *et al.*[31]). The key support that the browning of white adipocytes is dependent on β_3-adrenoceptor stimulation (for review, see Bartness *et al.*[26,27]) rests on the findings that cold-exposed β_3-adrenoceptor knockout mice compared with their wild-type controls have white adipocytes that: (a) do not express UCP-1, (b) have either reduced or no production of other factors associated with the 'thermogenic program' such as PGC1α, CIDEA and C/EBPβ and (c) have decreased morphological brown-type adipocyte characteristics such as decreases in the number of multilocular cells.[31–33]

Although it has been suggested that because chronic administration of the β_3-adrenoceptor agonist CL-316 243 differentially stimulates browning across WAT depots,[31] this does not necessarily imply that there is a cell autonomous process at work in browning independent of the CNS control of sympathetic drive as some suggest.[1] This effect could easily be explained in terms of the number and affinity of β_3-adrenoceptors that occur across WAT depots (for example, see Bowen *et al.*,[34] Leibel and Hirsch,[35] Umekawa *et al.*[36]) or the balance between lipolytic/browning β_3-adrenoceptors and antilipolytic (and perhaps anti-browning) α_2-adrenoceptors (for review, see Lafontan *et al.*[37,38]). The post-β-adrenoceptor signaling cascade responsible for either the transdifferentiation of white to brite/beige or *de novo* creation of brite/beige adipocytes will not be reviewed here. Instead, we will focus on what is known or appears to be highly likely concerning the neuroanatomical (and to a lesser extent neurochemical) underpinnings of brite/beige adipocytes. We also will include some of what is known about the neuroanatomical and neurochemical features of the more traditional white and brown

adipocytes that probably can be applied, at least in part, to brite/beige adipocytes.

What do we know about SNS innervation of brite/beige adipocytes?

Very little—but despite this and for a good reason, the topic has been included as a small part of a recent review of the browning of WAT.[1] At the level of the WAT depot, the clearest neuroanatomical evidence for the involvement of the sympathetic innervation of WAT in browning to date is that of C57BL/6J mice after cold exposure (6 °C) for 10 days versus their warm acclimated controls (28 °C[30]) and as such will be discussed in some detail here. Sympathetic nerves were immunolabeled with tyrosine hydroxylase (TH), an accepted marker of sympathetic nerves,[39] and the limiting enzyme in catecholamine synthesis including,[40] in several WAT depots and counted when in contact with adipocytes, but not blood vessels.[30] Cold exposure increased the number of TH-immunoreactive (ir) fibers in contact with unilocular white adipocytes (conventional white adipocytes) and, importantly for the topic at hand, TH-ir fiber contact was associated with triple the number of multilocular adipocytes possessed by the traditional WAT depots, especially IWAT and dorsosubcutaneous WAT (DWAT[30]). These depots also were associated with the largest increases in UCP-1-ir.[30] Importantly, TH-ir sympathetic fiber density and multilocular adipocytes that also were UCP-1-ir were positively correlated—a correlation that was greater in the cold-acclimated mice than in their warm-exposed controls.[30] Collectively, these data, along with the browning of WAT following cold exposure or chronic β3-adrenoceptor activation and its severe dampening in β_3-adrenoceptor knockout mice,[33] strongly infer that the sympathetic drive/NE stimulation of WAT β_3-adrenoceptor is important and causative in the WAT browning phenomenon. In addition, these data strengthen the similar notions of plasticity within the adipose organ[19] or of a continuum between white and brown adipocytes within WAT depots.[17]

Finally, it should be noted that it recently has been reported that isolated white adipocytes from β-less mice (knocked out $\beta_{1,2,3}$-adrenoceptors) exposed to cold versus normal room temperature (10 vs 22 °C) can brown and do so obviously without sympathetic stimulation.[25] In addition, by subjecting the murine cell line 3T3L1 cells to mild cooling (37 °C (quite warm) to 31 °C for 4 h) browning also occurs and does so independently of the typical cAMP/protein kinase A/cAMP response element-binding protein pathway that is downstream of post-β-adrenoceptor activation, an ability not shared by brown adipocytes.[25] The physiological *in vivo* significance of these findings is not clear, however.

Are there brain-specific sites controlling sympathetically mediated browning of WAT?

This is an important question that we are consistently asked because, as described in brief below, we were the first to demonstrate neuroanatomically the origins of the sympathetic outflow to WAT[41] and to BAT[42] using the viral transneuronal tract tracer, pseudorabies virus (PRV; for a review see Bartness *et al.*[26,43]). Unfortunately, because the pockets of brite/beige adipocytes are only visible microscopically and mixed among white adipocytes (for example, see Vitali *et al.*[30]), it is not possible to use the powerful ability of PRV to define the SNS outflow circuits from brain ultimately to these cells. We do not believe, however, that even if this was possible, perhaps with one exception discussed below, this would reveal anything startling, as it is our belief that it is not the origin of the CNS outflow to brite/beige cells within the general SNS outflow to adipocytes that is important. Instead, we believe it is the combination of increases in WAT SNS drive and, importantly, the propensity of the preadipocytes or adipocytes receiving the β-adrenoceptor stimulation by NE released by the sympathetic nerve endings that ultimately results in the browning of the adipocytes within the WAT depots. Clearly, the density of the sympathetic fibers is important for the *de novo* production/transdifferentiation of the browning effect based on the warm and cold exposure data of the Cinti laboratory,[30] but because it appears that all cells do not brown, the phenotype of the preadipocyte or adipocyte also is important.

To our knowledge, the only specific brain site that appears to exclusively cause browning specifically in selective WAT depots is the dorsomedial hypothalamic nucleus (DMH). More specifically, when neuropeptide Y (NPY) gene expression (and presumably translation into the NPY protein) within the DMH decreases (with assumed decreases in NPY release at its CNS targets), subcutaneous WAT depots brown.[44] This was demonstrated by injection of adeno-associated virus (AAV)-mediated RNAi (AAVshNPY) into the DMH of laboratory rats and resulted in a specific and marked knockdown of DMH NPY, but not arcuate NPY, compared with DMH injection of the scrambled short hairpin RNA (shRNA) control.[44] Importantly, DMH NPY knockdown decreased subcutaneous (IWAT and DWAT) fat mass and increased browning only in these fat pads, but not in EWAT, mesenteric WAT, retroperitoneal WAT or perirenal WAT.[44] The browning was strikingly apparent to the unaided eye and verified by UCP-1-ir, presence of brown adipocyte-like multilocular lipid droplets within these adipocytes, as well as reverse transcription-PCR and the western blot assay for UCP-1 gene expression and protein, respectively.[44] In addition, DMH NPY knockdown increased the gene expression of the 'thermogenic program' (that is, PPARγ and Pgc-1α mRNA) in IWAT[44]; DWAT was not assayed. To demonstrate that this effect was dependent on the SNS innervation of WAT, they used our specific, unilateral sympathetic denervation model,[45–48] where one IWAT depot received intra-WAT injections of the noradrenergic toxin 6-hydroxy-dopamine (6-OHDA) and its within-animal contralateral IWAT mate received vehicle control injections.[45–48] In the neurally intact IWAT pads of DMH NPY knockdown rats receiving intra-WAT vehicle injections, NE content (often a surrogate used for sympathetic drive, but not a replacement for NE turnover) was significantly increased compared with that of separate control rats receiving DMH injection of the scrambled shRNA. By contrast, the 6-OHDA-treated IWAT pads had significantly decreased NE content compared with its contralateral control IWAT depot and the IWAT depots of the separate group of control rats receiving DMH shRNA injections.[44] Moreover, the 6-OHDA-injected IWAT pads of the DMH NPY knockdown animals had markedly fewer multilocular adipocytes and prominent decreases in UCP1 gene and protein content.[44] Importantly, from a physiological standpoint, thermistors implanted in the browned IWAT of DMH NPY knockdown rats housed at room temperature (23 °C) had significantly increased IWAT temperature, especially at night compared with their controls that received the scrambled shRNA injections into the DMH.[49] To our knowledge, this is the first *in vivo* demonstration of heat production by a profoundly browned WAT depot. With respect to our PRV transneuronal defining of the SNS outflow to WAT, the DMH had more infected neurons after IWAT PRV injections compared with the DMH of animals given EWAT PRV injections.[41] Thus, latter finding suggests a richer SNS innervation from this nucleus to IWAT than EWAT, and perhaps provides neuroanatomical underpinnings for the DMH NPY knockdown effects on browning of subcutaneous WAT. Collectively, these data demonstrate the most specific browning of WAT depots (IWAT and DWAT) by a particular brain site, the effects of which are dependent on the SNS innervation and the only demonstration that browned fat *in vivo* increases WAT pad temperature.

Involvement of the sensory innervation of WAT in the function of brite/beige adipocytes

We previously demonstrated WAT sensory nerve innervation at the level of the fat pad for the proven sensory nerve markers

calcitonin gene-related peptide and substance P in parenchymal nerve fibers,[50] as others have done similarly for BAT.[51] Moreover, using another viral transneuronal tract tracer, the H129 strain of herpes simplex virus 1, we labeled the central sensory circuits from WAT[52,53] and from BAT[53] to the brain. In terms of the latter, sensory innervation of BAT is necessary for the BAT temperature increase in response to acute cold exposure, as evidenced by decreases in BAT temperature when interscapular BAT was specifically sensory denervated via intra-interscapular BAT injection of the sensory nerve toxin, capsaicin.[53] In terms of the function of WAT sensory nerves, they are responsive to intra-IWAT injection of leptin, as assessed by c-Fos-ir in the dorsal root ganglion neurons innervating WAT that were previously labeled with Fluorogold, a non-viral retrograde tract tracer.[54] In addition, the multiunit electrophysiological activity of the sensory nerves emanating from IWAT increases in response to these intra-IWAT leptin injections in Siberian hamsters.[54] Similar electrophysiological responses to intra-WAT leptin injection were first reported in laboratory rat EWAT injected with this cytokine.[55,56] Just as the tips of your fingers respond to pain, temperature, vibration and position, WAT sensory innervation also may respond to several different sensory stimuli including the products of lipolysis and perhaps temperature (for review, see Bartness *et al.*[26,43]). Similarly, brite/beige cells in white fat depots may respond to several different stimuli including those listed above for WAT and for BAT. If the physiological function of brite/beige cells is to increase thermogenesis, then it would seem important for these brownish adipocytes to convey temperature information to the brain to, perhaps in turn, control the SNS drive to the tissue depending on the environmental/physiological situation to ultimately alter heat production. At present, the study of the function of these sensory nerves from BAT or WAT is in its infancy, but we believe lessons learned from these tissues will likely apply, at least in part, to the potential feedback control of the SNS drive to brite/beige adipocytes. Indeed, SNS-sensory feedback loops are a neuroanatomical reality for both WAT and for BAT, as seen when both SNS-specific PRV and sensory-specific H129 viruses are injected into the same IWAT pad (Ryu and Bartness[57]) or into interscapular BAT (Ryu *et al.*[58]). This results in dually labeled (infected) neurons in several brain sites across the neural axis.

CONCLUSIONS AND SPECULATION

We have reviewed what little is known about the SNS innervation of brite/beige adipocytes and attempted to relate this, in brief, to some of what is known about the neuroanatomical and functional evidence of the sympathetic and sensory innervations of these tissues. Clearly from the work of Vitali *et al.*,[30] there are significant correlations between sympathetic nerves (TH-ir nerves) in WAT containing multilocular, UCP-1-ir adipocytes both in warm and cold environments in mice. In the unstimulated state, UCP-1 mRNA and protein are found in mesenteric WAT, EWAT, retroperitoneal WAT, IWAT and especially periovarian WAT, but they do not produce heat because in the unstimulated state UCP-1-bearing mitochondria do not show a natural leaky mitochondrial membrane to protons necessary for heat production.[59,60] This may seem in conflict to the data of Bi[49] who found temperature increases in the browned IWAT of the DMH NPY knockdown rats, but the latter animals were not housed at thermoneutrality and thus the animals were moderately thermogenically challenged. The study by Bi[49] is the only one we are aware of where the real physiology of brite/beige cells has been assessed *in vitro* in terms of their heat production. Given, however, that humans normally exist at thermoneutrality, or close to it, by altering the temperature of their living/working environments or by adding or subtracting clothes, this likely limits the number of naturally occurring brite/beige cells and consequently any significant role of these cells in everyday thermogenesis. The ability to harness the potential heat production by converting/producing white adipocytes to a brownish phenotype via specific increases in the sympathetic drive to these browned WAT depots seems remote, given the cross-innervation of WAT with other tissues (for example, see Kreier *et al.*[61]) including the heart and blood vessels that could lead to coronary/hypertension diseases. Whether this can be accomplished by by-passing the WAT SNS innervation to specifically cause conversion/production of brite/beige cells and subsequently to specifically activate them to increase energy expenditure such that obesity is reversed, diminished or prevented, seems no mean feat in our opinion.

In conclusion, perhaps the most important features of WAT for the production/conversion of white to brite/beige adipocytes is the density of the sympathetic nerves innervating WAT (and of course increases in their activity), as well as the adipocyte cell population surrounding this innervation for their genetic ability to brown. With increases in SNS drive to WAT, lipolysis is triggered and if the adipocyte population is capable, browning occurs. Whether lipolysis is necessary for browning remains to be demonstrated.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLAIMER

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