Aparacrine role for white thermogenic adipocytes in innervation: an evidence-based hypothesis

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Abstract
White adipose tissue (WAT) stores energy and also plays an important endocrine role in producing adipokines for communication with the peripheral and central nervous system. WAT consists of the major lipogenic unilocular adipocytes and the minor populations of beige and brite multilocular adipocytes. These multilocular adipocytes express thermogenic genes and have phenotypic similarity with thermogenic brown adipose tissue. According to a current paradigm, multilocular adipocytes have a thermogenic function in WAT. In this mini review, we discuss data revealing heterogeneity among multilocular cell subsets in WAT and their functions beyond thermogenesis. We propose a hypothetical neuroendocrine role for multilocular adipocytes subsets in the formation of adaptive sensory-sympathetic circuits between the central nervous system and adipose tissue, which activate lipolysis and thermogenesis in WAT in high energy demand situations.

Key Words: obesity; thermogenesis; innervation; vitamin A; aldehyde dehydrogenase; paracrine; efferent; afferent; brown adipose tissue

Endocrine Lessons Learned from Obesity Epidemics
The obesity epidemic, affecting 35.7% of adults in the United States (CDC.gov), draws an exponential growth in interest to the role of white adipose tissue (WAT). The understanding of WAT’s function was advanced beyond insulation and energy storage to its central endocrine role regulating energy homeostasis. The global impact of WAT is achieved after convergence of endocrine signals in the central nervous system (CNS). One of the first mechanisms underlying neuro-endocrine causes of obesity was linked to the adipokine leptin, or its receptor (LepR or ObR) (reviewed in Rosen and Spiegelman (2014)). Deficient leptin/LepR signaling deregulates pro-opiomelanocortin neurons in the hypothalamus controlling appetite, glucose metabolism, thermogenesis, and overall energy homeostasis. The endocrine function of WAT and its impact on CNS is now undisputable. Conditions such as metabolic syndrome, type 2 diabetes, cardiovascular disease, stroke, certain cancers, and diseases of the CNS, including depression and dementia, all appear to be linked to specific endocrine proteins, pro-inflammatory cytokines, and metabolites that are produced in WAT and influence CNS. We have performed a PubMed literature search of articles published in the period 1960–July 2018 on adipokines, neuropeptides, endocrine, beige, brown, brite, thermogenic, thermogenesis, lipolysis, and innervation of adipose tissue.

Paracrine Communication between WAT and Sympathetic Nervous System
The endocrine output depends on the WAT composition [reviewed in Rosen and Spiegelman (2014)]. Preadipocytes and adipocytes produce different endocrine molecules, cytokines, and chemoattractants in response to diet. In obesity, hypertrophic adipocytes and inflammatory cells recruited into hypertrophic WAT secrete additional pro-inflammatory factors into circulation that lead to systemic insulin and leptin resistance and glucose intolerance. High-fat diet, hyperglycemia and oxidative stress in WAT, induce neurodegeneration of sympathetic nervous system (SNS), influencing the norepinephrine/β-adrenergic receptor pathway. Deficiency in SNS signaling reduces lipolysis and thermogenesis in WAT (Figure 1). Blockage of α/β-adrenergic pathways markedly reduces (60%) lipolysis during exercise in lean people, compared to obese participants (100%) (Verboven et al., 2018), highlighting reduced contribution of SNS-dependent lipolysis in obesity. However, in response to exercise, both lean and obese participants activate another lipolytic pathway in WAT that is independent of β-adrenergic/SNS axes (Verboven et al., 2018). Thus, partial neurodegeneration in WAT as a result of obesity reduces the contribution of β-adrenergic pathways to energy mobilization.

The transition from storage to lipolysis in WAT during an energy demand state requires remodeling of sympathetic innervation. This plasticity in WAT SNS/β-adrenergic response has been demonstrated in mouse models (Ruohonen et al., 2018), where high-fat diet-induced neurodegeneration decreased lipolytic and thermogenic responses, and exercise restored them in WAT (Ruohonen et al., 2018). Although these responses are indicative for restored innervation, mechanisms regulating innervation plasticity in WAT are not well understood. Paracrine function of white adipocytes
contributes to innervation in WAT; however, this function is associated with lipid storage rather than activation of β-adrenergic pathways for lipolysis. Preadipocytes and adipocytes express nerve growth factor (NGF) [discussed in Shen et al. (2018)], which is augmented in response to inflammatory cytokines. NGF secretion appears to support an obesogenic type of innervation, highlighted by a positive relationship between plasma NGF levels and patients’ BMI. Paracrine secretion of leptin by WAT appears to induce sensory innervation that could support vasodilation, though progressive leptin resistance in obese patients may diminish its contribution to innervation; this function of leptin has not been tested in vivo. Since white adipocytes cannot produce factors stimulating de novo innervation, we investigated paracrine output of a subset of adipocytes from WAT on neurons (Shen et al., 2018).

**Minor Cell Populations in WAT**

Understanding the role of minor population of adipocytes in WAT and their endocrine contribution has been challenging technically due to the long-lasting misconception of their origin. Abdominal and inguinal WAT contains small interspersed population of multilocular adipocytes of the same mesodermal Myf5-lineage as the surrounding main population of unilocular, lipogenic white adipocytes [reviewed in Carobbio et al. (2018)] (Figure 1). However, these cells express thermogenic uncoupling protein 1 (UCP1), and higher levels of lipolytic and mitochondrial genes. This gene expression pattern is intermediate between brown and unilocular white adipocytes, therefore, these cells were termed ‘beige’, 'brite' (brown and white), UCP1 positive (UCP1+), multilocular, or thermogenic WAT adipocytes by different researchers (Wang et al., 2016). Ground-breaking studies showed that these cell subsets had a different origin than thermogenic brown adipose tissue (BAT) (Carobbio et al., 2018). However, historically, multilocular white adipocytes are attributed a similar thermogenic function as BAT.

The classical BAT originates from Myf5+ mesenchymal precursors; these cells constitutively express Ucp1+ and specific markers Prdm16+ and Zic1+ (Rosen and Spiegelman, 2014; Carobbio et al., 2018). BAT is constitutively innervated by sympathetic neurons during development (Figure 2). In response to cold and other sympathetic and β3-adrenergic receptor stimulations, BAT increases Ucp1 expression for heat production. In this oxygenated tissue, the role of UCP1 in direct defense against oxidative stress is moderate (Shabalina et al., 2006); moreover, increased levels of oxidation are necessary to activate the thermogenic function of UCP1. BAT is located in perivascular and supraclavicular regions in humans and in the subcutaneous scapular regions in mice (Rosen and Spiegelman, 2014). The anatomic location of BAT in humans and mice is permanent and consistent with this thermogenic function.

**Paracrine Mediators of BAT Assist SNS in Regulation of Thermogenesis**

BAT releases several endocrine factors to control thermogenic responses in conjunction with activation of SNS. BAT expresses and secretes bone morphogenetic protein (BMP7 and VEGFA that promote BAT differentiation and vascularization, respectively [reviewed in Carobbio et al. (2018)]. Both secreted molecules stimulate Ucp1 expression, energy expenditure, improve glucose tolerance, and weight loss. However, these effects are seen in context of SNS activation and do not occur at thermoneutrality. Sympathetic activation also mediates glucose flux into BAT that occurs even in the absence of thermogenic response in Ucp1−/− mice.

SNS also appears to control a paracrine feedback regulation of thermogenesis in BAT via activation of adenosine secretion [reviewed in Carobbio et al. (2018)]. Adenosine, at nanomolar concentrations, stimulates thermogenic function, whereas at high concentrations, adenosine blocks β-adrenergic-dependent lipolysis and thermogenesis. De novo innervation is seen mostly in artificial transplants of brown adipocytes, because BAT is innervated during development. Factors responsible for the innervation of BAT implants remain unknown, although in other tissues, BMP7 and VEGFA has been shown to promote innervation. The critical role of innervation in BAT was demonstrated during denervation of BAT in mice overexpressing Ucp1. Denervation disrupts thermogenesis and other systemic metabolic effects of BAT, including increase in energy expenditure and glucose uptake (Rosen and Spiegelman, 2014). This loss of function suggests that BAT acts predominantly downstream of CNS as a thermogenic organ and releases endocrine factors to assist SNS.

**Different Subsets of UCP1+ Adipocytes of WAT**

Multilocular UCP1+ adipocytes in WAT face different challenges than BAT. These multilocular UCP1+ adipocytes reside in WAT, which is a tissue of limited thermoconductivity and sympathetic innervation. Sympathetic response activates a specific subset of cells expressing Tmem26 and CD137. Upon SNS stimulation, these progenitors express Prdm16 and increased levels of adipose triglyceride lipase (ATGL)/ hormone sensitive lipase (HSL) and Ucp1/UCP1 to activate lipolysis and thermogenesis. The SNS-dependent subpopulation of UCP1+ adipocytes in WAT is commonly termed ‘beige adipocytes’ (Wang et al., 2016) (Figure 1). Progressive degeneration of peripheral sensory and sympathetic axons in obesity decreases the population of these cells and is associated with decreased lipolysis and thermogenesis in WAT. In the obesity state, the expression of Ucp1 in UCP1+ adipocytes in WAT is markedly lower compared to that in BAT, a property favoring energy storage in WAT. Under obeseogenic neurodegenerative conditions, interspersed beige adipocytes play a progressively decreased role in lipolysis and thermogenesis.

Other subsets of UCP1+ adipocytes can develop without β-adrenergic stimuli and can still respond to SNS stimulation. They are commonly termed ‘brite’ adipocytes. Stimulation of WAT with peroxisome proliferator-activated receptor (PPAR)γ ligands, cyclin-dependent kinase 5 inhibitors, and other stimuli leads to expression of specific genes Cdsn,
Another brite adipocyte subset in WAT could be generated by omitting genes suppressing thermogenic programs in WAT, known as thermogenic brakes [reviewed in Carobbio et al., (2018)]. The thermogenic suppressor pathways include Soluble low-density lipoprotein receptor relative LR/sorLA (sLP11)/BMP/transforming growth factor β or Zinc finger protein transcription factor 423 (Zfp423)/early B cell factor 2 (Ebf2) signaling axes. The inhibition of these pathways induces differentiation of precursors or conversion of white adipocytes into multilocular adipocytes UCP1\(^{+}\) with efficient ATGL-mediated lipolysis (Ahmadian et al., 2011), which activates PPARα, its target gene Ucp1, and mitochondrial biogenesis. These multilocular adipocytes are also UCP1\(^{+}\) and commonly express increased levels of Ucp2, to protect them from oxidative stress. CNS: Central nervous system. Aldh1a1: aldehyde dehydrogenase family 1 member A1 (Alias Raldh1).

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of UCPI^+ cells support basal lipolysis; however, it has been challenging to understand the specific function of minor cell populations. The energy demand signals from the working muscles (irisin and heart) and stress conditions (Rosen and Spiegelman, 2014) give rise to heterogeneous UCPI^+ populations. To meet chronic energy demand under these conditions, de novo innervation needs to be developed in WAT for an efficient lipolysis. The understanding of causality between innervation and development of brite and beige subsets holds a key to WAT remodeling.

**Vitamin A Metabolism Controls Transition between White and Brite Phenotype**

Different mechanisms have been proposed for induction of ‘brite’ phenotype in white adipocyte precursors expressing thermogenic blocker Zfp423 (Wang et al., 2016; Carobbio et al., 2018). In embryonic cells (Huang et al., 2009), Zfp423 expression is under control of retinoic acid receptor (RAR) activated by its ligand retinoic acid (RA), a vitamin A metabolite. Vitamin A is stored in lipid droplets in white adipocytes, which also express cytosolic enzymes converting retinol to retinaldehyde (Aldehyde dehydrogenase family of enzymes (ADH), retinol dehydrogenase (RDH), and short-chain dehydrogenase/reductase families) as well as retinaldehyde to RA (ALDH1 family, alias RALDH) (Box 1). Overexpression of any of Aldh1 enzymes induces expression of Zfp423 and its target Pparg (Reichert et al., 2011), promoting white phenotype in adipocytes. Deficient expression of Aldh1, particularly Aldh1a1, decreases expression of Zfp423 in vitro in white adipocytes and in vivo in WAT. Removal of this thermogenic brake (Reichert et al., 2011), induces lipolytic and thermogenic properties in WAT of Aldh1a1^−/− mice and render them resistant to obesity induced by high fat diets or ovariectomy (Yasmeen et al., 2013). Importantly, in vitro, Aldh1a1^−/− adipocytes maintained similar heritable lipolytic and thermogenic characteristics and constitutively expressed Ucp1, Ucp2, Pgc1a, Dio2, and cell death-inducing DFFA-like effector a (Cidea) (Shen et al., 2018). These autonomous lipolytic and thermogenic features of Aldh1a1^−/− adipocytes are consistent with properties of brite cells in WAT.

**Box 1 Pluripotent roles of retinoic acid (RA) in white adipose tissue (WAT)**

**Intracellular (endogenously-produced) RA has three independent roles:**

1. In mitochondria RA acts in conjunction with uncoupling protein 1 (UCP1) to uncouple H^+ transport and increase thermogenesis.
2. In cytosol, RA participates in post-translational modification of proteins during adipogenesis.
3. In nucleus, RA acts directly as a ligand for three retinoic acid receptor (RAR) (RARα, RARβ, and RARγ). RAR receptors are expressed in early, intermediate, and late stage of adipogenesis where they regulate expression of different transcription factors, including Zfp423, as well as Krüppel-like family of transcription factor (KLF) and Homeobox transcription factor (HOX) families of transcription factors. RAR receptors also mediate neurogenesis in vascular-resident adipose progenitor cells (APC). Overall, concentrations of RA do not predict its cellular function. The precise regulation of these pathways occurs via intracellular RA production by aldehyde dehydrogenase 1 (ALDH1) family of enzymes and transport of RA by binding proteins to different cellular compartments.

**Pharmacologic stimulation of RA can elicit different effects (inhibit adipogenesis, cause no effect, or promote obesity) dependent on early, intermediate, and late stage of adipogenesis in WAT or cultured cells, UCP1 expression, diet, or animal species. RA can potentially lead to neurogenesis of APC in WAT. RA induces neurogenesis during central nervous system (CNS) neuronal damage. In contrast, aldehyde dehydrogenase family 1 member A1 (Aldh1a1)^−/− cells promote growth of dorsal root ganglia axons of preexisting neurons in context of visceral WAT (Shen et al., 2018). This axon growth is inhibited by added RA.**

**Aldh1a1^−/− Adipocytes Can Induce Sensory-Sympathetic WAT Innervation**

The genomic comparison between Aldh1a1^−/− and wild type (WT) adipocytes followed by ingenuity pathway analysis revealed different expression of axon guidance molecules (Shen et al., 2018). The secreted molecules from Aldh1a1^−/− adipocytes (Aldh1a1^−/− secretome) induced marked outgrowth of neurites in sensory neurons compared to classic NT3 and NGF inducers. The molecules secreted from WT adipocytes did not influence axon growth consistent with gene expression. Axon guidance activity of Aldh1a1^−/− adipocytes was mediated in part by ephrinA5/ephrinA4 pathway and was repressed by RA (Shen et al., 2018). EphrinA5/ephrinA4 pathway could also be activated in classic white 3T3-L1 preadipocytes by inhibitors of RAR. This data suggests some of UCP^− subsets of adipocytes could serve as paracrine inducers of innervation. The paracrine regulation of sensory neurons is relevant, because in vivo they establish an afferent circuit from the WAT to the brain (Ryu and Bartness, 2014) (Figure 1). The comprehensive work by Bartness and
Ryu (Ryu and Bartness, 2014) showed that, following sensory stimulation, CNS establishes long sensory-sympathetic feedback loops that are involved in the control of lipolysis in WAT.

The formation of sensory-sympathetic circuits was tested in vivo in encapsulation model. Encapsulated Aldh1a1–/– adipocytes (500,000 per depot) were injected once into WAT of obese WT mice fed a high-fat diet (Host). In spite of obesogenic environment, encapsulated Aldh1a1–/– adipocytes stimulated outgrowth of peripherin-positive and tyrosine hydroxylase-positive sympathetic axons in host WAT (Shen et al., 2018), demonstrating de novo innervation. This WAT remodeling in host obese mice could be attributed to the paracrine action of encapsulated Aldh1a1–/– adipocytes communicating signals to the host WAT. Indeed, similar grafts containing encapsulated WT adipocytes did not influence innervation in obese host mice. Non treated obese mice had sparse innervation consistent with neurodegenerative processes in obesity.

The encapsulated Aldh1a1–/– subset maintained their ther-mogenic characteristics in vivo. In the proximity of sympathetic axons, host unilocular WAT was also remodeled into multilocular adipocytes expressing ATGL and UCP1. This lipolytic and thermogenic remodeling occurred outside of encapsulated Aldh1a1–/– adipocytes in conjunction with de novo sympathetic innervation (Shen et al., 2018). Once sympathetic innervation is established, subsequently released norepinephrine could induce lipolysis and thermogenesis producing subsets of adipocytes with beige characteristics. This proposed two-step remodeling mechanism (Figure 2) could potentially shed light on differences between acute thermogenesis and chronic adaptation to energy demand, presence of heterogenous populations of Ucp1+ adipocytes, and the clustered appearance of multilocular adipocytes in proximity of sympathetic axons. More studies would need to test the time course of generation of different UCP+ subsets. Cumulatively, WAT’s capacity for inducible sensory-sympathetic innervation appears to depend on paracrine function of Aldh1a1–/– subsets of thermogenic adipocytes, and probably on the other brite adipocytes.

Conclusion

Investigation of paracrine communication between thermogenic adipocytes and the nervous system could have important translational implications for fundamental understanding and treatment of metabolic diseases and their neurodegenerative complications, as well as injuries in the peripheral nervous system.

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References

Ahmadian M, Abbott MJ, Tang T, Hudak CS, Kim Y, Bruss M, Hellerstein MK, Lee HY, Samuel VT, Shulman GI, Wang Y, Duncan RE, Kang C, Sul HS (2011) Desnutrin/ATGL is regulated by AMPK and is required for a brown adipose phenotype. Cell Metab 13:739–748.

Carobbio S, Guenant AC, Samuelson I, Babri M, Vidal-Puig A (2018) Brown and beige fat: From molecules to physiology and pathophysiology. Biochim Biophys Acta Mol Cell Lipids doi: 10.1016/j.bbalip.2018.05.013.

Huang S, Loaukki J, Epping MT, Koster J, Holzel M, Westerman BA, Nijkamp W, Kata, A, Ashgr zachdeh S, Seeger RC, Versteeg R, Beijersbergen RL, Bernards R (2009) ZNF443 is critically required for retinoic acid-induced differentiation and is a marker of neuroblastoma outcome. Cancer Cell 15:328–340.

Reichert B, Yaseen R, Jeyakumar SM, Yang F, Thomou T, Alder H, Duester G, Maieyu A, Mihai G, Harrison EH, Rajagopalan S, Kirkland JL, Ziouzenkova O (2011) Concerted activation of adaldehyde dehydrogenases influences depot-specific fat formation. Mol Endocrinol 25:799–809.

Rosen ED, Spiegelman BM (2014) What we talk about when we talk about fat. Cell 156:20–44.

Ruohonen S, Valve L, Tuomainen K, Allinen L, Roytta M, Manz G, Baur N, Joos T, Savontaus E, Scheinin M (2018) Increased energy expenditure, lipolysis, and hyperinsulinemia confer resistance to central obesity and type 2 diabetes in mice lacking alpha2A-adrenoceptors. Neuroendocrinology doi: 10.1159/000492387.

Ryu V, Bartness TJ (2014) Short and long sympathetic-sensory feedback loops in white fat. Am J Physiol Regul Integr Comp Physiol 306:R886–900.

Shabalina IG, Petrovic N, Kramarova TV, Hoeks J, Cannon B, Nergaard J (2006) UCP1 and defense against oxidative stress. 4-Hydroxy-2-nonenol effects on brown fat mitochondria are uncoupling protein 1-independent. J Biol Chem 281:13882–13893.

Shen Q, Yaseen R, Marbourg J, Xu L, Yu L, Fadda P, Flechtner A, Lee LJ, Popovich PG, Ziouzenkova O (2018) Induction of innervation by encapsulated adipocytes with engineered vitamin A metabolism. Transl Res 192:1–14.

Verboven K, Stinkens R, Hansen D, Wens I, Frederix I, Eijnde BO, Jocken JW, Goossens GH, Bloak EE (2018) Adrenergic- and non-adrenergically-mediated human adipose tissue lipolysis during acute exercise and exercise training. Clin Sci (Lond) 132:1685–1698.

Wang H, Lu L, Lin LZ, Aprahamian TR, Farmer SR (2016) Browning of white adipose tissue with roscovitine induces a distinct population of UCP1+ adipocytes. Cell Metab 24:835–847.

Yaseen R, Reichert B, Deulitsl J, Yang F, Lynch A, Meyers J, Sharlach M, Shin S, Volke KS, Green KB, Lee K, Alder H, Duester G, Zechner R, Rajagopalan S, Ziouzenkova O (2013) Autocrine function of adipokines induces a distinct population of beige adipocytes. Cell Metab 24:835–847.

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