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Dermal white adipose tissue: a new component of the thermogenic response

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McArdle Laboratory for Cancer Research* and Department of Radiology,[§] University of Wisconsin School of Medicine and Public Health, Madison, WI; Department of Nutritional Sciences,[†] University of Wisconsin-Madison, Madison, WI; Department of Dermatology,** University of California, San Diego, CA; School of Biological and Biomedical Sciences,[††] Durham University, Durham, United Kingdom; Department of Molecular, Cellular, and Developmental Biology,[§§] Yale University, New Haven, CT; and Department of Molecular and Integrative Physiology,*** University of Michigan, Ann Arbor, MI

Abstract   Recent literature suggests that the layer of adipocytes embedded in the skin below the dermis is far from being an inert spacer material. Instead, this layer of dermal white adipose tissue (dWAT) is a regulated lipid layer that comprises a crucial environmental defense. Among all the classes of biological molecules, lipids have the lowest thermal conductance and highest insulation potential. This property can be exploited by mammals to reduce heat loss, suppress brown adipose tissue activation, reduce the activation of thermogenic programs, and increase metabolic efficiency. Furthermore, this layer responds to bacterial challenge to provide a physical barrier and antimicrobial disinfection, and its expansion supports the growth of hair follicles and regenerating skin. In sum, this dWAT layer is a key defensive player with remarkable potential for modifying systemic metabolism, immune function, and physiology.⬛ **In this review, we discuss the key literature illustrating the properties of this recently recognized adipose depot.**—Alexander, C. M., I. Kasza, C-L. E. Yen, S. B. Reeder, D. Hernando, R. L. Gallo, C. A. B. Jahoda, V. Horsley, and O. A. MacDougald. **Dermal white adipose tissue: a new component of the thermogenic response.** *J. Lipid Res.* 2015. **56: 2061–2069.**

Supplementary key words   adipocytes • diabetes • cytokines • skin • insulation • environmental defense • thermogenesis • antimicrobial • follicular development

BACKGROUND

Fat cells, or adipocytes, reside in specific locations or "depots," and although the adipocytes in each depot resemble one another, they are not identical (1, 2). Indeed, these depots have varied functions, and are therefore responsive to different cues. For example, some depots primarily provide high energy-calorie reserves [white adipose tissue (WAT), comprising on average 25% of total body weight for women); whereas other depots support homeostatically regulated thermogenesis, fueled by fatty acid oxidation in uncoupled mitochondria [brown adipose tissue (BAT)] (3, 4).

Here, we describe an understudied depot of WAT, embedded in the skin below the dermis and called dermal WAT (dWAT) (**Fig. 1**). There is a growing realization that dWAT is an important component of the defense provided by skin against a battery of environmental stressors. Although this layer is thin, it is not insignificant; simple calculation shows that for an average woman, a 1 mm-thick layer of dWAT would weigh 1.6 kg, comprising approximately 7% of total body fat. Although WAT does show some turnover (5), dWAT is perhaps uniquely kinetic among the adipose depots. Thus, dWAT expands in response to cold exposure, it coordinates with the expanding hair follicle during the hair cycle, and it reacts to wounding and to bacterial infection (6–10) (**Fig. 2**). Not only is dWAT regulated by these processes, this regulation turns out to be integral to the functional outcomes.

This dWAT depot is distinct from subcutaneous WAT (sWAT), and from the other depots around the body (2, 11, 12). In mice, dWAT is clearly defined as a layer of adipocytes between the muscle layer (the panniculus carnosus) and the dermis (Fig. 1); historically, this layer has been called the subcutis or hypodermis. A similar cell population almost certainly exists in humans, where developmentally,

Abbreviations: AMP, antimicrobial peptide; BAT, brown adipose tissue; Camp, cathelicidin antimicrobial peptide; dWAT, dermal white adipose tissue; Sdc, syndecan; SVF, stromal vascular fraction; sWAT, subcutaneous white adipose tissue; TLR, Toll-like receptor; WAT, white adipose tissue.

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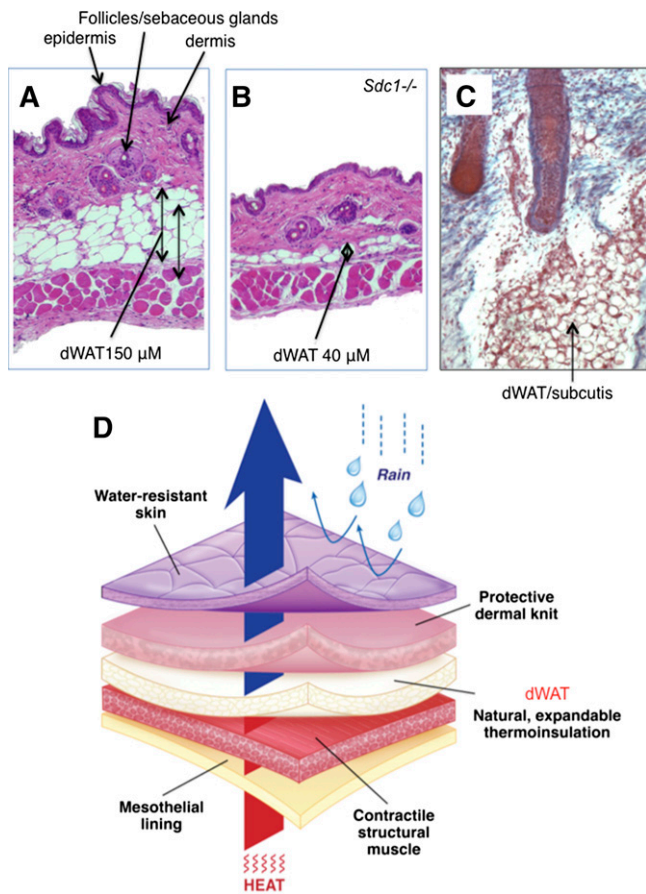   e-mail: alexander@oncology.wisc.edu

Fig. 1. dWAT in skin from mice and men. A: Morphology of mouse adult skin. Hematoxylin and eosin-stained section of belly skin from 11-week-old BALB/cJ female, housed at 21°C. B: Sample from corresponding $Sdc1^{-/-}$ mouse showing deficient dWAT. C: Human fetal skin (Azan-stained section, 30 μm) from the facial region at 19.5 weeks gestation showing the appearance of a cluster of adipose lobules very close to the base of a developing hair follicle. D: Diagram of the lamellar structure of skin. The multi-layered structure of skin is drawn by analogy to "Gore-Tex" to emphasize the different roles of each layer. Specifically, this illustration emphasizes the role that dWAT plays in the reduction of heat loss from a mammalian core body temperature (approximately 37°C) to the variable environmental temperature.

Fig. 2. Regulation of dWAT. Three separate regulators of dWAT expansion are indicated in red. This diagram indicates a cross-section of an average mammal, coated in skin with a subjacent layer of dWAT, expanded or not (adipocytes are shown as hexagons). The physiology of skin determines the physiology of all internal organs. dWAT expands in response to cold exposure to provide insulation, and in response to bacterial infection, where it counters microbial colonization, and in response to the hair follicle cycle, to support follicular invagination. Together, these responses comprise a comprehensive defensive strategy for the mammalian ectoderm.

"fat islands" are evident in the dermis (Fig. 1C). In humans, there is no continuous muscle layer separating dWAT from sWAT, although vestigial muscle remains to dermarcate sites such as the neck (13). This makes it difficult to distinguish sWAT from dWAT. Studies of mice suggest that this distinction may be important: dWAT and sWAT are developmentally, morphologically, biochemically, and functionally distinct (1, 14–20). Unfortunately, in previous literature, these depots have typically been referred to using the general "subcutaneous" descriptor, and without a specific visual indication, it is difficult to deduce the subject of these reports.

dWAT has recently been shown to respond to several independent sets of cues (Fig. 2). For example, a 2- to 10-fold expansion of dWAT (maximum nearly 500 μM) is ass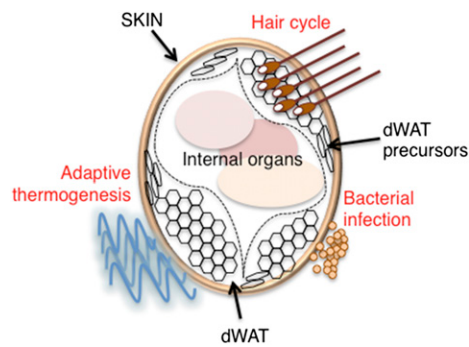ociated with the first synchronized hair cycle in mouse skin, commensurate with the downward invagination of the hair follicle (8, 9, 21, 22). Perhaps more importantly, this preadipocyte/adipocyte population appears to initiate the follicle activation, identifying these cells as the drivers of this process, rather than the other way around (8, 22). Another regulatory cue is provided by ambient temperature; thus, dWAT is thick when mice are housed at "room temperature" (21–24°C/70–75°F), and thins out when mice are transferred to warm housing conditions (29–33°C/84–91°F) (6). If dWAT does not thicken in response to cool ambient temperatures, mice show chronic activation of thermal defenses (6). The increase in dWAT that provides a defense against temperature change is also notable during the defense against microbial skin infection (Fig. 2). Differentiating preadipocytes are an abundant source of antimicrobial peptides (AMPs), including cathelicidin AMP (Camp), and the production of these antibiotics is triggered by exposure to bacteria (7). Gallo and colleagues found that when the hypertrophy of dWAT was inhibited, the severity of skin infection was greatly increased (7).

These findings and others suggest that it is important to understand this depot in more depth. Here, we review the various functions of dWAT as an insulator, as an antibiotic tissue, and as a regenerative component for wound repair and hair growth.

Thermal insulation

Although the dWAT layer of adipocytes is only 2–15 cells thick, when fully expanded, this layer of contiguous lipid is estimated to reduce heat loss from mice by at least 2-fold (for an average room temperature) (6). For mice housed in regular housing temperatures (20–25°C), this lipid comprises a sleeve underneath the waterproof epidermis (visualized by high resolution MRI in I. Kasza, et al., unpublished **Fig. 3**). Metabolic studies show that defense of body temperature can comprise the principal component
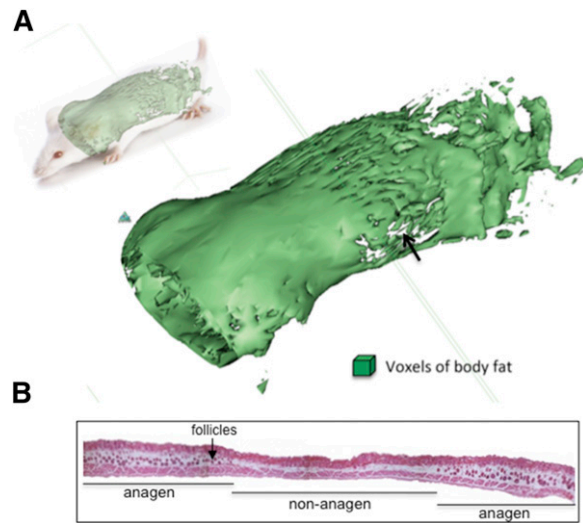
Fig. 3. Insulating sleeve of dWAT. A: dWAT was visualized (and quantified) in 3D using high resolution MRI (fat only) for an adult female BALB/cJ mouse. B. Typical adult mouse skin stained with hematoxylin and eosin to show the patched asynchronous pattern of anagen. I. Kasza et al., unpublished.

of the metabolic budget for a mammal at rest. This depends upon the body size and surface area, the difference between body temperature and ambient temperature, and the degree of insulation (4, 23–25).

The importance of physical insulation of mammals and birds has been a neglected topic since the early descriptions by Scholander and colleagues (26, 27). Together with his colleagues, he described a critical temperature for activation of so-called "chemical defenses" when animals were exposed to low environmental temperatures. Today, these chemical defenses are redefined as adaptive thermogenesis and include activation of BAT. By measuring the metabolic activity of various species of tropical and arctic animals as they responded to decreasing ambient temperatures, the threshold critical temperature for activating chemical defenses was shown to be dramatically different (26, 27). These studies attributed the stoic response of arctic huskies to their body insulation; indeed, their respiration did not increase even when challenged with exposure to $-30°C$. Since those studies, there have been few studies of natural physical insulation, and specifically, no studies of how alterations in insulation might impact metabolic efficiency and other aspects of normal physiology and pathophysiology. Recently, Nedergaard and Cannon (4) have reinterpreted phenotypes reported in published studies, based on the effect that specific gene mutations had on hair growth and/or skin structure and water resistance, and the impact these changes would have on insulation or waterproofing. As an example, nude mice, deficient in both dWAT and hair, are one of the most common disease models used to examine the growth of human xenografts, yet these mice are highly cold-stressed (24). Because the response to cold elicits a range of circulating factors and the activation of systemic checkpoints, this condition can dominate experimental findings.

Mammals can also be over insulated; in these animals, immune cells are rarely exposed to thermal defense program effectors and heat loss mechanisms are chronically activated. Indeed, because living tissues create heat, the ambient temperature at which mammals do no work to either heat or cool their bodies is below body temperature. This "thermoneutral" zone depends on their basal metabolic rate, recent food consumption, and variables relating to body size and surface area. It is defined by the minimum O_2 consumption for any animal challenged with a range of housing temperatures (23, 24). When the ambient temperature drops below thermoneutral, a heat-generating response is activated, and lipid stores are mobilized from WAT and BAT (**Fig. 4A**). Fatty acids mobilized from WAT are taken up by BAT, where oxidation in the uncoupled mitochondria provides "wood for the fire" and warms local capillary beds and systemic circulation. Whereas BAT activation is rapid (less than an hour) (28), dWAT expansion and reduction is a more chronic response. When the response to thermal stress is adequate, and the effects of cold exposure are mitigated, thermal defenses are deactivated. If dWAT expansion is inadequate or genetically adjusted to be thin, effectors of the thermal defenses may cycle on to a higher level or for a longer daily period, changing the mammal's physiology (Fig. 4B).

dWAT comprises the counterpoint to the active thermogenic responses. Thus, the thermogenic adipocyte depots coordinate with the more passive insulating dWAT depots to optimize the physiology of the organism, whether that is intended to emphasize metabolic efficiency or to increase the range of ecological opportunities. Thus, the ability of some species to stay warm when others cannot determines and expands their potential habitats. For example, rodents hunting nocturnally in desert environments have evolved more extreme physiological strategies to survive the dual stressors of food restriction and cold. One of these strategies is to lapse into a semi-coma, called "torpor", which is associated with decreased body temperature, low heart rate, and a 90% reduction in oxygen consumption rate. These "torpidator" species, such as mice, revive in warm temperatures and continue their normal feeding behaviors (29, 30). This natural behavior is a component of the cold stress signature defined by Kasza et al. (6) as a measure of deficient insulation in laboratory mice (**Fig. 5**).

Exploitation of the insulating properties of lipids may not be unique to mammals; instead, this may be a primal ancestral function that has been adapted to coordinate with active thermogenesis. For example, a heat conservation mechanism that depends on uniquely adapted adipose depots was illustrated recently for the Opah, a circumglobal fish species able to live in deeper colder waters. This fish uses 1 cm-thick fat-insulated gill arches and clever countercurrent circulation to retain heat from swimming muscles (31). Similarly, it is known that *Drosophila* not only have a fat body (serving as a calorie reserve for egg laying, among other demands), but also have peripheral and highly dispersed fat depots under the integument and close to the cerebrum (32). These fat depots are
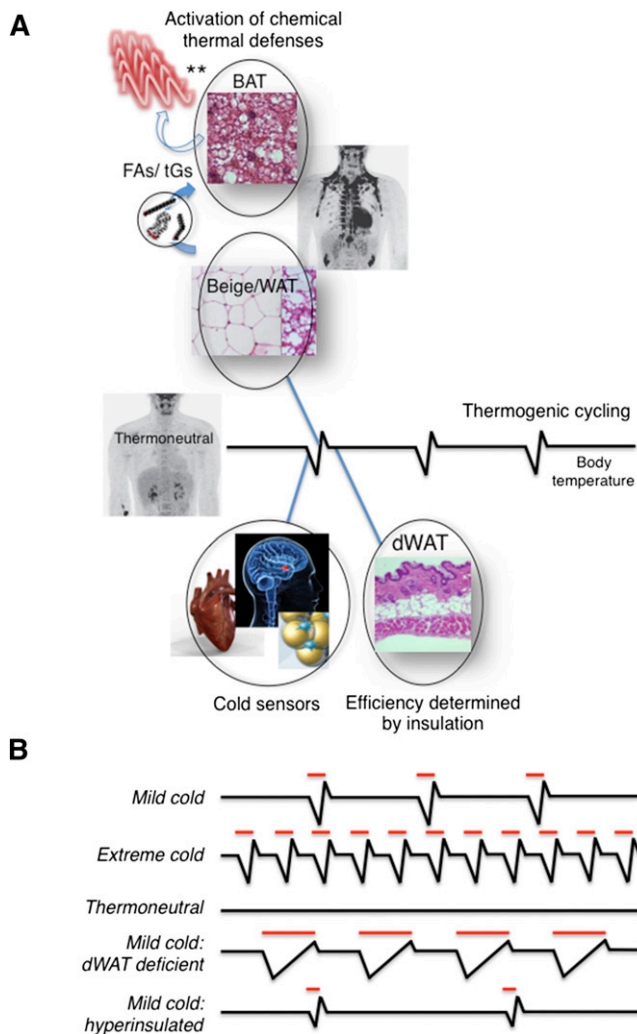
Fig. 4. The activation of thermogenic defenses. A: The integration of adipocyte depots that provide thermogenic homeostatis is diagrammed. Perceived body temperature is shown as a line in the center of the diagram. When body temperature drops, cold sensors are activated (including hypothalamic, cardiac, and perhaps local sources; shown here are macrophages in WAT tissues; see text for details). Effectors induce the activation of facultative thermogenic depots (including BAT and brite depots) that become lipolytic, generating heat (shown as pink wiggle lines) from uncoupled mitochondria and lipids to fuel the β-oxidation required for warming. The pattern of BAT activation, revealed by PET imaging, is shown for a human subject [reproduced from (74), with permission]. As the temperature challenge is remediated, thermogenesis is deactivated. The timeline for BAT activation in response to a (noxious) 4°C challenge is quick (less than 30 minutes) (28); otherwise, the periodicity of this cycle is not known. The efficiency of heat retention is determined by the total insulation, in part determined by the dWAT layer. This dWAT layer acts as a third component of this circuit, and responds to overall ambient temperature, but slowly (days). B: Hypothetical patterns of thermogenic activation. We propose that the overall time spent with thermal defenses activated is a function of the absolute temperature challenge, the efficiency of remediation with activation of thermal defenses, and the level of insulation. The body temperature of mice is shown as a black line and the activation of thermal defenses is shown above as a red line. A typical pattern for mice housed at 20–25°C is compared with those moved to 4°C (extreme cold) and the complete absence of thermogenesis observed under thermoneutral conditions. As a comparison, the lack of insulation in dWAT-deficient mice may slow remediation of cooling body temperature and activate

deduced to be under separate control, because they are targeted by different genetic drivers and respond to different cues. So far, there are no direct data that describe insulator activity in *Drosophila*; however, the metabolic phenotypes described for the syndecan $Sdc1^{-/-}$ insulation-defective mouse model (described in the following paragraph) are remarkably coincident with Sdc mutant *Drosophila* (33). Evidence points toward dWAT having a simple insulator function, with the support of hair covering as a relatively newer evolutionary innovation for this group of cells.

When dWAT expansion is deficient, there are dramatic changes in mouse physiology. For example, mice with a mutation of the heparan sulfate proteoglycan Sdc1 show 80% depletion of dWAT, without major effects on WAT. This depletion of DWAT is associated with chronic activation of BAT and development of "beige" or "brite" adipose tissue (6). The glycogen stored in the liver as short-term calorie reserves is depleted, and mice become susceptible to fasting-induced torpor. Perhaps more important, there is widespread activation of the p38α/MAPK14 stress-activated kinase, which is a powerful determinant of differentiation, division, and senescence in a variety of tissues (34) (Fig. 5). This signature of depleted insulation is reversed when $Sdc1^{-/-}$ mice are housed at thermoneutrality.

Several recent examples illustrate that the housing temperature of mice (and therefore the role of thermal defense activity) affects the function of immune cells. Repasky and colleagues showed that there is more recruitment and activation of CD8 helper T cells in response to breast tumor development and metastasis when host mice are housed in a warm environment. This study implicated these T cells as the effectors of the dramatically decreased tumor growth and dissemination observed under these conditions (35). Furthermore, the levels of norepinephrine that are present in mice housed in mild cold protect pancreatic tumor cells from cytotoxic therapies (36, 37). These examples illustrate how systemic effects downstream of cold exposure and insulation determine biological responses.

Defense against bacterial infection

The skin plays a vital role as the first barrier to pathogen entry. Keratinocytes, in concert with many other cell types, produce an array of AMPs that kill and clear microbes (38). AMPs are a diverse and potent mechanism of host defense and are produced in some form by all living organisms. In mammals, one family of AMPs, known as cathelicidins, has been shown to be essential for protection against invasive bacterial infections (39). Together with another gene family known as β-defensins, these AMPs are crucial for barrier function. Indeed, the common skin disease, atopic dermatitis, is associated with a chronic susceptibility of patients to bacterial and viral infections

the cycle more frequently, potentially leading to chronic activation. In contrast, high levels of dWAT observed in obese mice (and perhaps also in obese humans) leads to a hyper-insulated phenotype and little activation of thermogenesis.
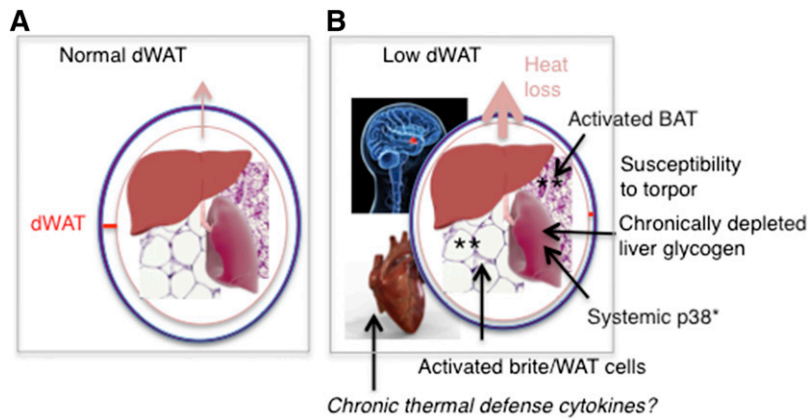
Fig. 5. The under-insulated phenotype. A: A diagrammatic representation of the cross-section of a mammal (as for Fig. 2) coated in skin and protected from heat loss (pink arrow) by a layer of dWAT. B: When dWAT is deficient, the thermogenic program is chronically activated, leading to systemic hyper-activation of key metabolic checkpoints, such as p38α. Symptoms of under-insulation include chronic WAT/brite/BAT activation at cool housing temperatures (room temperature for mice), depleted liver glycogen, and susceptibility to torpor in response to fasting. Note that total energy expenditure may not be increased in under-insulated mice; for example, energy expenditure was not affected in $Sdc1^{-/-}$ mice (6), and indeed the lack of response of the adipostat to thermogenic load has been discussed before (4). Therefore, this is not considered a core component of this phenotype.

(40), and it is caused by insufficient production of AMPs. These peptides are crucial because they serve as more than just natural antibiotics. Thus, several of the AMPs have been shown to have potent activity with respect to modifying other aspects of host defense, such as leukocyte recruitment, initiation of angiogenesis, and stimulation of epithelial proliferation (38). AMPs can both directly kill microbes and stimulate inflammation to activate other effectors of host defense.

Until recently, granulocytes and specialized epithelial cells were thought to be the major source of AMPs. Now dWAT has been implicated as an important player in the defense against bacterial infection. Gallo and colleagues (7) have shown that committed preadipocytes and adipocytes in mouse skin react to the infection of breached epidermis by *Staphylococcus aureus*. *S. aureus* is a common pathogen that causes dangerous acute infections and chronic inflammation conditions, such as cellulitis and fasciitis. In response to the subcutaneous injection of *S. aureus*, adipocytes in dWAT differentiate and hypertrophy to create a thicker layer. Furthermore, differentiating adipocytes in vitro and in vivo produce the mouse *Camp,* and this expression is further induced by *S. aureus* infection. This AMP is therefore at least partly responsible for reducing growth and colonization of skin by *S. aureus*, and because *Camp* is also pro-inflammatory, this peptide may have a role in other aspects of dWAT biology.

Not only is dWAT accumulation induced by infection, but this study also found that the adipogenic reaction is functionally important to the magnitude and effectiveness of the innate immune response mounted by the host (7). Thus, genetically mutant mouse strains with deficient adipogenesis could not resist systemic infection, measured three days after inoculation. Similarly, inhibiting the adipogenic response to infection with pharmaceutical agents (the PPARγ antagonists, BADGE and GW9662) increased the bacterial skin count. It will be interesting to know how

the antimicrobial properties of skin are modified in mice housed at different temperatures.

Support of hair growth and wound healing

dWAT remodeling also occurs during hair follicle cycling and wound healing. The postnatal development of a hair coat occurs synchronously in mice, presumably to provide insulation to newborn nude pups or "pinkies". The hair follicle then goes through rounds of death and regrowth, termed the hair follicle cycle. Although the first two postnatal hair cycles are synchronous, thereafter cycles are asynchronous, and anagen "patches" of variable sizes occur all over mammalian pelts (10). The dramatic regeneration of the hair follicle is paralleled by the expansion of dWAT (21), which is fueled by both hypertrophy of mature adipocytes and formation of new mature adipocytes by adipocyte precursor cells (8). The sleeve of skin-associated fat (Fig. 3A) is shown alongside a more detailed histological cross-section, to illustrate the patches of increased dWAT thickness that associate with anagen-stage skin (Fig. 3B).

Inhibition of adipogenesis can impair hair follicle regeneration, suggesting a role for immature adipocytes in hair follicle growth (8). Vice versa, epidermal Wnt signaling can activate adipocyte differentiation in dWAT, revealing that both cell types orchestrate their synchronous expansion (9). For many wintering mammalian species, cold exposure induces dWAT to thicken alongside the development of a more weatherproof coat, with thicker and longer hair. Given the interactions demonstrated so far, these seasonal processes are likely to be mechanistically interactive.

dWAT can regenerate following injury. Wound healing involves a stepwise process that coordinates the regeneration of the multiple cell types that comprise the skin. Following the closure and inflammation phase of wound healing, the proliferative phase includes both epithelial

and dermal cells. During this phase, adipocyte precursor cells are activated and adiponectin-expressing cells migrate into the wound bed. This is a functional recruitment: inhibition of adipogenesis during the proliferative phase abrogates the ability of fibroblasts to repopulate the wound bed, resulting in defective dermal healing (22, 41). The molecular basis for the role of adipocytes during wound healing has not yet been characterized. Predictably, perhaps wound healing is deficient in $Sdc1^{-/-}$ mice with deficient dWAT (42), but in general, the integration of the independent stimuli of cold, infection, and folliculogenesis, with respect to dWAT hypertrophy and involution, is not yet understood.

REGULATORY MECHANISMS

Comparison with other adipocyte depots

The dynamic expansion and involution of dWAT with every hair cycle in mice (at least every month) is quite distinct from the relative stability of tissue mass in traditional adipocyte depots such as sWAT (5). Indeed, the diversity of physiological processes that regulate dWAT adipogenesis and involution suggest that it is uniquely equipped with sensory and signaling molecules. These same effectors do not have the same (or any) impact on other WAT depots, even sWAT, which is so proximal to the dWAT (on the underside of the skin and outside the body wall).

sWAT depots share features with WAT tissues in general (1, 14, 43). Thus, the heterogeneous cell fractions extracted from distinct WAT depots [stromal vascular fractions (SVFs)], when compared by transcriptional profiling, show different and characteristic gene expression. Some of these differences have been implicated in the distinct properties of each WAT depot (18, 19, 44). For example, each depot has a characteristic capacity to store and mobilize fatty acids in response to specific physiological challenges. Different depots secrete specific adipokines and systemic effectors, and have characteristic patterns of innervation and vascularization (45). For example, the molecular signaling controlled by insulin, cortisols, and adrenergic agonists is different in various depots (19, 44, 46). It is not yet known which aspects of the molecular regulation of WAT are shared by dWAT, and which are unique.

Given the multiple roles of dWAT-associated adipocytes in defense and regeneration, it is possible that the SVF fraction of dWAT has a more plastic fate than corresponding SVF fractions from other WAT calorie store depots. Indeed, there are adipogenic cells in various tissues that appear to have plastic fates. For example, lipofibroblasts in the lung are regulated by their environment to become adipogenic and accumulate lipid, or to become fibroblastic, overproducing extracellular matrix components to become fibrotic (47). Likewise, studies have suggested that a fibroblast cell type embedded in skeletal muscle is typically pro-regenerative; however, these cells switch fate to become adipocytic when given an unhealthy microenvironment (48).

This plasticity is also observed for a developmental precursor in the dermis, which can become either fibroblastic or adipocytic (22). For example, when epidermal function is perturbed by genetic inhibition of Wnt signaling, dWAT is reduced and there is a commensurate increase in reticular fibroblasts (9). It is not yet clear whether this indicates that there are alternate progenitor pools or a respecification of cell fates. The answer to this question awaits a specific tool to label individual mesenchymal precursor cells in mice.

There are known molecular regulators of each of the known stagewise processes that govern adipogenesis, including cell specification, proliferation and commitment of preadipocytes, and adipogenic differentiation and hypertrophy of the triglyceride-enriched lipid globule (49, 50). Hypothetically, the process of dWAT expansion may be regulated at any one of these stages. As dWAT regresses during follicular telogen, or in mice in warmer temperature housing, the final thickness of dWAT could be governed by depletion of the lipid globule or death of differentiated adipocytes. This involution process is so far uncharacterized.

Certainly, canonical effectors can induce adipogenesis in dWAT. For example, this layer thickens when exposed to rosiglitazone, a PPARγ agonist (6, 51), and thins out when exposed to WY14643, a PPARα agonist (I. Kasza et al., unpublished observations) and the PPARγ antagonists, BADGE and GW9662 (8). An adipose-deficient model (A-ZIP/F1 mice) expressing a dominant negative CEBP transcription factor is also dWAT deficient (52). Similarly, engineering a gain of function for Wnt signaling in the adipocytes of transgenic mice induces a predictable deficiency of dWAT (53).

Regulation by cold exposure

In mammals stimulated by exposure to cool temperatures, dWAT responds differently than from other adipocyte depots. Thus, dWAT expands, accumulates triacylglycerols, and becomes lipogenic; whereas, BAT tissues become lipolytic, increasing their uptake of circulating lipids and mobilizing triacylglycerol stores to generate fatty acids for β-oxidation in uncoupled mitochondria. WAT depots and their embedded populations of brite/beige adipocytes also become lipolytic (54) (Fig. 4). So far, the mechanistic basis for the difference between the responses of dWAT and other WAT depots in cold-exposed mice is not known.

For example, in mice housed in warm temperatures (31°C/88°F), dWAT thins out (40 μM); whereas in normal housing conditions (21°C/70°F), which comprise a cold stress for such a small mammal, dWAT expands to a thickness of 200 μM. It is not yet known whether dWAT is regulated similarly in (mostly hairless) human subjects exposed to sub-thermoneutral temperatures. Indeed, human subjects tend to avoid the adaptation typical of wild mammals, adopting behavioral changes such as additional clothing or altered body posture. Intriguingly, scleroderma patients, who undergo a fibrotic transformation of dWAT, commonly report feeling continuously cold (J. Varga, unpublished observation). The ability to regulate this depot

appears to be reduced with age and increasing BMI (I. Kasza et al., unpublished observations and (55)).

Mammalian thermal defenses are activated by a number of circulating endocrine factors secreted, for example, by cardiac cells and in response to autonomic nervous responses (56–58) (Fig. 4). In fact, thermogenesis may also be locally regulated by short-range paracrine interactions with immune cells (59) or by adipocytes themselves, or even by any cell expressing temperature-sensitive channels (60–62). Recent studies have suggested that the direct perception of cold may be a more widely distributed function than previously acknowledged. For example, when preadipocytes were cultured at reduced temperatures in a culture dish, Spiegelman and colleagues observed induction of the uncoupling protein, UCP1, and other transcriptional responses typically associated with cold activation (60). There are a series of channel proteins, transient receptor potential receptors (TRPs), which act as "molecular thermometers" (61), which are activated at different temperature thresholds across the spectrum from noxious cold to noxious heat. At least one of these has been implicated as a mediator of thermogenic responses in adipocytes (63), and others are expressed in skin and internal organs (for example TRPM8). It is therefore possible that skin and the dWAT-associated preadipocytes could react directly to cold exposure without implicating the sympathetic nervous system.

Note that the macrophages present in adipose tissue in mice are known to be temperature-sensitive. Thus mice housed at sub-thermoneutral temperatures (21°C and 4°C) show high rates of alternative activation in WAT-associated macrophages, which results in secretion of catecholamines and the induction of lipolysis in local adipocyte depots in response to cold (64). The defenses activated by "noxious" temperature exposure are often different from those activated by "innocuous" changes (65), and these sensors and reactions may have different impacts on dWAT. When the thermal defense circuit is activated, lipids are continuously mobilized from WAT and BAT, and these circulating lipids are therefore candidates as functional modifiers of various tissues in cold-exposed mammals, including dWAT, lung, or liver (66).

Regulation by hair cycle

One of the differences between dWAT and other WAT depots is its local interaction with epithelium. In fact, there is a unique relationship between ectodermally derived epithelial cells and adipose tissues. For example, deposition of subcutaneous adipose can be the cue for development of skin-associated appendages. Thus, mammary glands start as an invagination of epidermis/ectoderm along the milk line, followed by the colonization of the (so-called) mammary fat pad to generate a functional gland with external secretions (67). In contrast to dWAT, adipocytes collapse with terminal differentiation of mammary epithelial cells at parturition, repopulating the gland after involution. The molecular regulators of this crosstalk are emerging, and indeed several factors secreted by adipocytes are now recognized to be epithelial cell growth factors; for example, leptin is produced by human follicular papillae (68).

Do keratinocytes start the dWAT expansion, or is the anagen stage of hair growth initiated by dWAT? In mice engineered to have a defect in follicle morphogenesis (K14-ΔNLef1), the adipocytes in dWAT are depleted over time; instead, the hypodermal layer becomes populated with cells depositing reticular collagen (9). On the flip side, over-activation of Wnt signaling (K14-ΔNβcatenin/estrogen receptor fusion protein) in keratinocytes induces the progression toward anagen, together with precocious folliculogenesis; alongside the induction of epithelial expansion, there is commensurate adipogenesis (9). Wnt signaling is known to inhibit adipogenesis in mesenchymal stem cells (69), suggesting that an adipogenic effector that is not a Wnt protein is secreted as part of a paracrine "conversation" from Wnt-stimulated keratinocytes (50).

Regulation by bacterial infection

The proliferation of preadipocytes and expansion of dWAT in response to infection likely invoke some of the same regulatory pathways that are active in response to temperature changes and during follicular morphogenesis. Furthermore, adipocytes directly detect bacterial products and respond with an increase in their production of AMPs (7). This response is likely mediated, in part, by Toll-like receptors (TLRs), and in particular TLR2. Not only do TLRs act as microbial pattern-recognition receptors, they also detect endogenous products released during tissue injury, such as hyaluronic acid fragments and double-stranded RNAs. These so-called "danger associated molecular patterns" may define elements that regulate dWAT across multiple species.

SUMMARY AND FUTURE

The functions associated with dWAT so far reflect its barrier function, which may have primitive origins. Thus, activation of dWAT adipogenesis in response to bacterial infection is a component of innate immunity, presumably enhanced by more sophisticated acquired immunity mechanisms. Furthermore, hypertrophy of dWAT in response to cold exposure provides basic protection of body viability and metabolism. We have provided examples that suggest that the ability to accumulate fat as insulation could be an ancient mechanism (70), and indeed the insulating properties of fat may be exploited to keep fish swimming in cold oceans, fruit flies on the wing in cool orchards (71), or roundworms scavenging as the seasons change (72). For mammals, dWAT provides a counterpoint in the thermogenic circuit, as a passive insulator protecting the heat generated chemically by the activation of BAT and WAT. This process of adaptive thermogenesis is orchestrated by the hypothalamus and by other tissues, which, together with behaviors like shivering, are ultimately responsible for maintaining thermal homeostasis. By controlling hair cycling and wound healing, dWAT serves as a primary responder to enable the protective barrier function for skin.

The interdependence of epithelium and adipose tissue is nowhere more evident than in skin, where dWAT is an important player in regeneration and wound repair. How these separate responses are integrated to facilitate barrier function will be a topic for the future.

There is little known about dWAT in human subjects as yet, and many questions remain. For example, what is the correlation of dWAT with gender, obesity, average/minimum ambient housing temperature, rate of wound healing, and aging? Is dWAT thickness genetically determined, and does it interact with other factors to determine the onset of disease? How do skin diseases such as psoriasis, scleroderma, alopecia, and atopic dermatitis affect dWAT and human insulation? For the skin conditions that affect the permeability barrier, how does trans-epidermal water loss affect cold perception and dWAT development? Is the vascularity of dWAT controlled differently from other adipocyte depots, where thermogenesis is accompanied by vasodilation?

If we humans can control the thickness of our own dWAT (for example, by using a specific diurnal pattern of hot/cool environments) (73), we may be able to exploit the activation of thermal defenses to promote health and wellness. This factor could impact metabolic conditions such as diabetes, obesity, and the development of other pathologies that are impacted by the hyper-activation of stress checkpoints, such as fibrosis and cancer.⬛

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