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Microenvironmental Control of Adipocyte Fate and Function

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Abstract

The properties of tissue-specific microenvironments vary widely in the human body and demonstrably influence the structure and function of many cell types. Adipocytes are no exception, responding to cues in specialized niches to perform vital metabolic and endocrine functions. The adipose microenvironment is remodeled during tissue expansion to maintain the structural and functional integrity of the tissue and disrupted remodeling in obesity contributes to the progression of metabolic syndrome, breast cancer, and other malignancies. The increasing incidence of these obesity related diseases and the recent focus on improved in vitro models of human tissue biology underscore growing interest in the regulatory role of adipocyte microenvironments in health and disease.

Keywords

Adipose Development; Metabolic Disease; Extracellular Matrix; Biomechanics; Signal Transduction; Tissue Engineering

Adipose Depots and Functions

Adipose tissue is distributed over multiple subcutaneous and visceral depots typically accounting for 15% to 30% of total human body weight [1]. Although plasticity between canonical phenotypes is observed [2,3], adipose tissue is generally considered “white”, characterized by adipocytes with a single lipid droplet for efficient energy storage, or “brown”, characterized by adipocytes with multiple lipid droplets, numerous enlarged mitochondria expressing *UCPI* for uncoupled oxidative phosphorylation and non-shivering

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thermogenesis, and increased vasculature for heat dissipation [4,5]. In adult humans, the vast majority of adipose is white, while brown adipose is present in periscapular and perispinal depots [6]. Human perivascular fat may also contain brown adipocytes [7], which help control arterial blood pressure and temperature in mice [8,9]. All adipose types secrete hormones to regulate systemic metabolism [10,11], and, at least in the case of white adipose depots, absorb mechanical shock to protect wear-prone tissues [12], and provide insulation to maintain body temperature [13]. The adipose microenvironment both supports and modulates adipocytes in the execution of these functions by providing regulatory cues in the forms of mechanical stimulation [14], mono- and heterotypic cell-cell interaction [15], nutrient availability [16], and interaction with extracellular matrix [17] (Figure 1). In this review, we discuss how microenvironmental cues are transduced in adipose tissue and the functional implications of altered adipocyte microenvironments associated with obesity and adipose related diseases. Less is known about brown adipose in humans, so of necessity we focus on the predominant white adipocyte and add observations in brown adipocytes where possible.

The Developing Adipocyte Niche

Lineage tracing experiments in animals indicate different adipose depots, and sometimes even different adipocytes within the same depot, arise from a variety of mesenchymal precursors of neural crest or mesodermal origin [18-20]. Brown adipocytes are derived from progenitors expressing Myogenic Factor 5 that also have the potential to form skeletal muscle [21]. The complete set of white adipose precursors has not been fully characterized, but includes multipotent mural cells expressing the zinc-finger transcription factor Zfp423 [22]. Despite arising from disparate lineages, a common microenvironment supports adipose development. Preceding adipogenesis in humans, vascularization of loose connective tissue promotes the migration and aggregation of mesenchymal cells and their differentiation into preadipocytes [23]. Lipid-bearing adipocytes appear in subcutaneous and visceral adipose depots by the end of the second trimester and variably expand from the 15-micron diameter preadipocyte up to 80 microns by birth [24]. Innervation of adipose tissue also follows inductive signals from blood vessels, but the exact developmental stages when neural projections reach different fat depots have not been characterized and may occur postnatally [25]. After birth, adipose also forms and progressively dominates in bone marrow, although little is known about its function [26]. Into adulthood, visceral and subcutaneous adipose depots grow at variable rates dependent upon sex hormones, nutrition, and other factors reviewed previously [27,28].

Adipocyte Interactions with Extracellular Matrix

Typical of the loose areolar connective tissue in which it develops, adipose is supported by an isotropic matrix of collagen and elastic fibers. Extracellular fibronectin and laminin form networks with collagen fibers [29] and provide attachment points for integrins anchored in the adipocyte membrane [30] (Figure 2). Integrins are heterodimers with alpha and beta subunits, the combination of which dictates ligand specificity [31]. Like receptors for paracrine signals, integrins transduce cues from the extracellular matrix to regulate gene expression and function. During adipogenesis, alpha integrin expression shifts from

predominantly alpha5 in preadipocytes to alpha6 in mature adipocytes, signifying release from alpha5-binding fibronectin and attachment to alpha6-binding laminin [32,33]. Blocking this substrate switch with an alpha6 antibody prevents the aggregation and subsequent differentiation of preadipocytes *in vitro* [32] likely due to sustained anti-adipogenic RhoA activity [34] downstream of fibronectin engaged alpha5-integrin complexes [35]. Similarly, enriching fibronectin deposition from cultured preadipocytes by Secreted-Protein-Acidic-and-Rich-in-Cysteine treatment inhibits adipogenesis and the alpha5 to alpha6 integrin switch [36]. The contribution of alpha6-integrin signaling to adipogenesis has not been studied and may deviate from focal adhesion kinase pathways in pluripotent stem cells or endothelial colony forming cells that engage alternate laminin isoforms [37,38]. Along with their role in adipogenesis, integrins may also provide the adipocyte a means to sense its own size. As one might expect, the beta integrin found in adipocytes, beta1, is upregulated during hypertrophic growth as is the activity of downstream effector kinases [39]. Thus, integrin signaling links adipocyte size and function.

Total protein and carbohydrate concentration in adipose extracellular matrix is 10-70 mg/mL, with 25-35% collagen, 15-22% elastin, and 0.2-0.8% sulfated glycosaminoglycan by dry weight [29,40,41]. As with integrin function, blocking collagen synthesis in preadipocytes inhibits differentiation again underscoring the importance of matrix interactions to adipogenesis. Collagen VI is abundant in adipose tissue and attachment to Collagen VI is sufficient to restore adipogenic potential in preadipocytes with blocked collagen synthesis [42]. Paradoxically, humans lacking Collagen VI have increased body fat and dystrophic fatty infiltration of the muscle, suggesting Collagen VI restricts adipose expansion [43]. In addition to synthesis, adipocytes also remodel extracellular matrix as a requirement for differentiation. The membrane-type matrix metalloproteinase MT1-MMP is anchored in the adipocyte membrane and hydrolyzes the previously mentioned alpha5 and alpha6 integrin ligands, fibronectin and laminin, as well as entactin, vitronectin, and collagen [30]. Adipose does not develop *in vivo* without MT1-MMP, and adipocyte differentiation efficiency within three-dimensional collagen I hydrogels is dependent on MT1-MMP activity and collagen concentration [44], again supporting the hypothesis that extracellular matrix physically restrains hypertrophic growth. Conversely, tissue inhibitors of metalloproteinases also regulate adipose development and function by checking matrix remodelers in contexts that would otherwise lead to tissue destruction and inflammation [45]. In sum, adipocyte interactions with extracellular matrix are balanced to provide both the flexibility needed for cell migration and hypertrophy as well as the stability needed for the structural and functional integrity of the tissue as a whole.

Adipose Cytoarchitecture and Adipocyte-Adipocyte Interaction

Accompanying changes to extracellular matrix, the cytoskeleton is also remodeled as spherical adipocytes form from stellate progenitors [46,47]. Decreased RhoA activity at the onset of adipogenesis triggers disassembly of actin stress fibers, freeing globular actin to sequester the anti-adipogenic transcriptional co-activator Megakaryoblastic Leukemia-1 [48]. To accommodate expanding lipid droplets, vimentin transcription increases to proportionally expand the vimentin cage surrounding each droplet [49], while actin and tubulin expression are decreased [50]. Demonstrating the significance of cytoskeletal and

morphological transformations, imposing particular cell shapes on preadipocytes with microprinted adhesion proteins modulates their differentiation potential in a manner dependent upon the arrangement of the cytoskeleton [34,51].

At the tissue level, large (up to 290 μm diameter) spherical adipocytes are typically arranged in an imperfect hexagonal packing architecture resembling honeycomb, with smaller pre-adipocytes and interweaving capillaries and nerves filling the interstitial space [52-54]. Adipocytes themselves are interconnected via gap junctions and thereby share cytoplasm and respond in concert to electrical stimuli [55]. The gap junction protein Connexin43 is expressed and present on adipocyte membranes and is required for adipogenesis [56]. Preadipocytes are also interconnected via gap junctions, but Connexin43 is transiently down-regulated during differentiation [57] necessitating the formation of new gap junctions in hyperplasia and the normal ten percent annual turnover of the tissue [58]. Whether gap junctions interconnect entire depots or smaller, independent subunits within each depot has not been explored. Likewise, the specific role of gap junctions in adipose function is unknown, but as in other tissues these junctions enable distribution of intracellular molecules smaller than 485 daltons (e.g. fatty acids, glucose, etc.) to synchronize electrophysiology and metabolism within coupled cells [59]. Together, these studies suggest that adipose function is coordinated by adipocyte cytoarchitecture and coupling.

Mechanotransduction in Adipocytes

The expansion of lipid droplets and cytoskeletal rearrangements in adipogenesis alter the mechanical properties of the tissue. For example, cell stiffness, the resistance of an object to deformation by an applied force, was measured during in vitro differentiation of mouse 3T3-L1 preadipocytes. Stiffness increased from 300-900 Pa in preadipocytes to ~2 kPa in white adipocytes, attributable to the fact that lipid droplets are stiffer than the surrounding cytoplasm and occupy an increasing majority of the cell as differentiation progresses [60]. Demonstrating the significance of cell stiffness to adipose function, stiffness was previously shown to regulate adipocyte insulin sensitivity [61]. By definition, stiffer cells are more resistant to deformation and thus impose strain on the surrounding cells and extracellular matrix. The static strain imposed by adipocytes on neighboring preadipocytes promotes differentiation and prompted the hypothesis that obesity and inactivity are a self-reinforcing biophysical feedback loop [60,62,63]. By the same token, cyclic strain, as experienced during exercise, suppresses adipocyte differentiation [62,64-67]. Mechanical regulation of adipogenesis has similarly been demonstrated in humans, where static stretching for ten weeks nearly doubled breast adipose tissue mass [68] and massages repeated for several weeks reduced thigh circumference and increased lipolysis in femoral adipose tissue [69,70]. Despite consistency in the observed gross impacts of mechanical strain on adipogenesis, different studies have attributed adipocyte mechanotransduction to different molecular pathways [62,64-67,71] and in one case suggest the same signal (mitogen activated protein kinase activation) is both stimulatory and inhibitory [62,64].

Mechanical regulation of adipogenesis may stem from the role adipose plays in protecting other organs from physical insult and the contribution of marrow fat to the mechanical properties of bone [72]. Adipogenesis would restore and relax the protective fat layer spread

thin and taut in stretched tissue, reminiscent of ventral grooved blubber in Rorqual whales that undergoes extreme stretching during lunge feeding [73]. Strain from expanding adipocytes pushing against the extracellular matrix would also be an appropriate cue for matrix remodeling to accommodate hypertrophic growth [74,75]. Supporting this notion, the forces adipocytes exert on the surrounding extracellular matrix per cell area are uniformly distributed and maintained throughout hypertrophic growth [76]. Moreover, static compression inhibits adipogenesis [77] while the reduced adipose tensile strength in Collagen VI knockout mice is associated with abnormally large adipocytes [75], again suggesting mechanically coordinated matrix remodeling during adipocyte maturation. The impacts of mechanical cues on brown adipocyte structure and function have not yet been explored. Complete mapping of the molecular pathways induced in white adipocytes by mechanical forces as well as the extent to which mechanical cues modulate brown adipocyte structure and function is an exciting opportunity for future research and could lead to the application of physical therapy to adipose related diseases.

Heterotypic Cell Interactions within Adipose Depots

Adipocytes share their microenvironment with multiple cell types that interact to coordinate adipose functions. A few examples are given below to demonstrate the role and prevalence of heterotypic cell interactions in adipose depots, but more comprehensive reviews exist that expand upon adipose vasculature [78], neurons [79], and leukocytes [80] among other cell types [81,82]. In addition to the previously mentioned inductive role during adipose depot development (see “The Developing Adipocyte Niche” section), vasculature transports nutrients and hormones both to and from adipose tissue for maintenance of systemic energy homeostasis. Lipids associate with lipoproteins during blood transport and are released for uptake into adipose tissue by lipoprotein lipase. In an insulin-dependent mechanism [83], endothelial cells induce adipocytes to secrete lipoprotein lipase into proximal capillaries for its anchorage to endothelial cell membranes [84]. Lipid accumulation expands adipose tissue, a cooperative process that both directs and requires vascular remodeling [85,86]. Beyond vasculature, neurons within adipose tissue regulate adipocyte function via released neurotransmitters. Noradrenaline from postganglionic sympathetic nerves stimulates brown adipocytes to metabolize stored lipids for thermogenesis and similarly mediates leptin-induced lipolysis in white adipocytes for the release of free fatty acids [87,88]. Adipocyte interaction with resident leukocytes also contributes to adipose structure and function. Resident M2 macrophages and T cells are required for adipose tissue remodeling and expansion [89]. Browning of white adipose tissue in response to cold exposure is also regulated by macrophages and eosinophils [90-92]. Immune cells also can affect the browning of white adipose in response to obesity and inflammation [93]. Cellular composition can vary between adipose depots and may help explain the differential impact of fat accumulation in different locations [27]. For example, mesothelial cells surround visceral but not subcutaneous depots and may contribute to the metabolic differences between visceral and subcutaneous depots by modulating inflammatory responses among other functions [82]. In sum, cues from vasculature, nerves, immune, and other cell types contribute to the regulatory role of the adipocyte microenvironment.

Adipocyte Microenvironments in Disease

Given the regulatory roles for the microenvironment in normal adipose development and function, it is no surprise that altered adipose microenvironments are associated with disease. While the classification of obesity in and of itself as a disease is debated [94], there is significant risk for cardiovascular and other diseases with obesity [95] that may be explained by changes to the adipose microenvironment during tissue expansion. As discussed previously, adipose tissue expansion requires coordinated remodeling of the extracellular matrix. In a comparison of obese women, properties of the adipose extracellular matrix distinguished healthy obese individuals from those with compromised metabolism [75]. Adipose from metabolically compromised individuals exhibited decreased tensile strength relative to healthy obese individuals suggesting a lack of coordination between matrix remodeling and tissue expansion in diseased individuals [75]. Increased fibrotic content, particularly Collagen VI, is also associated with compromised metabolism in obese humans [96], whereas some metabolic improvements have been observed in obese mice lacking Collagen VI [97]. Furthermore, overexpression of a secreted cleavage product of the $\alpha 3$ chain of Collagen VI called endotrophin can exacerbate fibrosis, inflammation, and metabolic dysfunction in mice on a high fat diet, although its mode of action remains unclear [98]. Potentially a cause or consequence of aberrant matrix remodeling, M1 macrophages infiltrate visceral adipose in obesity and exacerbate adipose inflammation [99,100] and increased numbers of myofibroblasts are also observed in obese adipose tissue [101]. Myofibroblasts contribute directly to fibrotic extracellular matrix elaboration in obese adipose tissue [102] and may be recruited by inflammatory cytokines [103] or differentiated from multipotent progenitors influenced by the stiffer extracellular matrix [102].

While the order, necessity, and sufficiency of these events to promote type 2 diabetes mellitus, cardiovascular disease, or other diseases associated with obesity are unclear, mechanisms of pathogenesis have been proposed. Obesity is thought to mediate the progressive loss of insulin sensitivity by increasing circulating levels of free fatty acids (hyperlipidemia) and altering energy homeostatic mechanisms normally mediated by adipocyte hormone secretion in a multi-organ cascade [104-108]. Accordingly, a compromised adipose microenvironment would disrupt the discussed matrix interactions and remodeling, cytoskeletal arrangement, and intercellular junctions and interactions required to coordinate adipogenesis, lipid uptake and metabolism, and endocrine functions undermined in disease. Similarly, the same adipose microenvironment found in metabolically compromised obese individuals also promotes progression of breast and other types of cancers [109]. Obesity associated fibrosis was observed in human mammary fat depots and promoted the growth and migration of a premalignant human breast epithelial cell line in vitro [102]. Further, Endotrophin is enriched in mammary tumors and stimulates their growth and metastasis in mice [110]. Despite the discussed connections with metabolic syndrome and cancer, no cases of degenerative adipose tissue or lipodystrophy can clearly be attributed to altered adipose microenvironments, although the causes of some lipodystrophies remain unknown. Altogether, these studies underscore the importance of the adipose microenvironment to normal adipose function and its contribution to diseases for which obesity is a risk factor.

Applying Regulatory Principles of Adipocyte Microenvironments

How are these principles of microenvironmental control of adipocyte function being applied? Currently, *in vitro* adipocyte (and most cell) culture is performed on generic two-dimensional polystyrene that lacks the regulatory features of the *in vivo* microenvironment. In an effort to improve the physiological relevance of *in vitro* studies, cell culture technology is in development to mimic the microenvironments of multiple organs including heart [111], skeletal muscle [112], eye [113], lung [114], kidney [115], breast [116], brain [117], liver [118], and gut [119,120]. Likewise, biomimetic platforms to accommodate three-dimensional adipocyte expansion would improve *in vitro* modeling of adipose tissue. For example, during differentiation and lipid accumulation adipocyte buoyancy increases due to decreasing cell density and causes adipocytes to detach from planar culture surfaces and lyse [121] before reaching the typical cell sizes observed in adult humans (Table 1). Several platforms have been already been developed that could surmount the limitations two-dimensional culture imposes on adipocyte size. Scaffolds for three-dimensional adipogenesis have been made from particulate leached polyglycolic acid [122] or silk fibroin [123], meshed microfibers [124,125], esterified hyaluronic acid sponges [126], electrospun polycaprolactone [127,128] or poly L-lactic acid [129], or freeze dried mixtures of nanocellulose and alginate [130]. Preadipocyte aggregates have also been differentiated on two-dimensional surfaces coated with elastin-like polypeptide and polyethyleneimine [131] or on stirred [132] or levitated beads [133]. Hydrogel encapsulation has also been used for three-dimensional *in vitro* adipogenesis [44,134-137]. While all these substrates support adipogenesis, the potential of any of these platforms to mature human adipocytes beyond sizes that can already be achieved with conventional methods has yet to be demonstrated.

Beyond the three-dimensional structure of scaffolds for adipogenesis, accommodating the natural interplay with vasculature [83], neurons [138,139], and leukocytes [93] as well as integrin engagement of extracellular matrix proteins [140] also increases the accuracy of *in vitro* adipose models. A single platform that combines all these microenvironmental features remains to be built. Another consideration for *in vitro* modeling of adipose tissue is that properties of the adipocyte microenvironment vary among different adipose depots, including the protein components of the extracellular matrix [141]. Toward depot-specific modeling, adipocytes cultured in a collagen-based hydrogel are more similar to visceral versus subcutaneous adipose [142]. Incorporation of depot-specific extracellular matrix proteins and other microenvironmental features into three-dimensional adipocyte culture systems are needed to mechanistically explore the nuanced contributions of different adipose depots to human biology.

Concluding Remarks

In summary, our growing appreciation for the adipocyte microenvironment in adipose development and function provides motivation for comprehensive characterization of the adipocyte microenvironment as well as experiments to determine the potential regulatory role of as yet unidentified and untested microenvironmental cues (see Outstanding Questions box). The resulting data will enable design of physiologically relevant culture conditions for

in vitro human adipose disease modeling, drug testing, and other in vitro studies and inform reconstructive tissue engineering approaches or other in vivo applications.

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Trends Box

- Subcutaneous and visceral adipose depots are innervated, vascularized endocrine organs composed of multipotent progenitor cells and differentiated adipocytes.
- Brown adipocytes differ from white adipocytes in their morphology, functional capacities, and depot locations, but “beige” or “brite” adipocytes, which share characteristics of both white and brown adipocytes, are found in some white and brown depots.
- Adipocytes can expand several-thousand fold in size during cellular maturation and are electrically and metabolically coupled by gap junctions.
- Biophysical cues from the microenvironment modulate adipocyte differentiation, growth, and function.
- Altered adipocyte microenvironments in obesity are associated with type 2 diabetes mellitus, breast cancer, and other diseases suggesting microenvironmental factors in adipose tissue can be pathogenic.

Outstanding Questions Box

- What microenvironmental features distinguish subcutaneous and visceral adipose depots? What features distinguish subcutaneous or visceral depots in different locations?
- How do gap junctions facilitate adipose function? To what extent do they coordinate entire depots?
- What microenvironmental factors regulate cell size? How do they influence growth by hyperplasia versus hypertrophy?
- When are adipose depots innervated? What is the significance of sensory neurons in adipose?
- What factors fine tune vasculature and extracellular matrix remodeling during hypertrophy? What is their role in hypoxic and fibrotic adipose tissue?
- Do mechanical cues influence brown fat differentiation or browning? How are mechanical cues transduced in adipocytes? What signaling pathways respond to laminin engagement?

Figure 1 |. Regulatory Cues in the Adipocyte Niche.

The contents (yellow sphere = lipid droplet, gray triangle = nucleus, purple ovals = mitochondria, white circle = cytosol), extracellular matrix (gray lines) and surrounding cell types (gold spheres = white adipocytes, gray stars = preadipocytes, crimson tubes = capillaries, cream tubes = nerves) of a white adipocyte are depicted. Red boxes represent membrane sections where regulatory cues from the microenvironment are sensed.

Figure 2 I. Dynamics of integrin attachment to the extracellular matrix during adipocyte maturation.

The matrix-membrane interface is depicted in a time series spanning the transition from a preadipocyte (a) to a mature adipocyte (c). Structural support is provided by type I (100nm-10um diameter, brown banded fiber) and type VI (50nm diameter, brown beaded filament) collagen networks. Microfilaments (7nm diameter, purple) and microtubules (25 nm diameter, red) are abundant in the cytosol (pink) of preadipocytes, but are gradually displaced by the expanding lipid droplet, caged in vimentin (10nm, brown filament) (b,c). At the intermediate stage (b), the attachment of integrin alpha-5/beta-1 complexes (orange) to extracellular fibronectin (green) is replaced by alpha-6/beta-1 attachment to extracellular laminin (light blue). The membrane-type matrix metalloproteinase MT1-MMP (magenta)

and other proteinases facilitate this switch by cleaving integrin attachments to fibronectin. The nucleus (blue) moves to the cell periphery and is deformed by the lipid droplet in mature adipocytes (c).

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Table 1 |

Adipocyte Sizes in Developing and Adult Humans versus in vitro Culture

Cell Size	Subcutaneous & Visceral Fat			Subcutaneous Fat		Visceral Fat		2D Culture
	Fetus ^a	Neonate	Infant ^b	Lean	Obese	Lean	Obese	
Diameter Range (µm)	40-50	50-80	90-130	50-130	90-270	45-110	90-200	30-70
Mean Cell Volume (µm³)	48,000	144,000	697,000	382,000	3,054,000	244,000	1,596,000	65,000
Reference	[24]	[24]	[24]	[52]	[54]	[52]	[53]	[143,144]

^a25-30 weeks gestation^b1-3 months postpartum