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Review article

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Lipid droplet biogenesis and functions in health and disease

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Supplementary Information:

Supplementary box 1: Adipose heterogeneity: size (hyperplasia) vs number (hypertrophy)

Unified by the ability to store energy in lipid droplets, adipose tissue is a remarkably heterogeneous organ varying in colour, anatomical distribution, and function (**Supplementary table B1**). It is comprised of lipid droplet occupying adipocytes among a stromal vascular fraction of fibroblasts, nerve tissue, blood vessels and immune and endothelial cells[1]. Though, adipocyte turnover stabilises to 8.3 years in adulthood[2], adipose tissue expansion may occur by increasing adipocyte size (hypertrophy), number (hyperplasia) or both. In the former, existing adipocytes become engorged through enhanced lipid droplet growth and accumulation, before supporting the development of the latter, formation of new adipocytes through increased adipogenesis of precursors[3]. As angiogenesis precedes hyperplasia[4], and thereby enables sufficient vascularisation and adipokine and cytokine signalling upon expansion, hyperplasia is considered an adaptive response[5]. Hypertrophy, on the other hand, is linked to an onslaught of negative metabolic outcomes. It is positively correlated with cell senescence, strongly implicated in the development of inflammation, associated with mitochondrial dysfunction, elevated basal lipolysis, hypoxia, fibrosis, and increased leptin but reduced adiponectin production and secretion[6,7]. Interestingly, hypertrophy predominates visceral adipose tissue expansion, while hyperplasia drives the growth of subcutaneous depots[8]. By regulating the vascular supply of adipose tissue, the sex hormone, estrogen may influence the mode of hypertrophic or hyperplasic expansion, in addition to modulating the typical gynoid or android adipose tissue distribution of premenopausal women or men, respectively[9]. According to the adipose tissue expandability hypothesis, which concurs with both the adipokine and inflammation hypotheses[10,11], it is this capacity of expansion, rather than the absolute amount of adipose tissue one possesses that may lead to such adverse metabolic complications. This is because beyond the critical point where further expansion is limited, lipotoxicity may ensue through increased circulating lipids and ectopic lipid accumulation in non-adipose insulin-sensitive tissues such as the liver, heart, and skeletal muscle[12].

Supplementary table B 1: lipid droplet diversity across mammalian cells in health and disease

Mammalian cell type	Lipid droplet size (μm)	Composition/ human anatomical distribution	Primary function(s)	References
Adipocyte				
- white	Nascent (< 0.2), initial (0.3 - 0.5), expanding (1), giant (> 100)	Unilocular lipid droplet > 90 % cell volume/ subcutaneous (80 %) or visceral (10-20 %)	Energy homeostasis, lipotoxicity prevention	[13,14]
- brown		Multiple small lipid droplets among pool of mitochondria/ supraclavicular, cervical, paraspinal, mediastinal, axillary, and abdominal	Uncouple oxidative phosphorylation from adenosine triphosphate production to enable energy to dissipate as heat	[15,16]
- beige		Indistinguishable from white during basal conditions but resembles brown upon cold or other β-adrenergic stimulant exposure	Dissipate energy as heat	[17,18]
- pink (mammary epithelial cell)		White adipocytes that are pink upon microscopic inspection/ alveoli	Produce milk lipid droplets	[19,20]
Hepatocyte	60 nm - over 5 μm	Cytosolic lipid droplets in close proximity to	Adaptive response to imbalanced fatty acid levels but can progress to various	[21]

		mitochondria and the endoplasmic reticulum	stages of non-alcoholic fatty liver disease upon chronic imbalance	
Pancreatic β-cell	< 1.2 μm	Both inter- and intralobular deposition	Regulate glucose-stimulated insulin secretion, both protect from and contribute to β-cell demise	[22–24]
Cardiomyocyte	< 1 μm	Small lipid droplets associated with clusters of mitochondria	Cardioprotective but accumulation can lead to cardiac lipotoxicity, cardiomyopathy and heart failure	[25,26]
Enterocyte/ intestinal epithelial cells	2.7, 4.4, 3.5 μm after 1.5, 3 and 6 hr olive oil treatment in C57BL/6J mice	Triacylglycerol, diacylglycerol, free cholesterol, cholesterol esters are the major lipids, but sphingomyelins, phosphatidylcholines and ceramides were also present	Contribute to postprandial triacylglycerol concentration regulation, store/metabolise lipophilic vitamins and digested food	[27,28]
Dermal adipocyte	Various	Dermal cones superficial to subcutaneous white adipose tissue	Mechanical cushioning, thermoregulation, hair follicle cycling, wound healing	[5]
Macrophage	Variable/ dependant on context	Depending on stress conditions, lipid droplets can either be composed of triacylglycerol (e.g., hypoxia exposed) or cholesterol esters	Contribute to the pathogenesis of several infectious and non-infectious diseases	[29]

		(e.g., in presence of cholesterol)		

Supplementary box 2: lipid droplet biogenesis vs adipogenesis: mutual factors and mechanistic differences

Broadly involved in the anabolic arm of lipid metabolism, lipid droplet biogenesis and adipogenesis are mechanistically distinct processes united by several mutual factors. Unlike lipid droplet biogenesis which constitutes the creation of neutral lipid storage organelles, adipogenesis involves the generation of new adipocytes via two main steps. In the initial commitment phase, mesenchymal precursors commit to the adipocyte lineage, becoming preadipocytes with unaltered morphology[30]. During differentiation, preadipocytes then change conformation to enable lipid accumulation and fatty acid binding protein 4, glucose transporter 4, leptin and adiponectin expression[31]. Countless extranuclear factors regulate this process, including local insulin, triiodothyronine, glucocorticoid, brain natriuretic peptide, Wnt, transforming growth factor-β and hedgehog signalling, as well as systemic influences from circadian rhythms, reactive oxygen species and chronic inflammation[5,32]. However, the most critical factor for white, brown, and beige adipocyte differentiation remains the transcriptional master regulator, PPAR-γ[33–35]. Whilst PPAR-γ is both necessary and sufficient for adipogenesis[36], it can be functionally synergised by its coactivator, C/EBPα[37], and potently inhibited by phosphatidic acid and its derivative, cyclic-phosphatidic acid[38,39].

Originally identified for its role in adipogenesis[40], and subsequently linked to lipid droplet formation[41,42], seipin has emerged as the central factor unifying the mutual influencers of these processes. Not only does it interact with phosphatidic acid among other phospholipids[43], its interaction with key enzymes involved in lipogenesis has provided mechanistic insights into its dual roles. Through binding to and putatively inhibiting the rate-limiting enzyme in glycerolipid synthesis, GPAT3/4[44], Seipin may reduce phosphatidic acid levels, preventing PPAR-γ inhibition and enabling adipogenesis. Moreover, its simultaneous and direct interaction with the PA producing, AGPAT2 and the phosphatidic acid phosphatase, lipin 1[45] might provide a mechanism for both phosphatidic acid clearance and lipogenesis regulation by seipin. Although AGPAT2 functions to synthesise phosphatidic acid, its deficiency causes a nearly three-fold increase in the level of phosphatidic acid. The recently identified direct interaction between AGPAT2 and CDS1/2[46] explains this phosphatidic acid increase under

AGPAT2 deficiency and also supports previous findings linking CDS proteins with the regulation of both cellular (lipid droplet formation) and systemic (adipogenesis) lipid storage[47].

Supplementary box 3: Lipolysis, lipophagy and chaperone-mediated autophagy

Activated during energy deprivation or stress, lipolysis, lipophagy and chaperone-mediated autophagy (CMA) are three distinct yet cooperative processes essential for regulating the degradation, and in turn size, of cLDs. In cytosolic lipolysis, triacylglycerols are sequentially hydrolysed by the neutral lipases, patatin-like phospholipase domain-containing protein 2 (PNPLA2), hormone sensitive lipase (HSL) and monoglyceride lipase[48]. In both lipophagy and CMA, lysosomal degradation catalysed by lysosomal acid lipase (LAL), or the chaperone, heat shock cognate protein 70 occurs, respectively[49]. Initially considered parallel processes, recent findings have revealed several functional and synergistic links between them. In particular, CMA has been shown to promote neutral lipolysis through the direct binding of heat shock cognate protein 70 to the KFERQ pentapeptide motif on perilipin 2 and 3[50], allowing PNPLA2 unrestricted lipolytic access to the surface of cLDs. Moreover, whilst lipophagy has been found to precede lipolysis during starvation[51,52], lipolysis of large cLDs have been shown to supply small cytoplasmic lipid droplets for subsequent lipophagic digestion[21]. Furthermore, mammalian target of rapamycin complex 1 not only acts as the prominent negative regulator of lipophagy through its Ser142 and S211 phosphorylation and cytoplasmic sequestration of transcription factor EB[53,54], it is also implicated as an inhibitor of PNPLA2-stimulated lipolysis via its upregulation of early growth response protein 1[55].

Interestingly, both activation and inhibition of these interconnected processes is strongly and amply connected with disease. Unrestrained basal lipolysis, for example, is well established in the development of insulin resistance sequelae and cancer cachexia[12,56]. In turn, pharmacological inhibition[57,58] or genetic depletion[59–61] of PNPLA2, HSL and monoglyceride lipase, have been shown to improve insulin sensitivity. Despite this, humans deficient in PNPLA2 or its coactivator, abhydrolase domain containing 5 experience systemic triacylglycerol accumulation in the form of neutral lipid storage disease with myopathy (NLSDM) or ichthyosis (NLSDI), respectively[62,63]. Likewise, LAL deficient mice demonstrate enhanced glucose tolerance[64], and humans with mutations in its encoding gene exhibit Wolman or cholesterol ester storage disease, depending on the extent of lost LAL activity[65]. Finally, whereas downregulation of CMA is linked to increased lipogenesis, lipid uptake and synthesis,

leading to fatty liver disease and atherosclerosis[66,67], upregulation of CMA may also contribute to the pathogenesis of the autoimmune disorder, lupus[68].

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