Autophagy—a key player in cellular and body metabolism

Kook Hwan Kim and Myung-Shik Lee

Abstract | Knowledge gained over the past 10 years about the mechanisms that underpin autophagy has provided a universal framework for studies of diverse physiological and pathological processes. Of particular interest is the emerging role of autophagy in the maintenance of energy homeostasis, both at the cellular level and within the organism as a whole. Dysregulation of autophagy might contribute to the development of metabolic disorders, including insulin resistance, diabetes mellitus, obesity, atherosclerosis and osteoporosis. The authors of this Review highlight research findings on the regulation of cellular autophagy by nutrients. They also describe the role of autophagy in various tissues in the regulation of energy metabolism and the development of diseases related to altered metabolism. Finally, the potential of pharmacological modulation of autophagy as a treatment for human metabolic disorders is discussed.

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Introduction

Autophagy defines an evolutionarily conserved process of recycling, whereby intracellular macromolecules are broken down into their constituent parts within the lysosomes. Three kinds of autophagy have been described to date: macroautophagy, microautophagy and chaperone-mediated autophagy. This Review focuses on the role of macroautophagy (referred to as 'autophagy' hereafter) in the regulation of metabolism.

Autophagy is characterized by the rearrangement of subcellular membranes to sequester a portion of cytoplasm and organelles into a structure called the autophagosome, which is then transported to the lysosome for proteolysis of the sequestered materials.[1] Autophagy can be constitutive or adaptive. The main functions of constitutive autophagy are removal of damaged or senescent organelles and maintenance of basal energy balance. By contrast, adaptive autophagy is characterized by the mobilization of intracellular nutrients to meet energy requirements in the event of nutrient deficiency. Thus, autophagy is essentially a metabolic process that can control energy balance. Adaptive autophagy might have evolved from ancient mechanisms designed to supply nutrients during energy crisis in primordial unicellular organisms.[2] In multicellular organisms, the metabolic effects of autophagy within an individual cell type or tissue can extend beyond local boundaries. Therefore, autophagy can control energy homeostasis of both single cells and the whole organism.

The aim of this Review is to summarize research findings on the role of autophagy in the development of metabolic disorders and discuss the possible therapeutic role of autophagy modulators for their treatment.

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Molecular mechanisms of autophagy

The process of autophagy is tightly controlled from initiation to termination through the coordinated activity of diverse regulatory components. To date, >30 autophagy-related (ATG) proteins have been identified and characterized, most of which exhibit marked homology between yeast and mammalian genomes. Highly-conserved key steps that occur during autophagy include initiation, vesicle nucleation (formation of a cup-shaped double-membrane structure, termed an isolation membrane), vesicle elongation, fusion and degradation.[3] Current understanding of the molecular mechanisms underlying autophagy is briefly summarized in Figure 1; further details of these mechanisms are beyond the scope of this article but have been reviewed elsewhere.[3,4]

Initiation of autophagy

Autophagy is initiated by the serine–threonine protein kinase ULK1 (Figure 1). Autophagy inducers, such as nutrient deprivation and exposure to rapamycin, cause dephosphorylation of ULK1; the ULK1 complex comprising ULK1, ATG13, ATG101 and FIP200 then dissociates from the mTORC1 complex.[5–7] ULK1 is rendered enzymatically active and phosphorylates ATG13 and FIP200 to commence the process of autophagy.

Vesicle nucleation

As shown in Figure 1, the Beclin-1–Vps34 complex, which comprises Beclin-1, Vps34, Vps15 and ATG14L, participates in the process of vesicle nucleation.[8] Under conditions that induce autophagy, the activated ULK1 complex recruits the Beclin-1–Vps34 complex to the site of autophagosome formation through phosphorylation of Ambra1.[9] In this process, Ambra1 interacts with TRAF6, functioning as an E3 ubiquitin ligase for ULK1, which leads to self-association and stabilization

Key points

- Autophagy has a crucial regulatory role in energy metabolism; consequently, dysregulation of autophagy can contribute to the development of metabolic disorders
- Autophagy maintains energy balance in situations of nutrient deficiency by degradation of energy stores, such as proteins, lipid droplets and glycogen
- Amino acids, fatty acids and glucose modulate the core components of the autophagy machinery; hence, overnutrition can lead to dysregulation of this process
- Autophagy influences energy metabolism both locally (tissue-specific effects) and globally (endocrine effects)
- Pharmacological modulation of autophagy could prove feasible for the prevention and treatment of metabolic disorders

of ULK1 through K63 ubiquitination.[10] The activated ULK1 complex also enhances the activity of the ATG14L-containing Vps34 complex through direct phosphorylation of Beclin-1.[11] In addition, AKT and EGFR modulate autophagy through phosphorylation of Beclin-1, independent of mTORC1.[12,13] Then, the phosphatidylinositol-3-phosphate produced by Vps34 recruits an effector protein (DFCP1) to promote the initiation of double-membrane vesicle nucleation.[14] The protein WIPI, which is encoded by a member of the *ectopic P-granule* (*epg*) subset of the metazoan-specific autophagy gene family,[15] is also involved in this process.[16]

Vesicle elongation

The expansion or completion of the autophagosome is mediated by ATG proteins, assembled into two ubiquitin-like conjugation systems, ATG12–ATG5–ATG16L and ATG8 (LC3)–phosphatidylethanolamine. In the first system, ATG12 is conjugated to ATG5 through the concerted action of ATG7 (an E1-like ubiquitin-activating enzyme) and ATG10 (an E2-like ubiquitin-conjugating enzyme).[17,18] The ATG12–ATG5 conjugate binds to ATG16L, forming the ATG12–ATG5–ATG16L complex,[19] which then participates in LC3–phosphatidylethanolamine conjugation.[20] LC3, generated from Pro-LC3 by the ATG4 protease,[21,22] is conjugated to phosphatidylethanolamine by ATG7, ATG3 (another E2-like enzyme) and the ATG12–ATG5–ATG16L complex (acting as an E3-like enzyme complex).[20,23] After processing, the lipid-conjugated LC3 (LC3–phosphatidylethanolamine), which is localized to the autophagosomal membranes, participates in the formation and elongation of autophagosomes.

Fusion and degradation

Tethering of target proteins and organelles destined for degradation to a developing or engulfing autophagosome is facilitated by autophagy receptor or adaptor proteins (sequestosome-1 [also called p62],[24] optineurin,[25] NDP52,[26] NBR1[27] and Alfy[28]) functioning as a bridge. Mature autophagosomes become fused with lysosomes to form autophagolysosomes (autolysosomes), the structures where materials or organelles tethered by autophagy receptor proteins are digested by lysosomal enzymes (Figure 1).

Termination of autophagy

Termination of autophagy can be achieved through reactivation of mTOR—an essential component of the mTORC1 complex—by nutrients generated by the

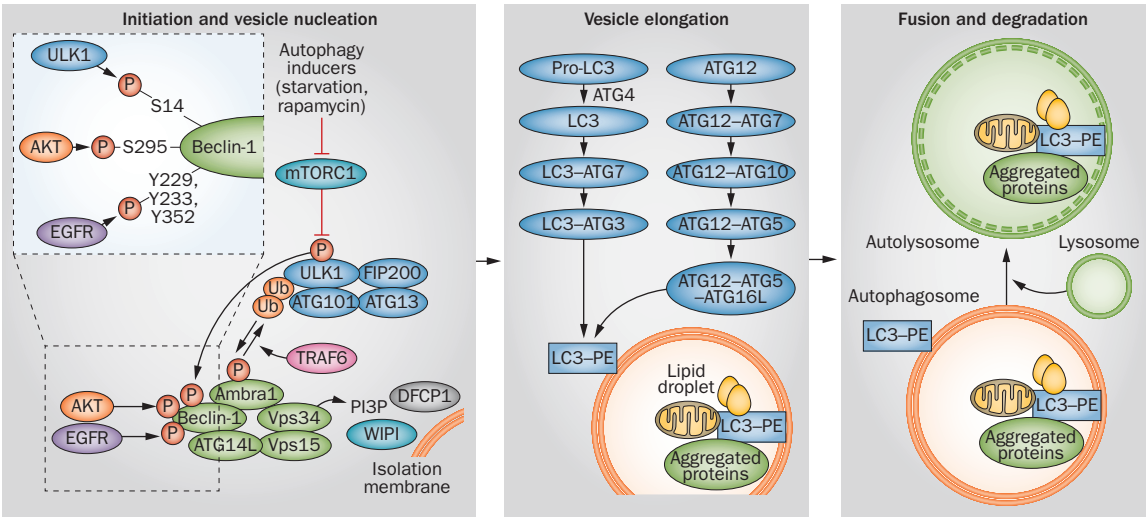

Figure 1 | Autophagy is regulated by multiple signalling pathways. Autophagic stimuli, such as nutrient deprivation or rapamycin, activate the ULK1 complex, which leads to increased activity of the Beclin-1–Vps34 complex through phosphorylation of Ambra1 and Beclin-1. In this process, Ambra1 induces autophagy by regulating the stability and kinase activity of ULK1 through interaction with TRAF6. AKT and EGFR induce autophagy through phosphorylation of Beclin-1 independently of mTORC1. PI3P produced by Vps34 recruits effector proteins, such as DFCP1 and WIPI, to promote autophagosome formation. Elongation of the autophagosome is mediated by two conjugated systems comprising ATG12–ATG5–ATG16L and LC3–PE. After formation of complete autophagic vesicles, the mature autophagosome becomes fused with a lysosome to create an autolysosome, where sequestered molecules and organelles are degraded. Abbreviations: P, phosphorylation; PE, phosphatidylethanolamine; PI3P, phosphatidylinositol-3-phosphate; Ub, ubiquitin. Permission obtained from Springer © Kim, H. K. & Lee, M.-S. *Rev. Endocr. Metab. Disord.* **15**, 11–20 (2014).

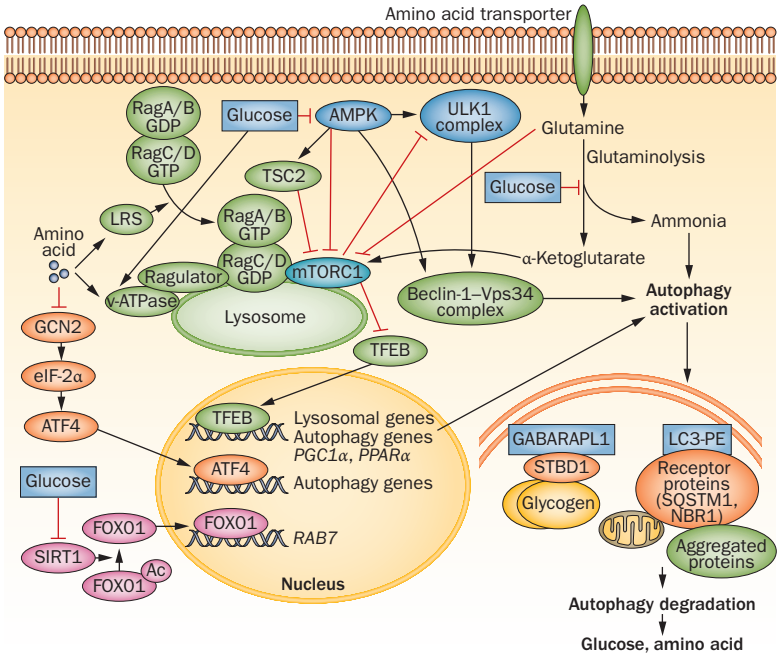

Figure 2 | Regulation of autophagy by amino acids and glucose. In the presence of sufficient amino acids, the v-ATPase–Ragulator–Rag GTPase or LRS–Rag GTPase complexes activate mTORC1. Consequently, ULK1 and Beclin-1–Vps34 complexes are inhibited and autophagy suppressed. In conditions of amino acid deprivation, these pathways are inactivated and autophagy is induced. ATF4 activated by GCN2–eIF-2α also contributes to this induction. TFEB induces genes associated with lysosomal biogenesis, autophagy and lipid catabolism. Glutamine produced by FOXO-induced glutamine synthetase can activate autophagy by inhibiting mTORC1 translocation to the lysosome; however, glutamine can also inhibit autophagy through activation of mTORC1 via α-ketoglutarate production. Glucose starvation increases autophagic activity through transcriptional regulation of autophagy-related genes or post-translational modification. Ammonia promotes autophagy in glucose-deprived conditions. Glucose and amino acid produced by autophagy provide energy sources for ATP production or building blocks for protein synthesis. Abbreviations: Ac, acetylation; LRS, leucyl-tRNA synthetase; SIRT1, sirtuin-1; SQSTM1, sequestosome-1; TSC2, tuberin.

autolysosomes. This feedback mechanism inhibits excessive activation of autophagy during periods of starvation. Reactivated mTOR generates protolysosomal tubules or vesicles; these structures extrude from the autolysosomes and mature into functional lysosomes, thereby providing the full complement of the autophagy machinery.[29]

Autophagy and cellular metabolism

At the cellular level, autophagy is the primordial system for energy production during nutrient deficiency. Here, the relationship between autophagy and cellular metabolism is reviewed as this information is fundamental to understanding the role of autophagy in the overall control of body metabolism.

Amino acids

Amino acids are crucial regulators of cellular autophagy across a broad spectrum of eukaryotes, from yeast to mammals. Disturbances in amino acid homeostasis are implicated in the pathogenesis of insulin resistance, diabetes mellitus and obesity, in addition to the well-known role of glucose and lipids in these conditions.[30,31]

The mechanisms by which amino acids regulate autophagy are outlined in Figure 2. Essential amino acids, such as leucine, that activate mTORC1 and inhibit autophagy enter cells through a bidirectional system that coordinates efflux of intracellular glutamine and influx of essential amino acids.[32] Although the exact means by which amino acids are sensed and activate mTORC1 are not fully understood, novel components involved in amino acid sensing related to mTORC1 activation have been reported. In conditions of amino acid sufficiency, these molecules accumulate in the lysosomal lumen (rather than in the cytoplasm) and recruit mTORC1 to the lysosomal membrane through a lysosomal v-ATPase–Ragulator–Rag GTPase complex that acts as an amino acid sensor.[33,34] The GTP-binding protein Rheb can then activate mTORC1 on the lysosomal surface and suppress autophagy. In addition, autophagy receptor protein sequestosome-1 serves as a regulator of an amino-acid sensing complex that induces mTORC1 activity through interaction with Rag GTPase.[35] Leucyl-tRNA synthetase (LRS) acts as a GTPase-activating protein for Rag GTPase and can mediate leucine-induced mTORC1 activation and inhibition of autophagy.[36]

Glutamine can stimulate GTP-loading of Rag by producing α-ketoglutarate through glutaminolysis, resulting in activation of mTORC1 and inhibition of autophagy; however, the mechanism of α-ketoglutarate-induced activation of mTORC1 is unclear.[37] In this process, leucine stimulates conversion of glutamate produced from glutamine to α-ketoglutarate by activating glutamate dehydrogenase. Conversely, glutaminolysis might induce mTORC1-independent autophagy through production of ammonia.[38] By contrast, glutamine produced by FOXO3-mediated and/or FOXO4-mediated induction of glutamine synthetase seems to promote autophagy through mTORC1 inhibition.[39] The diverse and seemingly inconsistent effects of glutamine in the regulation of autophagy might in part stem from differences in cell types and experimental procedures used between investigations. The detailed mechanisms of mTORC1 regulation related to autophagy control have been reviewed elsewhere.[40,41]

In addition to modulation of autophagy through post-translational mechanisms such as Rag GTPase–mTORC1 signalling, amino acids can influence the process of autophagy at the transcriptional level. For example, GCN2, a sensor of amino acid status, can induce transcription of autophagy-related genes in a manner dependent on the eIF-2α–ATF4 axis.[42] During amino acid deprivation, TFEB, a master regulator of lysosomal biogenesis, translocates from the lysosome into the cell nucleus to induce transcription of autophagy genes and lipid catabolism genes, such as PPARα and PGC1α.[43,44]

Amino acids are not only modulators of autophagy but also end-products of this process. The amino acids produced by lysosomal degradation serve as substrates for ATP production[45] or *de novo* protein synthesis,[46] and can also participate in the regulation of gluconeogenesis.[47,48] Thus, autophagic proteolysis functions in the regulation of energy and nutrient balance through the maintenance of the amino acid pool.

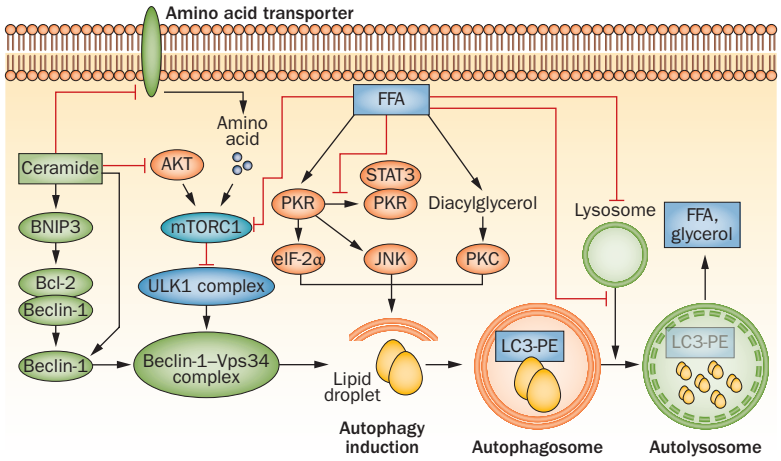

Figure 3 | Regulation of autophagy by lipids. FFAs and ceramide influence autophagy through the modulation of multiple steps. FFAs increase autophagic activity as they can inhibit mTORC1, promote activation of eIF-2α by PKR released from STAT3, activate the PKR–JNK pathway or enhance activation of PKC through diacylglycerol. Conversely, FFAs inhibit autophagic activity through the impairment of lysosomal function or by blocking fusion of the autophagosome and lysosome. Ceramide stimulates autophagy (or autophagic cell death) through inhibition of amino acid uptake, induction of Beclin-1, inhibition of AKT activity or promotion of the dissociation of Beclin-1 from Bcl-2 by increasing BNIP3 expression. In nutrient-deficient conditions, FFAs and glycerol produced by autophagic degradation of lipid droplets are used as energy sources. Abbreviations: BNIP3, Bcl-2/adenovirus E1B 19 kDa protein-interacting protein 3; FFA, free fatty acid; PKC, protein kinase C.

Glucose

Glucose metabolism is intimately related to cellular autophagy (Figure 2). In conditions of glucose deprivation, cellular levels of ATP decrease and the AMP:ATP ratio increases, leading to activation of the energy sensor AMP-activated protein kinase (AMPK).[49] This enzyme then inhibits mTORC1 and induces autophagy through phosphorylation of Raptor or suppression of Rheb via phosphorylation of tuberin.[50,51] Independent of mTORC1 inhibition, AMPK also directly phosphorylates ULK1[52,53] and Beclin-1,[54] which positively regulates Vps34 activity and autophagy. Intriguingly, glucose might also enhance mTORC1 activity via formation of a lysosomal v-ATPase–Ragulator–Rag GTPase complex, independent of AMPK.[48] This observation suggests that glucose, like amino acids, might control autophagy by Rag-dependent activation of mTORC1. In addition, sirtuin-1-mediated deacetylation of the transcription factor FOXO1 and FOXO1-induced expression of the GTPase-activating protein RAB7 have been reported to mediate autophagy in response to glucose deprivation in cardiac myocytes.[55] Similar to regulation of autophagy by ammonia generated from glutaminolysis, ammonia derived from catabolism of amino acids during glucose deprivation may trigger autophagy independent of ULK1 or mTORC1.[56] Thus, induction of autophagy when glucose levels become critically low proceeds through pathways that are both distinct from, and similar to, those observed in conditions of amino acid deprivation. Diversity of the pathways involved in the regulation of autophagy by glucose might be attributable to differences in cell types (for example, origin of tissue or primary versus immortalized cells) or experimental design (for example, the duration of nutrient deprivation).

Autophagy can also affect glucose metabolism or energy status through the regulation of gluconeogenesis, utilizing amino acids released by proteolysis via the aforementioned mechanisms.[47,48] During starvation, autophagy might also participate directly in the degradation of glycogen, in addition to the process of classic degradation of glycogen by glycogen phosphorylase and glycogen debranching enzyme. The starch-binding domain-containing protein 1 (STBD1) tethers glycogen to autophagic membrane structures for lysosomal degradation.[57] This step is mediated by interaction between this protein and the ATG8 family member GABARAPL1. Thus, STBD1 could function as a receptor protein for selective degradation of glycogen, similar to other autophagy receptor proteins involved in selective autophagy.

Lipids

The interaction between cellular autophagy and lipids is less well characterized than that between autophagy and amino acids or glucose. Nonetheless, some studies have suggested that as well as undergoing degradation in response to cytosolic lipases, triglycerides stored in lipid droplets can be broken down in an autophagic process called 'lipophagy'.[58] In the latter scenario, lysosomal lipase degrades intracellular lipid droplets and triglycerides.[59] Autophagy also regulates reverse cholesterol transport, a process that mobilizes release of cholesterol from lipid droplets in peripheral tissues to the plasma, and then to the liver.[60] Conversely, a role for LC3-mediated autophagy in the formation of cytoplasmic lipid droplets has been reported.[61,62]

The effects of lipids on autophagy are outlined in Figure 3. Excessive lipid concentrations are reported to inhibit autophagy by blocking the process of fusion between the autophagosome and the lysosome[63] or by impairing lysosomal acidification and hydrolase activity.[64] By contrast, free fatty acids, such as palmitic acid and oleic acid, increase the degree of autophagy through inhibition of mTORC1[65] or via the PKR–JNK pathway.[66] In addition, palmitic acid disrupts interactions between STAT3 and PKR, which leads to PKR-dependent phosphorylation of eIF-2α and subsequent induction of autophagy.[67] Palmitic acid-induced autophagy involves increased diacylglycerol content and protein kinase C activation independent of mTORC1 in mouse embryonic fibroblasts.[68] These discrepancies in the observed effect of free fatty acids on autophagy could reflect differences in the cell types used or the experimental methods to measure the steady-state autophagy level or dynamic autophagic flux. In addition, the degree of saturation of free fatty acids can exert differential effects on autophagy.[69]

Ceramide comprises sphingosine linked to a fatty acid and is a putative participant in obesity-induced insulin resistance.[70] Several studies have demonstrated that ceramide is able to induce autophagy or autophagic cell death by various mechanisms, including inhibition

of amino-acid uptake,[71] suppression of AKT activity,[72] induction of Beclin-1 expression,[72] and dissociation of Beclin-1 from the apoptosis regulator Bcl-2.[73]

Autophagy and *in vivo* metabolism

The role of autophagy in the regulation of *in vivo* metabolism has been investigated primarily using gene modification technology (Table 1). In metazoan species, autophagy that occurs in a metabolically active organ can affect nutrient homeostasis not only within that specific organ but also in remote tissues or the body as a whole through endocrine mechanisms.

Pancreas

Insulin-producing pancreatic β cells were among the first tissues in which autophagy was disrupted by gene targeting. The role of β-cell autophagy *in vivo* has been investigated in β cell-specific *Atg7*-knockout ($Atg7^{\Delta\beta\ cell}$) mice.[74,75] These mice characteristically display hyperglycaemia, glucose intolerance and hypoinsulinaemia owing to reduced β-cell mass and insulin secretion but not diabetes mellitus. In autophagy-deficient β cells, large inclusion bodies that contain ubiquitinated material and co-localize with sequestosome-1 accumulate. Mitochondrial swelling or distension of the endoplasmic reticulum (ER) is observed as well. Contrary to the expectation that the expression of unfolded protein response genes would be induced owing to ER stress accompanying autophagy deficiency, β cells derived from $Atg7^{\Delta\beta\ cell}$ mice show markedly reduced expression of almost all unfolded protein response genes. These cells are also susceptible to cell death after treatment *in vitro* with compounds that induce ER stress, such as thapsigargin or free fatty acids.[76] Together, these observations suggest that autophagy is important for an adaptive unfolded protein response to ER stress. The mechanism by which unfolded protein response gene expression is inhibited in the autophagy-deficient β cells is unclear, although reduced expression of the noncatalytic regulatory subunits of PI3K (p85α and p85β) that bind to XBP1 and are important for the expression of diverse unfolded protein response genes[77,78] might play a part.[76]

The functional implications of the observed increase in susceptibility of autophagy-deficient β cells to ER stressors has been investigated in $Atg7^{\Delta\beta\ cell}$ mice crossed with leptin-deficient obese (*ob*/*ob*) mice, as obesity causes ER stress in β cells *in vivo*.[79] The resultant $Atg7^{\Delta\beta\ cell}$-*ob*/*ob* mice develop severe diabetes mellitus accompanied by markedly increased β cell apoptosis, reduced β cell mass, cumulative oxidative stress in pancreatic islets and severely impaired β cell function.[76] These results are in line with a previous report that $Atg7^{\Delta\beta\ cell}$ mice have defects in the adaptive increase of β-cell mass in response to a high-fat diet.[74] Thus, autophagy deficiency in β cells could influence the progression from obesity to diabetes mellitus. These data showing susceptibility of autophagy-deficient β cells to ER stress are consistent with other reports of a protective role of autophagy against ER stress caused by misfolding of proinsulin or insulin secretory defects.[80,81]

The effect of obesity or diabetes mellitus on β-cell autophagy has also been studied in obese mice and in individuals with type 2 diabetes mellitus. Increased numbers of autophagosomes[74,76] and lipid droplets[82] are observed in the pancreatic islets of obese mice. Transgenic mice expressing a *GFP* construct conjugated to *LC3* (*GFP-LC3*$^{+}$) have been used for autophagy research, as green fluorescent protein (GFP) puncta observed by fluorescent microscopy indicate autophagosomes and cleaved 'free' GFP indicates the lysosomal event of the autophagy process, thus autophagic flux or activity;[83] the numbers of GFP-LC3 puncta (representing autophagy level) and cleaved GFP (representing autophagic flux) are increased in islets of *GFP-LC3*$^{+}$-*ob*/*ob* mice compared with those of *GFP-LC3*$^{+}$-*ob*/*wt* mice.[76] Despite apparently increased autophagic flux in the β cells of obese mice, sequestosome-1 levels are increased because the autophagy machinery clusters around lipids to execute lipophagy, and proteolysis—the eventual step of autophagy—is decreased as a trade-off.[76] Although GFP cleavage clearly shows that the lysosomal steps of autophagy have occurred, the possibility of cleaved GFP accumulation by the inhibition of the later steps of lysosomal proteolysis has been raised,[84] suggesting that cleavage of GFP alone might not provide definitive evidence for the existence of increased autophagic flux. Autophagosome numbers are increased in the pancreatic islets of patients with type 2 diabetes mellitus; however, autophagic flux has not been determined.[85] Thus, further studies are necessary to evaluate autophagic flux in β cells and pancreatic islets of obese mice and patients with type 2 diabetes mellitus.

The role of β-cell autophagy in diabetes mellitus has been studied in cells and animal models that express human islet amyloid polypeptide (hIAPP), which forms toxic oligomers and amyloid fibrils, both intracellulary and extracellularly. Islet-associated amyloid is found in up to 90% of human patients with type 2 diabetes mellitus but not in their murine counterparts owing to between-species differences in the amino acid sequence of IAPP.[86] Studies on the pathogenic role of hIAPP in human type 2 diabetes mellitus have shown that this protein triggers islet inflammation, β-cell loss and functional impairment of β cells.[87] Furthermore, hIAPP (but not rodent IAPP) impedes the autophagic process, whereas augmentation of autophagy ameliorates hIAPP-induced β-cell injury *in vitro*,[88] which suggests that the modulation of autophagy could have therapeutic value in the management of human type 2 diabetes mellitus associated with islet amyloid deposition. Nevertheless, the mechanisms by which hIAPP impairs autophagy and the *in vivo* role of autophagy in hIAPP-induced type 2 diabetes mellitus await further investigation.

Skeletal muscle

The skeletal muscle is a major target tissue of insulin-mediated glucose utilization;[89] consequently, the presence of insulin resistance in skeletal muscle is a key contributor to the pathogenesis of type 2 diabetes mellitus.[90] The role of skeletal muscle autophagy in glucose utilization

Table 1 | Metabolic phenotypes of mice with genetic modifications of autophagy-related genes

Genotype	Target tissue	Metabolic phenotype
RIP-*Cre*-*Atg7*$^{F/F}$ (*Atg7*$^{\Delta\beta\ cell}$)	Pancreas (β cells)	Hyperglycaemia, glucose intolerance and hypoinsulinaemia owing to reduced β-cell mass[74,75] Development of diabetes mellitus after breeding with leptin-deficient *ob/ob* mice[76]
Mlc1f-*Cre*-*Atg7*$^{F/F}$ (*Atg7*$^{\Delta sm}$)	Skeletal muscle	Muscle atrophy[91,92] and reduced muscle power[92] Protection against diet-induced obesity and insulin resistance[91]
Alb-*Cre*-*Atg7*$^{F/F}$ (*Atg7*$^{\Delta hep}$)	Liver (hepatocytes)	Increased hepatic lipid content in both fed and fasted states[58] Decreased hepatic lipid content after starvation[61,91] or HFD[91] Protection against diet-induced obesity and insulin resistance[91]
Alb-*Cre*-*Vps34*$^{F/F}$ (*Vps34*$^{\Delta hep}$)	Liver (hepatocytes)	Increased lipid content and reduced glycogen content in the liver[100]
Alb-*Cre*-*Tfeb*$^{F/F}$ (*Tfeb*$^{\Delta hep}$)	Liver (hepatocytes)	Increased hepatic lipid content after starvation or HFD[44]
Alb-*Cre*-*FIP200*$^{F/F}$ (*FIP200*$^{\Delta hep}$)	Liver (hepatocytes)	Reduced hepatic lipid content after starvation or HFD owing to decreased *de novo* lipid synthesis[101] Aggravated liver injury and/or fibrosis after HFD[101]
Mx1-*Cre*-*Atg7*$^{F/F}$ [after poly(I:C) injection]	Liver	Low blood glucose levels owing to reduced gluconeogenesis[47] Decrease[2] or no difference[47] in hepatic glycogen content
Adenovirus-*Cre*;*Atg7*$^{F/F}$	Liver	Increased hepatic lipid content[44]
Adenovirus-sh*Atg7*	Liver	Increased endoplasmic reticulum stress and aggravated insulin resistance[102] No difference in hepatic lipid content[102]
Adenovirus-*Atg7*;*ob/ob*	Liver	Improvement of obesity-induced endoplasmic reticulum stress and insulin resistance[102]
Adenovirus-*Tfeb*;*ob/ob*	Liver	Improvement of obesity-induced insulin resistance[44]
GFAP-*Cre*-*Atg7*$^{F/F}$	Liver (HSCs)	Amelioration of chemically-induced liver injury and fibrosis owing to HSC inactivation caused by lack of free fatty acids[104]
aP2-*Cre*-*Atg7*$^{F/F}$ (*Atg7*$^{\Delta Ad}$)	Adipose tissue (WAT, BAT)	Reduced fat mass owing to defects in adipogenesis[106,107] 'Browning' of WAT (development of BAT-like characteristics)[106,107] Protection against HFD-induced obesity and insulin resistance[106,107]
Myf5-*Cre*-*Atg7*$^{F/F}$	BAT, skeletal muscle	Defective differentiation and function of BAT[108] Worsened insulin resistance owing to impaired insulin signalling in muscle and reduced muscle mass[108]
Lentivirus-sh*Atg7* to the hypothalamus	Hypothalamus	Obesity owing to increased food intake and decreased energy expenditure[114]
AgRP-*Cre*-*Atg7*$^{F/F}$ (*Atg7*$^{\Delta AgRP}$)	Hypothalamus (AgRP)	Reduced food intake and leanness owing to increased levels of α-MSH[115]
POMC-*Cre*-*Atg7*$^{F/F}$ (*Atg7*$^{\Delta POMC}$)	Hypothalamus (POMC)	Increased food intake and obesity owing to reduced α-MSH level,[116] α-MSH-positive fibre density[117] and leptin signalling[117,118] Reduced peripheral lipolysis owing to a decrease in central sympathetic tone[116]
Lyz-*Cre*-*Atg5*$^{F/F}$	Myeloid system (macrophages)	Aggravated atherosclerosis owing to overproduction of IL-1β in macrophages after crossing with *LDLR*[130] or *ApoE*-knockout mice[131]
Lyz-*Cre*-*Atg7*$^{F/F}$ Lyz-*Cre*-*Atg5*$^{F/F}$	Bone (osteoclasts)	Increased bone mass owing to defective bone resorption[135]
Dmp1-*Cre*-*Atg7*$^{F/F}$	Bone (osteocytes)	Decreased bone mass owing to reduced numbers of osteoclasts and osteoblasts[136]
Osx-*Cre*-*FIP200*$^{F/F}$	Bone (osteoblasts)	Osteopaenia owing to defects in the terminal differentiation of osteoclasts[137] Decrease in body weight[137]
Bcl2 AAA (T69A, S70A, S84A)	Whole body	Impaired exercise-induced autophagy and/or metabolic improvement and reduced exercise endurance[96] No difference in HFD-induced metabolic changes[96]
Becn1$^{+/-}$	Whole body	Impairment in exercise-induced improvement of endurance capacity[97] Increased hepatic lipid content at 16–24 months of age[145] No difference in body weight, glucose clearance and insulin tolerance at 12 weeks of age[147]
CAG-*Cre*-*Atg5*$^{F/F}$ (mosaic *Atg5* knockout)	Whole body	Increased hepatic lipid content at 19 months of age[146]
Transgenic *Atg5*	Whole body	Protection against diet-induced obesity and insulin resistance[151] Extension of lifespan[151]
Becn2$^{+/-}$	Whole body	Obesity and insulin resistance owing to increased cannabinoid signalling[147]

Abbreviations: AgRP, agouti-related peptide; Alb, Albumin; aP2, adipocyte protein 2; ApoE, apolipoprotein E; BAT, brown adipose tissue; CAG, cytomegalovirus immediate early enhancer, chicken β-actin promoter and rabbit β-globin intron; Dmp1, dentin matrix protein 1; HFD, high-fat diet; HSCs, hepatic stellate cells; LDLR, LDL receptor; Lyz, Lysozyme M; Mlc1f, myosin light chain 1 fast; Myf5, myogenic factor 5; Osx, osterix; poly(I:C), polyinosinic-polycytidylic acid; POMC, proopiomelanocortin; RIP, rat insulin promoter; WAT, white adipose tissue.

or insulin sensitivity has been investigated using skeletal-muscle-specific *Atg7*-knockout (*Atg7*$^{\Delta sm}$) mice.[91] These mutant mice have reduced lean body mass and fat mass together with enhanced glucose clearance and energy expenditure compared with wild-type mice. Furthermore, *Atg7*$^{\Delta sm}$ mice are protected from obesity and insulin resistance induced by a high-fat diet, probably owing to increased lipolysis and β-oxidation, and acquisition of the characteristics of brown adipose tissue by white adipose tissue (the process of 'browning'). These metabolic changes are attributed to the endocrine effects of FGF21, a mitokine that is released from autophagy-deficient muscle in response to mitochondrial dysfunction. This process is dependent on the induction of the transcription factor ATF4, a master regulator of the integrated stress response.[91] Regarding muscular function, *Atg7*$^{\Delta sm}$ mice exhibit muscle atrophy and decreased muscle force.[92] These results suggest a role for constitutive autophagy in the maintenance of muscle mass, which is distinct from muscle wasting caused by excess autophagy.[93,94]

Exercise is reported to stimulate autophagy in skeletal muscle.[95] The autophagy status of muscle can, in turn, affect an individual's exercise capacity and exercise-induced improvement of their metabolic profile. Basal autophagy is intact but exercise-induced autophagy and exercise endurance are impaired in mice with *Bcl2* knock-in mutations of three different alanine residues (*Bcl2* AAA) that inhibit dissociation of the Bcl-2–Beclin-1 complex and activation of autophagy.[96] Similarly, exercise-trained mice haploinsufficient for *Beclin-1* (*Becn1*$^{+/-}$) show limited improvement in endurance capacity and mitochondrial biogenesis after exercise.[97] Furthermore, metabolic parameters do not improve appreciably after exercise in the *Bcl2* AAA mice, suggesting an essential role of autophagy in the metabolic effects of training.

Lipid accumulation in skeletal muscle is a prominent feature of obesity-induced insulin resistance and type 2 diabetes mellitus.[98] Given that autophagy participates in lipid removal, evaluation of autophagy in skeletal muscle of animals and humans with obesity could be clinically relevant. One study found that the expression of components of the autophagy machinery (Beclin-1 and LC3–phosphatidylethanolamine) is upregulated in the skeletal muscle of *ob*/*ob* mice;[99] however, neither the level nor flux of autophagy has been determined in these mice. Whether obesity inhibits autophagy in skeletal muscle and thereby promotes lipid accumulation, or autophagy is increased to compensate for lipid overload in skeletal muscle, remains to be determined.

Liver

As the liver is a major organ involved in diverse metabolic processes, the role of hepatocyte autophagy in body metabolism and metabolic disorders has been extensively investigated. Lipid content in the liver is increased in both the fed and fasted states in hepatocyte-specific *Atg7*-knockout (*Atg7*$^{\Delta hep}$) mice, which is compatible with the concept of lipophagy.[58] Mice with hepatocyte-specific deletion of *Vps34*, a central regulator of autophagy (*Vps34*$^{\Delta hep}$), consistently display lipid accumulation in the liver, which is accompanied by hepatomegaly.[100] More pronounced lipid accumulation is also observed in mice lacking hepatic *Tfeb* (*Tfeb*$^{\Delta hep}$) after starvation or consuming a high-fat diet when compared with wild-type mice.[44] These results underscore the functional importance of lipophagy due to TFEB-mediated induction of lysosome biogenesis and autophagy genes; however, a role for TFEB-induced lipid catabolism in the observed drop in lipid content cannot be excluded. By contrast, decreased lipid content after starvation or consumption of a high-fat diet has been reported in *Atg7*$^{\Delta hep}$ mice.[61,91] Such a finding could be attributed to enhanced lipid catabolism by FGF21 secreted from autophagy-deficient liver and reduced hepatic synthesis of fatty acids or triglycerides, which is similar to the findings in *Atg7*$^{\Delta sm}$ mice that have autophagy deficiency in their skeletal muscle.[91] Mice lacking hepatic *FIP200* (*FIP200*$^{\Delta hep}$) also show reduced lipid accumulation after starvation or a high-fat diet owing to reduced *de novo* lipid synthesis.[101]

Disruption of autophagy in the liver by adenovirus-mediated *in vivo* gene delivery has also been conducted. The liver of *Atg7*-floxed (*Atg7*$^{F/F}$) mice infected with adenovirus expressing *Cre* recombinase has increased lipid content,[44] whereas hepatic lipid content is not changed following gene knockdown by adenovirus-mediated short-hairpin *Atg7* RNA (sh*Atg7*) delivery.[102] Thus, the role of hepatic autophagy in lipid metabolism is more complicated than expected. These inconsistencies might reflect differences in the genetic background of experimental animals, diet, experimental procedures or analytical methods. In particular, the difference in target genes examined (*Atg7*, *TFEB*, *Vps34* and *FIP200*) seems to underpin these discrepancies because autophagy-related genes have additional cellular functions other than just the regulation of autophagy. Furthermore, the techniques used for gene disruption (adenovirus-mediated delivery versus knockout) could result in different metabolic outcomes.

Autophagy also influences hepatic glucose metabolism through regulation of gluconeogenesis and glycogen storage. Fasting blood glucose levels are reduced following injection of polyinosinic-polycytidylic acid into myxovirus resistance 1 (Mx1)-*Cre*-*Atg7*$^{F/F}$ mice to activate the interferon-inducible Mx1 promoter and thereby to delete *Atg7* in the liver.[47] This observation might reflect reduced levels of gluconeogenesis owing to a lack of amino acids released by autophagic proteolysis. In addition, contrary to the expectation that glycogen content in autophagy deficiency would be increased owing to reduced glycogen degradation, polyinosinic-polycytidylic-acid-injected Mx1-*Cre*-*Atg7*$^{F/F}$ mice have an altered distribution of glycogen,[2] and *Vps34*$^{\Delta hep}$ mice display no glycogen in the liver.[100] Nonetheless, the same Mx1-*Cre*-*Atg7*$^{F/F}$ mice showed no change in hepatic glycogen content in another study.[47] Clearly, further studies are required to understand the differential effects of autophagy on glycogen metabolism.

The role of hepatic autophagy in insulin sensitivity and type 2 diabetes mellitus has also been explored. Hepatic autophagy is defective in mice with obesity-induced

insulin resistance owing to either reduced expression of autophagy-related genes,[103] or the post-translational cleavage of core autophagy proteins by the protease calpain.[102] Reduced expression of *Atg7* in the liver induced by adenovirus-mediated delivery of sh*Atg7* leads to increased ER stress and worsened insulin resistance in lean mice, whereas adenovirus-mediated overexpression of wild-type *Atg7* relieves these features in obese mice.[102] Similarly, adenovirus-mediated overexpression of *TFEB* ameliorates insulin resistance in mice fed a high-fat diet or in *ob*/*ob* mice.[44] By contrast, *Atg7*$^{\Delta hep}$ mice fed a high-fat diet have reduced hepatic steatosis, increased insulin sensitivity and enhanced glucose clearance, probably owing to reduced fat mass and induction of FGF21.[91] These various metabolic outcomes correspond to differences in experimental techniques used, the severity or duration of gene disruption and the target genes for abrogation of autophagy.

Autophagy of hepatic stellate cells (pericytes in the perisinusoidal space of the liver that are involved in fibrosis) is activated in mice with chemically induced liver injury. Glial fibrillary acidic protein (GFAP)-*Cre*-*Atg7*$^{F/F}$ mice with deletion of *Atg7* in hepatic stellate cells, where the GFAP promoter is active, display increased lipid accumulation in these cells and attenuated liver fibrosis.[104] Furthermore, reduced fibrogenesis in autophagy-defective hepatic stellate cells is partially reversed by supplementation with free fatty acids to increase production of ATP.[104] This observation suggests that free fatty acids resulting from lipophagy in the hepatic stellate cells are required for maintenance of hepatic stellate cell activation and progression of liver fibrosis. Thus, a strategy for selective inhibition of autophagy in hepatic stellate cells might be potentially beneficial for the treatment of fibrotic liver diseases. By contrast, hepatocyte-specific *FIP200* deletion aggravates liver injury and fibrosis in mice fed a high-fat diet,[101] which indicates a protective role of hepatocyte autophagy in fibrogenesis. As various cells in the liver—hepatocytes, hepatic stellate cells, Kupffer cells and sinusoidal endothelial cells—contribute to the progression of liver fibrosis,[105] further investigation is required to understand the role of autophagy in this process.

Adipose tissue

The role of autophagy has been investigated in the adipose tissue, which is another target of insulin action. In mice with a targeted deletion of *Atg7* in the adipose tissue (*Atg7*$^{\Delta Ad}$), fat mass is markedly reduced owing to defects in the differentiation of white adipocytes.[106,107] Intriguingly, the white adipose tissue of *Atg7*$^{\Delta Ad}$ mice presents features characteristic of brown adipose tissue.[106,107] When challenged with a high-fat diet, the *Atg7*$^{\Delta Ad}$ mice are resistant to obesity, with increased insulin sensitivity and glucose clearance, possibly owing to the induction of thermogenic uncoupling or β-oxidation.[106] In addition to white adipose tissue, a role for autophagy in brown adipose tissue has been explored.[108] Mice with a deletion of *Atg7* in the Myf5-positive progenitor cells that give rise to the brown adipose lineage[109] show defective differentiation and impaired function of this adipose depot in response to cold exposure.[108] Despite increased levels of lipolysis, β-oxidation and browning of the white adipose tissue, these mice have impaired glucose clearance and worsened insulin resistance, probably as a result of perturbation of insulin signalling in the muscle and reduced muscle mass.[108] The decreased muscle mass reflects autophagic defects in muscle cells, which also originate from Myf5-positive progenitors. Thus, studies of other mouse models are needed to determine the exact role of brown adipose tissue autophagy in the regulation of energy metabolism.

Changes in adipose tissue autophagy have been examined in the context of obesity. Some studies reported that the level of autophagy that occurs in the adipose tissue correlates with the degree of adiposity in both patients with obesity and obese mice, which presumably corresponds to compensatory increases in the expression of autophagy-related genes.[110,111] Autophagic activity also positively correlates with adipose-tissue inflammation that is characterized by macrophage infiltration.[111] Furthermore, inhibition of autophagy results in a marked increase in the expression of proinflammatory cytokines in adipose tissue of obese mice or patients with obesity,[111] which suggests that autophagy has a compensatory role to attenuate both obesity-induced proinflammatory cytokine expression and insulin resistance. Studies of mice that develop adipocyte-specific disruption of autophagy as adults but not during development would be useful to further characterize the role of adipose tissue autophagy in lipid-associated inflammation and insulin resistance.

ER stress could be a factor in obesity-induced insulin resistance in adipose tissue.[112] One study found that autophagy plays a part in the downregulation of insulin receptor expression in adipocytes after treatment with chemical stressors of the ER.[113] Whether this effect is directly dependent on autophagy or whether degradation of insulin receptors via autophagy is related to obesity-induced ER stress and insulin resistance remains to be determined.

Hypothalamus

The hypothalamus is a central regulator of appetite, energy expenditure and whole-body metabolism. Several research groups have addressed the role of hypothalamic autophagy in the control of whole-body energy homeostasis.[114–118] Impaired autophagy is observed in the hypothalamic arcuate nucleus when mice are challenged with a high-fat diet.[114] Knockdown of *Atg7* in the mediobasal hypothalamus of mice leads to increased body weight owing to hyperphagia and decreased energy expenditure.[114] Consequently, these mice develop obesity and insulin resistance in response to a high-fat diet, indicating that hypothalamic autophagy has an important role in maintenance of energy homeostasis. A neuron-specific deletion of *IKKβ* abolishes the metabolic phenotypes in these mice, which suggests a role for inflammation in obesity associated with hypothalamic autophagy deficiency.[114] However, which of the diverse hypothalamic

neurons are predominantly affected by mediobasal *Atg7* shRNA administration remains unclear. To help answer this question, knockout of autophagy genes in the two main neuronal populations of the hypothalamic arcuate nucleus has been conducted.

Agouti-related peptide (AgRP)-expressing neurons

AgRP neurons produce orexigenic hormones, such as AgRP and neuropeptide Y. Mice with an *Atg7* deletion in this neuronal population ($Atg7^{\Delta AgRP}$) fail to induce expression of AgRP in response to free fatty acids released during starvation.[115] By contrast, these mice express increased levels of the anorexigenic hormones proopiomelanocortin (POMC) and α-melanocyte-stimulating hormone (α-MSH), and consequently display a lean phenotype.[115]

Proopiomelanocortin (POMC)-expressing neurons

Mice with an *Atg7* deletion in the POMC neurons ($Atg7^{\Delta POMC}$) exhibit increased food intake and an obese phenotype.[116–118] These mice display impaired glucose tolerance and insulin resistance after consumption of a high-fat diet.[116] Deficiency of autophagy in the POMC neurons leads to elevated food intake owing to decreased production and secretion of α-MSH.[116] Furthermore, reduced α-MSH-positive fibres in the paraventricular nucleus of the hypothalamus owing to defective POMC axonal projections are observed, which would contribute to the defective neural control of food intake.[117]

Impaired leptin signalling in the autophagy-defective POMC neurons also influences feeding behaviour of the *Atg7* mutant mice.[117,118] Food intake during refeeding after long-term fasting is suppressed by intracerebroventricular leptin administration in wild-type mice but not in the mutant mice.[118] Thus, weight loss induced by leptin is lower in *Atg7* mutant mice than in the controls, which is indicative of leptin resistance.[117,118] In parallel, leptin induces activation of the transcription factor STAT3 to a lesser degree in POMC neurons of the *Atg7* mutant mice compared with those of the control mice,[118] although the total number of viable POMC neurons does not differ between the two groups of mice.[116–118] The mechanism behind the defective activation of STAT3 in the autophagy-deficient POMC neurons is unclear, although inflammatory changes such as activation of NF-κB, increased ER stress, reduced intracellular ATP content or accumulation of sequestosome-1-positive ubiquitinated aggregates might contribute to this effect. Given that leptin-induced sympathetic activation is reported to be abrogated by ablation of the leptin receptor in the hypothalamic arcuate nucleus,[119] impaired leptin signalling in POMC neurons of the *Atg7* mutant mice might also reduce peripheral lipolysis via a decrease of central sympathetic tone.[116] Taken together, these results suggest that metabolic influence of hypothalamic autophagy is dependent on the types of neurons affected and is regulated by complicated interactions between these neurons.

Myeloid system

Convincing evidence exists that inflammation and innate immunity are crucial components in the pathogenesis of type 2 diabetes mellitus and the metabolic syndrome.[120,121] Inflammasome activation in macrophages through the NLRP3 receptor seems to play an important part in the development of type 2 diabetes mellitus in animal models and human patients.[122–125] Autophagy negatively regulates inflammasome activity through quality control of mitochondria in a process called mitophagy that removes dysfunctional mitochondria[126,127] and also via elimination of active inflammasome complexes.[128,129] Obesity or administration of free fatty acids causes NLRP3-mediated hyperactivation of the inflammasome and overproduction of the cytokine IL-1β in macrophages, which in turn leads to impairment of insulin signalling in the adipose tissue, liver and skeletal muscle.[123] These findings potentially link defective autophagy in macrophages with the metabolic syndrome and type 2 diabetes mellitus; however, further studies will be necessary to evaluate the *in vivo* role of macrophage autophagy in this process.

Autophagy status also influences the development and progression of atherosclerosis. Autophagy in macrophages is impaired within the atherosclerotic lesions of the LDL receptor or apolipoprotein E knockout mice fed a diet high in fat and cholesterol.[130,131] Macrophage production of IL-1β is increased when such mice models also harbour a macrophage-specific deletion of *Atg5*.[130,131] Consequently, IL-1β released from macrophages could accelerate the progression of atherosclerosis in these mice, probably by triggering the expression of additional cytokines and chemokines in surrounding cells, such as neutrophils and T lymphocytes. These results are consistent with findings that atherosclerosis induced by cholesterol crystals is ameliorated in mice lacking *Nlrp3* or *Il-1β*.[132] Thus, autophagy protects against the development of atherosclerosis by reduction of inflammasome activation in macrophages.

Bone

Human genome-wide association studies have found a link between single-nucleotide polymorphisms of autophagy-related genes and stature[133] or BMD,[134] which implies that autophagy might participate in bone development and bone disorders. An *in vivo* role for autophagy in the development and homeostasis of bone has been investigated using gene knockout technology to create mice with specific deletions of *Atg7* or *Atg5* in the myeloid cells that give rise to osteoclasts. These mutant mice exhibit defects in lysosomal trafficking and formation of the ruffled border of osteoclasts.[135] Secretion of lysosomal enzymes to digest the organic matrix of bone is thereby impaired and bone mass becomes increased,[135] which suggests that osteoclast autophagy is important for bone resorption. The *Atg7* mutant mouse with autophagy deficiency in the osteocytes, which comprise approximately 90–95% of all bone cells, exhibits low bone mass, which is associated with reduced osteoclast and osteoblast numbers and a lack of balance between bone resorption and bone formation.[136] Thus, autophagy in osteocytes has an important role in the maintenance of bone mass and bone remodelling.

Box 1 | Autophagy and cancer metabolism

Paradoxical role of autophagy in cancer biology

Autophagy is upregulated in cancer cells to meet increased demands for nutrients and to adapt to metabolic stresses.[189] Although autophagy might be expected to promote survival of cancer cells, it tends to suppress tumorigenesis through reduction of oxidative stress and genomic damage via removal of dysfunctional cellular organelles and protein aggregates.[189] Thus, the role of autophagy in cancer cells is complex and varies depending on the stage of cancer or the cellular context.

Mouse models

A role for autophagy in the suppression of cancer has emerged from investigation of autophagy knockout mouse models. Mice deficient in beclin-1 (*Becn1*$^{+/-}$) develop multiple spontaneous tumours.[143,144] Other autophagy-deficient mice, such as *Atg7*$^{\Delta hep}$ and mosaic *Atg5*-deficient strains, also display high tumour incidence.[146,190]

Therapeutic implications

The accumulation of sequestosome-1 that occurs during autophagy deficiency promotes tumorigenesis, probably through induction of oxidative stress and genomic instability.[191] Thus, autophagy inducers or inhibitors of sequestosome-1 might be potentially useful for the treatment of autophagy-deficient tumours. Conversely, autophagy inhibitors could be considered as potential therapeutic agents for cancers with increased autophagic activity. Further research into the fundamental role of autophagy in tumour development and progression will provide opportunities for the design of novel cancer drugs.

An *in vivo* role for autophagy in osteoblasts has also been reported. Mice with an osteoblast-specific deletion of *FIP200* exhibit osteopaenia owing to defective terminal differentiation of osteoblasts and decreased bone formation.[137] These mice also display reduced body weight, which is attributable in part to a reduction in adipose-tissue mass.[137] Some evidence indicates that the skeleton is able to produce hormones (for example, osteocalcin and osteopontin) that regulate glucose and lipid metabolism.[138–140] As these hormones are produced by osteoblasts, it will be of interest to investigate their contribution to the phenotype of the mice with autophagy deficiency in osteoblasts. Taken together, these results suggest that control of autophagy in all three types of bone cells is important in maintenance of bone homeostasis.

Reduced autophagic activity during ageing is associated with development and progression of age-related diseases such as osteoporosis.[141] Decline of autophagy with ageing might, therefore, be a causal factor in the development of osteopaenia or osteoporosis. However, this hypothesis requires further investigation to fully understand the effects of ageing on autophagic activity in the bone.

Whole body

As autophagy has a role in the development and differentiation of mammals, conventional knockout mice with global deletion of autophagy-related genes predominantly display embryonic or neonatal lethality.[142] However, a few mouse strains with systemically altered autophagy of the whole body survive to adulthood, which provides an opportunity to study the role of autophagy in the whole organism. Mice heterozygous for *Beclin-1* (*Becn1*$^{+/-}$) have defective global autophagy and show a high incidence of spontaneous tumours (Box 1)[143,144] and increased lipid accumulation in the liver compared with wild-type mice at 16–24 months of age.[145] A similar profile is observed in 19-month-old mice with a mosaic deletion of *Atg5*, characterized by a proportion of mutant cells throughout the body,[146] which suggests that systemic autophagy is important in the regulation of lipid metabolism. Body weight, glucose clearance and insulin tolerance in *Becn1*$^{+/-}$ mice fed a regular diet do not differ from those in wild-type mice, despite increased hepatic lipid accumulation.[147] No specific alterations of metabolic parameters are detected with or without a high-fat diet in *Bcl2* AAA mice with defects in stimulus-induced autophagy,[96] whereas exercise-induced improvements in metabolic parameters are impaired in this model. Hypomorphic mice with partial loss of Atg16l1 function,[148] *LC3b*-null mice[149] and *Atg4b*-null mice[150] have been generated but body metabolism has not been studied in these models. One study has shown that *Atg5* transgenic mice with a global increase in autophagic activity exhibit a lean phenotype in old age.[151] Consequently, these mice have reduced hepatic levels of triglycerides and increased glucose clearance and insulin sensitivity, together with a substantial extension of lifespan.[151] Together, these mouse strains present potentially useful models for studies of autophagy in the global regulation of energy homeostasis.

Integrated regulation of autophagy

The previous sections discussed the importance of autophagy within specific organs in the regulation of energy metabolism and in the development of metabolic diseases within the whole organism as summarized in Figure 4. Metabolic outcomes of organ-specific autophagy deficiency are mediated by local (cell-autonomous) effects, such as increased lipid accumulation owing to defective lipophagy, augmented metabolic stress owing to impaired organelle function and dysregulated differentiation and development of the affected tissues. In addition, systemic (non-cell-autonomous) effects, such as induction or suppression of secreted factors and changes in sympathetic nerve activity, contribute to the metabolic changes of the whole organism, which suggests an important role for autophagy in the integrated regulation and cross-talk between different organs.

Diverse metabolic effects of autophagy deficiency in the regulation of whole-body metabolism are dependent on the relative contribution of affected organs. For example, pancreatic β cells are virtually the sole source of insulin, the primary hormone responsible for both the uptake of glucose into peripheral tissues and the trafficking or anabolism of other nutrients. Thus, autophagy deficiency in pancreatic β cells has irrevocable effects on body metabolism that cannot be compensated by other means or other tissues. Conversely, insulin-mediated metabolic changes occur in parallel within the skeletal muscle, liver and adipose tissue. Insufficiency of autophagy in one of these insulin target tissues exerts metabolic effects that depend upon the relative dominance of each tissue in the metabolism

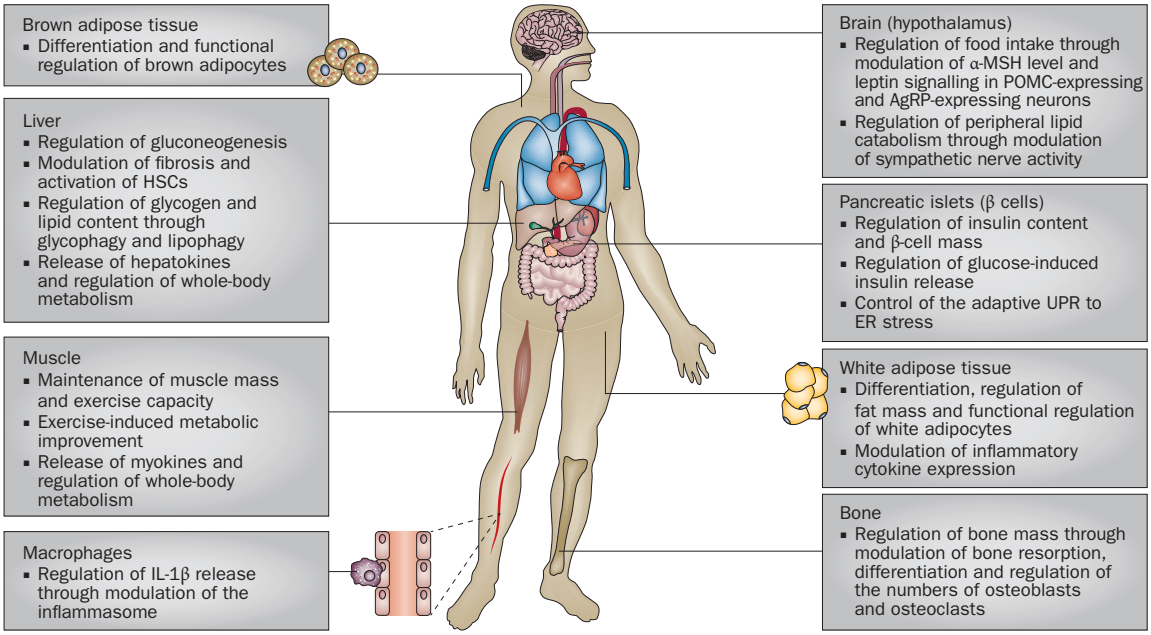

Figure 4 | Autophagy functions in multiple organs. The role of autophagy within metabolic organs is summarized from the results of animal experiments. Hepatokines and myokines are soluble factors released from the liver and muscle, respectively, which exert diverse actions on metabolism, inflammation, growth and other processes in an autocrine, paracrine or endocrine manner. Abbreviations: AgRP, agouti-related peptide; ER, endoplasmic reticulum; HSCs, hepatic stellate cells; POMC, proopiomelanocortin; UPR, unfolded protein response.

of nutrients. Furthermore, severe autophagy deficiency in insulin target tissues can lead to the induction of the mitochondrial stress response, which is similar to the stress response associated with amino acid deficiency,[91] and the release of FGF21 that has profound non-cell-autonomous effects on whole-body metabolism, particularly lipid metabolism.

By contrast to the changes that occur in parallel within the skeletal muscle, liver and adipose tissue, the relative hierarchy of a tissue with autophagy deficiency in the vertical metabolic cascades can also dictate metabolic outcome. For instance, mice with a deletion of *Atg7* in the POMC neurons are obese, whereas mice with an *Atg7* mutation in the AgRP neurons are lean. The obesity and insulin resistance characteristic of mice with a hypothalamic *Atg7* knockdown suggest that the action of POMC neurons could be distal to that of AgRP neurons, which is consistent with previous reports of the inhibition of POMC neurons by γ-aminobutyric acid from AgRP neurons.[152]

Although the relative dominance or hierarchical position of the tissues with autophagy deficiency will determine the metabolic outcome in the whole body, endogenous factors (for example, ageing and genetic predisposition) or exogenous factors (for example, drugs and environmental changes) that cause changes in autophagy status are likely to affect the whole body but not specific tissues. Metabolic changes in response to such factors or drugs will, therefore, reflect integration of the responses of individual tissues and the cross-talk between them. However, it is still possible that some measures, such as exercise of a specific muscle or administration of drugs to specific sites, affect autophagy status of specific tissues rather than the whole body. Endogenous factors that influence autophagy status might not have the same effects on diverse tissues because of differences in the expression level or post-translational modification of the autophagy machinery, other transcriptional or translational apparatus that are specific for each tissue, and the intracellular environment where autophagy or proteolysis takes place. Thus, diverse factors should be considered for the development of compounds that modify autophagy as potential treatments of metabolic disorders.

Autophagy as a therapeutic target

Growing evidence has suggested that dysregulation of autophagy occurs among patients with metabolic disorders such as diabetes mellitus,[85] obesity[110,111] and atherosclerosis.[153] Nonetheless, no mutations or single-nucleotide polymorphisms of autophagy-related genes have been reported among such patients, although variants of autophagy-related genes have been identified in individuals with neurodegenerative disease,[154–157] cancer[158–160] and Crohn disease.[161–163] Notwithstanding, some investigations have revealed clues to such a connection. Variation within *FTO* is linked to obesity;[164,165] the protein encoded by this gene acts as an amino-acid sensor upstream of LRS and induces activation of mTORC1.[166] Consequently, autophagic flux is increased in *FTO*-deficient cells.[166] In addition, the 1q43 chromosome locus that is linked to susceptibility to obesity and diabetes mellitus in humans[167,168] comprises *BECN2*, an autophagy regulator gene with sequence homology to *BECN1*.[147] Mice haploinsufficient for *Beclin-2* (*Becn2*$^{+/-}$) develop obesity and insulin resistance, probably owing

to impaired turnover of G-protein-coupled receptors (for example, cannabinoid receptors), although these phenotypes might not involve defective autophagy.[147] Such findings suggest that variants of autophagy-related genes could contribute to the development of metabolic diseases, although the pathogenetic links remain to be characterized.

Given the critical roles of autophagy in the development of various human diseases, several investigations have been undertaken to develop therapeutic agents targeting autophagy.[169,170] To date, efforts to discover drugs that modulate autophagy have mainly focused on cancer, neurodegenerative diseases and infectious diseases. Although clinical trials using drugs targeting autophagy for the treatment of metabolic disorders have not been yet performed, emerging evidence suggests that several drugs or compounds that are already available or being developed for metabolic diseases might exert therapeutic effects by modulation of autophagy. Despite the complex and conflicting effects of autophagy deficiency in various tissues that participate in the regulation of energy metabolism, activation of autophagy could be a beneficial approach for the prevention and treatment of metabolic diseases.[170]

Several of these potential therapies for metabolic disorders modulate autophagy by targeting components of its core machinery. For example, rapamycin (or its analogue everolimus) induces autophagy through inhibition of mTORC1 activity. Although mTORC1 inhibitors are generally considered to impair glucose tolerance, one study has shown that rapamycin can exert beneficial metabolic effects when long-term treatment is administered.[171] Rapamycin and everolimus also attenuate the progression of atherosclerotic plaques in animals fed a high-fat or cholesterol-rich diet,[172,173] and reduce the risk of restenosis and major cardiovascular events among patients with coronary artery disease.[174] Further studies are needed to confirm the obligate requirement for autophagy in these therapeutic effects.

The antidiabetic drug metformin also inhibits mTORC1 and activates autophagy[175] through either an AMPK-dependent[176] or an AMPK-independent pathway.[177] Exendin-4, a glucagon-like peptide-1 agonist used to treat type 2 diabetes mellitus, has been reported to ameliorate hepatic ER stress and steatosis after consumption of a diet high in fat and/or fructose by increasing the expression of Beclin-1 and inducing autophagy.[178] Beyond antidiabetic effects, metformin and glucagon-like peptide-1 agonists can reduce cardiovascular-related mortality and morbidity among patients with diabetes mellitus,[179–182] and suppress atherosclerotic plaque formation in animal models.[183,184] Thus, it is plausible that autophagy mediates the observed beneficial metabolic and cardiovascular effects of these drugs. In addition, beneficial effects of resveratrol (an activator of sirtuin-1) in diet-induced obesity and insulin resistance[185] could be mediated by sirtuin-1-induced autophagy.[186]

Finally, autophagic activity declines with age in the tissues of experimental animals, an effect that probably also occurs in humans.[141] Whether age-related changes in autophagy are causal or coincidental to the development of age-related metabolic diseases represents an important topic for future research.

Conclusions

Research findings suggest that dysregulation of autophagy plays an important part in the development and progression of cancer, neurodegenerative disorders, ageing and pathogenic infections. A growing body of evidence also reveals important roles in the regulation of whole-body energy metabolism and the development of metabolic diseases. However, the precise role of autophagy in the development of metabolic disorders in humans remains inconclusive, as most studies have been conducted using knockout animal models that are deficient in autophagy. Furthermore, studies of autophagy in metabolic diseases using human tissues samples are particularly difficult owing to technical problems in the measurement of autophagic activity. Thus, further investigation of the metabolic effects of autophagy insufficiency within the physiologically relevant range will be necessary to answer questions about the role of autophagy in human physiology or human metabolic disorders.

Novel compounds and cell-permeable peptides that modulate autophagy are in development as therapeutic agents for diverse human diseases.[187,188] Given that metabolic diseases such as obesity and diabetes mellitus involve dysregulation of multiple metabolic pathways—insulin signalling, insulin secretion, glucose utilization, thermogenesis, mitochondrial function and autophagy, among others—it is hard to predict whether modulation of autophagic activity alone will appreciably improve metabolic profiles. However, autophagy modulators would probably have at least additive therapeutic effects when combined with other currently available drugs for the treatment of metabolic disorders. Another point to consider in the clinical application of autophagy modulators to patients with metabolic diseases is that global alterations in autophagy might exert diverse effects on systems other than the endocrine system or body metabolism (for example, carcinogenesis, inflammation, infection, senescence and neurodegeneration). Hence, autophagy modulators could have unanticipated *in vivo* effects that might be either beneficial or harmful to the patient. Further studies are, therefore, necessary to develop safe and clinically effective autophagy modulators that target metabolic disease.

Review criteria

Articles selected for this Review were identified by searching PubMed and Google Web using combinations of the following terms: “diabetes”, “metabolic disease”, “obesity”, “insulin resistance”, “bone disease”, “cancer”, “atherosclerosis”, “autophagy” and “knockout mouse”. The articles cited were published in English from 1983 to 2013 and were full-text papers. Several references of identified articles were retrieved and cited in this Review.

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