Autophagy in health and disease. 2. Regulation of lipid metabolism and storage by autophagy: pathophysiological implications

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Czaja MJ. Autophagy in health and disease. 2. Regulation of lipid metabolism and storage by autophagy: pathophysiological implications. Am J Physiol Cell Physiol 298: C973–C978, 2010. First published January 20, 2010; doi:10.1152/ajpcell.00527.2009.—Autophagy is a lysosomal degradative pathway critical for the removal and breakdown of cellular components such as organelles and proteins. Despite striking similarities in the regulation and function of autophagy and lipid metabolism, the two processes have only recently been shown to be interrelated. This review details new findings of critical functions for autophagy in lipid metabolism and storage. Studies in hepatocytes and liver have demonstrated that macroautophagy mediates the breakdown of lipids stored in lipid droplets and that an inhibition of autophagy leads to the development of a fatty liver. In contrast, in adipocytes the loss of macroautophagy decreases the amount of lipid stored in adipose tissue through effects on white and brown adipocyte differentiation. Other investigations have indicated that the relationship between autophagy and lipids is bidirectional, with changes in cellular lipid content altering autophagic function. These newly described links between autophagy and lipid metabolism and storage have provided new insights into the mechanisms of both processes. The findings also suggest possible new therapeutic approaches to the problems of lipid overaccumulation and impaired autophagy that occur with aging and the metabolic syndrome.

Lipids and Lysosomes

Lipid droplets are cytosolic structures in which lipids are stored as a central core of TG and cholesterol esters surrounded by a phospholipid monolayer and a number of lipid droplet-associated proteins (21). Lipid droplets have biological functions that have led them to be considered as cellular organelles, suggesting that their disposition may be regulated by standard pathways of lysosomal or proteasomal degradation. These organelles allow storage of potentially toxic fatty acids as TG, which can then be broken down by lipolysis into free fatty acids for mitochondrial β-oxidation to supply the cell with ATP. Even in the absence of limited nutrients, stored TG have a rapid turnover, but the fatty acids released by lipolysis are preferentially reesterified back into TG with only a small proportion oxidized or packaged into lipoprotein for secretion (16). This degradative process is thought to be strictly controlled by cytosolic lipases. However, the mechanisms and site of lipid droplet mobilization and lipolysis remain unclear, particularly in liver where even the lipases involved are poorly defined (23).

Lysosomes are known to contain acid lipases for lipid breakdown, and remnants of TG-rich lipoproteins are taken up by receptor-mediated endocytosis and hydrolyzed in lysosomes (4, 36). Studies employing nonspecific lysosomal inhibitors, such as ammonium chloride or chloroquine or agents intended to target acid lipases and not cytosolic lipases, have suggested...
that lysosomes may also participate in the breakdown of endogenous cellular lipids. These agents inhibit fatty acid β-oxidation and ketone production in hepatocytes from fasted rats (7) and newborn rabbits (10), consistent with a function for lysosomes in lipolysis. However, a mechanism for the lysosomal degradation of stored cytosolic TG was not established until recently.

**Autophagy Mediates Hepatic Lipolysis**

Although lipids had not been described as a substrate for autophagy, the parallel between the processes of lipolysis and macroautophagy and the existence of lysosomal lipases prompted studies to investigate whether the two pathways may be interrelated. Findings from these investigations have delineated a novel function for macroautophagy in hepatocyte lipid metabolism (29). An inhibition of macroautophagy by genetic knockdown of the autophagy gene atg5 or the pharmacological inhibitor 3-methyladenine in hepatocytes challenged with a lipid load from fatty acid supplementation or culture in methionine- and choline-deficient medium significantly increased cellular TG content. If supplemented with cholesterol, macroautophagy-inhibited hepatocytes also accumulated more cholesterol. This function of macroautophagy was not limited to hepatocytes because murine embryonic fibroblasts (MEFs) from atg5-null mice similarly accumulated greater amounts of TG than did wild-type cells in response to a lipid challenge. The increased lipid in hepatocytes was present in lipid droplets as assessed by fluorescence and electron microscopy and oil red O staining, all of which demonstrated increased lipid droplet size and number in cells with an inhibition of macroautophagy. Excessive lipid storage resulted from a failure to break down stored lipid because hepatocytes lacking macroautophagy had decreased rates of lipolysis and fatty acid β-oxidation. Lipid movement through the autophagic pathway was detected by the colocalization of neutral lipid with markers of autophagic vacuoles and lysosomes as well as electron microscopic findings of lipid droplets within autophagic vacuoles. Immunogold staining for the autophagosome-associated protein LC3 revealed its association with lipid droplets in the hepatocyte or the animal indicate that mechanisms exist for the selective recognition and engulfment of lipid droplets. The mechanism by which lipid droplets are recognized as substrate

**Fig. 1.** Function of macroautophagy in hepatocyte lipid metabolism. Small lipid droplets in the hepatocyte are engulfed whole either by themselves or in combination with other substrates such as mitochondria in autophagosomes. Portions of larger lipid droplets are removed and also sequestered in autophagosomes. Autophagosomes fuse with lysosomes for cargo degradation. Breakdown of triglycerides in lipid droplets by lysosomal lipases yields free fatty acids (FFA) which can undergo β-oxidation in mitochondria to supply the hepatocyte with ATP. Macroautophagy therefore performs a critical function in the regulation of liver lipid stores.
These findings have been confirmed in studies of transporter 4 in cells with an inhibition of macroautophagy. C/EBPβ-null adipocyte differentiation (peroxisome proliferator-activated receptor-γ, CCAAT/enhancer-binding protein-α (C/EBP-α), and C/EBP-β) and of markers of differentiated adipocytes (fatty acid synthase, stearoyl-coenzyme A desaturase 1, and glucose transporter 4) in cells with an inhibition of macroautophagy. These findings have been confirmed in studies of atg5-null MEFs that were similarly induced chemically to undergo adipocyte differentiation (1). Consistent with the findings in 3T3-L1 cells, atg5-null cells failed to differentiate and accumulate lipid (1). Levels of macroautophagy were reported to increase in MEFs during the induction of differentiation, but this conclusion was derived from steady-state measures of LC3-II and autagagosome number, whereas studies of autophagic flow in 3T3-L1 cells demonstrated no change in flow when these cells were induced to differentiate (30). Thus, macroautophagy is essential for preadipocytes to differentiate into white adipocyte in vitro, but it remains unclear whether this is a function of basal or induced levels of autophagy.

These in vitro findings were confirmed by in vivo studies of adipocyte-specific atg7-knockout mice. Null mice lacking macroautophagy in adipocytes were lean and more insulin sensitive owing to a marked decrease in white adipose tissue mass (30). Although the knockout mice had altered adipocyte differentiation, the in vivo effects of the loss of macroautophagy on adipocyte differentiation were more complex than a simple failure of white adipocytes to differentiate. Along with the decrease in white adipocyte mass, the remaining cells in these fat depots had increased morphological and biochemical features of brown adipocytes. In addition, fat depots normally composed of brown adipocytes were increased in size. The two types of adipocytes differ in function in that white adipocytes store large amounts of TG for breakdown and release in times of nutrient deprivation, whereas brown adipocytes have increased numbers of mitochondria that consume lipid through β-oxidation for heat generation (32). Adipocyte-specific Atg7-knockout mice with an enlargement of their brown adipocyte mass therefore had increased rates of fatty acid β-oxidation that consumed TG and prevented lipid accumulation in non-adipose organs such as liver and heart that would have otherwise occurred because of the reduction in white adipocyte storage capacity. Loss of adipocyte macroautophagy therefore promotes a more favorable metabolic phenotype of decreased white adipose mass and increased brown adipose tissue, resulting in elevated levels of β-oxidation and a lean mouse with increased insulin sensitivity. Although humans have been thought to lose their brown adipocytes after birth, recent findings of the presence of significant numbers of brown adipocytes in human adults (24, 34) have spurred interest in therapies for obesity directed at the conversion of white adipocytes into brown ones. Manipulation of adipocyte macroautophagy may provide a unique approach to achieve this goal.

The investigations to date have yet to elucidate the mechanism by which adipocyte differentiation is regulated by macroautophagy. Macroautophagy is known to affect cell differentiation based on findings that global knockouts of autophagy genes in mice result in developmental defects in organs such as the nervous system (2). One possible mechanism for the effect of the loss of autophagy on adipocyte differentiation may be that brown adipocyte precursor cells have a growth advantage in the absence of macroautophagy. However, evidence against this possibility is that adipocyte-specific Atg7-knockout mice have normal numbers of white adipocyte progenitor cells (30). In addition, the adipocyte-specific Cre promoter used for the in vivo studies should have been exclusively expressed in differentiated adipocytes and therefore not have affected stem cell populations. Alternatively, the absence of macroautophagy may have altered the process of white and brown adipocyte transdifferentiation. Autophagy may perform a critical cellular remodeling function, such as through the removal of the large numbers of mitochondria present in brown adipocytes that mediates transdifferentiation from brown to white adipocytes. These possibilities require further study (Fig. 2).

An additional critical question is whether the effects of macroautophagy on adipocyte differentiation occur only in the early formative perinatal period of adipose development or in adult mice as well. Adipose tissue was previously thought to be a static organ, but recent studies indicate that there is a 10% yearly turnover in human adipocytes (31), suggesting the potential for autophagy to regulate adipocyte tissue later in life. Whether macroautophagy regulates adipocyte differentiation in adult tissue remains unknown. Alternatively, can autophagy regulate lipid metabolism in differentiated adult adipocytes in a manner similar to what has been reported in hepatocytes? This question could not be addressed in the studies to date because the in vitro and in vivo models of an inhibition of macroautophagy had profound effects on differentiation-dependent lipid storage that precluded studies of lipid metabolism in these cells. Further studies of an inhibition of autophagy in a fully differentiated adipocyte are needed to answer this question.

Lipid Stores Regulate Autophagic Function

Along with findings that autophagy affects lipid storage, it has been demonstrated that the inverse relationship also ex-
ALTERATIONS IN CELLULAR CHOLESTEROL LEVELS HAVE ALSO BEEN DETERMINED TO AFFECT AUTOPHAGIC FUNCTION. IN PARTICULAR, INCREASED HEPATOCELLULAR LIPID ACCUMULATION THAT OCCURS WITH EXCESSIVE LIPID LEVELS HAS BEEN SHOWN TO IMPAIR MACROAUTOPHAGY. STUDIES OF CULTURED HEPATO CYTES WITH A KNOCKDOWN OF ATG5 REVEALED THAT ALTHOUGH CELLS INDUCED TO ACCUMULATE LIPID BY CULTURE IN A METHIONINE- AND CHOLINE-DEFICIENT MEDIUM HAD INCREASED LEVELS OF MACROAUTOPHAGY, CELLS CHALLENGED ACUTELY WITH THE FA TTY ACID OLEATE HAD IMPAIRED MOVEMENT OF LIPID THROUGH THE AUTOPHAGIC PATHWAY (29). IN VIVO STUDIES OF WILD-TYPE MICE FED 16 WK OF A HIGH FAT DIET DEMONSTRATED A SIMILAR REDUCTION IN HEPATIC AUTOPHAGIC FUNCTION FROM THIS LIPID CHALLENGE. ALTHOUGH THE HIGH FAT DIET-FED MICE HAD A GREATER ASSOCIATION OF LC3 WITH LIPID DROPLETS IN THE FED STATE, THE INCREASE IN THIS ASSOCIATION THAT OCCURRED IN REGULAR DIET-FED MICE FOLLOWING THE AUTOPHAGIC STIMULUS OF STARVATION FAILED TO OCCUR IN HIGH FAT DIET-FED MICE. BY ELECTRON MICROSCOPY, LIPID DROPLETS FAILED TO MOVE INTO AUTOPHAGIC VACUOLES IN THE HIGH FAT DIET-FED MICE IN RESPONSE TO STARVATION, INDICATING AN IMPAIRMENT IN MACROAUTOPHAGIC FUNCTION IN THESE MICE WITH DIET-INDUCED FATTY LIVER (29). FURTHER SUPPORT FOR AN IMPAIRMENT IN AUTOPHAGY BY INCREASED CELLULAR LIPID COMES FROM PRELIMINARY FINDINGS OF AN INHIBITION OF HEPATIC MACROAUTOPHAGIC FUNCTION IN A GENETIC MODEL OF OBESITY, THE ob/ob MOUSE (18). THUS, CELLULAR LIPID ACCUMULATION MAY INITIATE OR EXACERBATE HEPATOCELLULAR DEFECTS IN MACROAUTOPHAGY, WHICH HAS IMPORTANT IMPLICATIONS FOR THE NORMAL PHYSIOLOGY OF AGING AND PATHOPHYSIOLOGY OF STEATOHEPATITIS AS DISCUSSED SUBSEQUENTLY.

ALTERATIONS IN CELLULAR CHOLESTEROL LEVELS HAVE ALSO BEEN DEMONSTRATED TO AFFECT AUTOPHAGIC FUNCTION. IN SEVERAL CELL TYPES, CHOLESTEROL DEPLETION ACTIVATES MACROAUTOPHAGY, AS DETERMINED BY INCREASES IN LC3-II AND NUMBERS OF AUTOPHAGOSONES, ALTHOUGH THERE HAS ALSO BEEN SOME INDICATION THAT AUTOPHAGOSOME MATURATION MIGHT BE IMPAIRED (3). CHOLESTEROL MAY AFFECT AUTOPHAGY THROUGH EFFECTS ON THE mTOR PATHWAY BECAUSE CHOLESTEROL DEPLETION HAS BEEN REPORTED TO DECREASE (3), AND HYPERCHOLESTEROLEMIA TO ACTIVATE (17), mTOR SIGNALING. NOVEL STUDIES IN CHAPERONE-MEDIATED AUTOPHAGY (CMA), THE FORM OF AUTOPHAGY IN WHICH PROTEINS WITH A SPECIFIC PEPTIDE MOTIF ARE TARGETED FOR UPTAKE AND DEGRADATION IN LYSOSOMES (22), HAVE DEMONSTRATED ANOTHER MECHANISM BY WHICH LIPID ALTERATIONS MAY AFFECT AUTOPHAGIC FUNCTION. CMA FUNCTION IS LIMITED BY THE RATE AT WHICH SUBSTRATES MAY BIND TO THE LYSOSOME-ASSOCIATED MEMBRANE PROTEIN TYPE 2A (LAMP-2A) FOR UPTAKE INTO THE LYSOSOME (22). LAMP-2A HAS BEEN IDENTIFIED TO RESIDE IN DISCRETE LIPID MICRODOMAINS OF THE LYSOSOMAL MEMBRANE THAT REGULATE LAMP-2A FUNCTION AND THEREFORE CMA ACTIVITY. CHOLESTEROL LOADING ALTERED THE LIPID MEMBRANE COMPOSITION OF THE LYSOSOME AND DECREASED CMA ACTIVITY IN MOUSE FIBROBLASTS (20). SINCE CMA IS DECREASED IN AGING, THESE FINDINGS PROVIDE ANOTHER MECHANISM BY WHICH THE INCREASED CELLULAR LIPID CONTENT THAT ACCOMPANIES AGING MAY ALSO LEAD TO IMPAIRED AUTOPHAGY. SIMILAR ALTERATIONS IN AUTOPHAGOSOME OR LYSOSOME MEMBRANE LIPID COMPOSITION MAY UNDERLIE THE EFFECTS OF FA TTY ACIDS AND HIGH FAT DIET ON MACROAUTOPHAGY.

Fig. 2. Macroautophagy in adipocytes regulates cellular differentiation. Inhibition of macroautophagy reduces white adipose tissue (WAT) mass and increases the amount of brown adipose tissue (BAT). This effect may be mediated through an inhibitory effect on white adipocyte stem cells or a stimulatory effect on brown adipocyte progenitor cells. Alternatively, the loss of autophagy may alter white/brown adipocyte transdifferentiation to favor brown adipocyte differentiation. The loss of WAT mass together with an increased rate of fatty acid β-oxidation resulting from the increase in brown adipocytes leads to decreased lipid storage and a reduction in fat mass. This decrease in fat has the beneficial effects of reducing body weight and improving insulin sensitivity.

**Autophagy, Aging, and the Metabolic Syndrome**

The newly described interrelationship between autophagy and lipid metabolism suggests that alterations in autophagic function may play a role in the pathophysiology of human disorders that result from excessive cellular lipid accumulation, such as manifestations of the metabolic syndrome. The metabolic syndrome is a major world health problem that includes the clinical features of obesity, glucose intolerance, dyslipidemia, and fatty liver disease (11). This disorder is associated with aging because the prevalence increases from 7% among individuals aged 20–29 yr to 44% in those aged 60–69 yr (14). With aging, both macroautophagy and CMA decline in parallel with development of the metabolic syndrome and an increase in lipid accumulation in both adipose and nonadipose tissues (5). The impairment in autophagy that accompanies aging may therefore contribute to the excessive cellular lipid storage that underlies the metabolic syndrome. In particular, findings of an essential function for macroautophagy in the control of hepatocyte lipid storage suggests a role for any impairment in autophagy in the development of fatty liver disease or hepatic steatosis. Interestingly, the phenotype of the hepatocyte-specific Atg7-knockout mouse is similar to that of human fatty liver disease because both are characterized by increased hepatic TG accumulation and decreased VLDL secretion (15). Nonalcoholic fatty liver disease is a continuum from simple lipid accumulation (steatosis) to steatosis plus cellular injury and inflammation (steatohepatitis). An impairment in hepatocyte autophagy may not only promote steatosis but also lead to progression to liver injury and steatohepatitis due to loss of autophagy’s protective function against cell death (35). The sensitivity of hepatocytes with impaired autophagy and resultant lipid accumulation to death stimuli needs to be examined to address this question. Finally, whether an inhibition of macroautophagy promotes lipid accumulation in cells other than hepatocytes remains to be determined because MEFs are the only other cells in which this function of autophagy has been
described (29). However, it seems likely that macroautophagy will be found to mediate lipid breakdown in other cell types, although one notable exception may be the adipocyte.

Findings in adipose tissue suggest a potentially different role for autophagy in this organ in the setting of the metabolic syndrome. In contrast to the liver, a loss of macroautophagy in adipose tissue may be of benefit by reducing harmful white fat mass, promoting the development of metabolically active brown adipocytes and thereby reducing body weight and increasing insulin sensitivity. Thus, a decrease in autophagy with aging may actually be beneficial to the metabolic state of the whole animal. However, little is known about the effects of aging on autophagic function in adipose tissue, which may not suffer the aging-related decrease in autophagy that occurs in the liver. Alternatively, as discussed above, it remains unclear as to how macroautophagy functions in the fully differentiated adipocyte. One way to unify the current findings in hepatocytes and adipocytes is that autophagy may function to protect nonadipose organs such as liver from damaging lipid accumulation by increasing lipid breakdown and at the same time promoting sufficient and safe storage of lipid in white adipocytes (30). The cell type-specific effects of autophagy on lipids need to be better defined to resolve the global effects of autophagy before manipulations of this pathway can be considered as a potential therapy for disorders such as fatty liver.

The finding that lipids regulate autophagic function suggests a new mechanism for the decrease in autophagy that occurs with aging. Lipid-induced effects on autophagy may underlie other problems associated with both aging and obesity such as the development of cancer. The beneficial effects of calorie restriction on longevity have been attributed to an increase in autophagy (9, 19). A decrease in cellular lipid content from such a drastic diet may be a mechanism for the improvement in autophagy that occurs, and an additional health benefit of this effect need to be delineated as do those that underlie the effects of autophagy on adipocyte differentiation. The effects of autophagy on other aspects of lipid metabolism also require further investigation. For example, degradation of apolipoprotein-B, a major component of secreted lipoproteins, is regulated by macroautophagy (25, 26), suggesting that autophagy may modulate lipoprotein assembly or secretion. Studies to date have focused on macroautophagy, and whether CMA functions in lipid metabolism needs to be examined. Finally, defects or alterations in the autophagic pathway may underlie human metabolic diseases such as fatty liver disease. The multiple and cell type-specific functions of autophagy may make therapeutic manipulations of this pathway to alter human lipid metabolism problematic, but for the first time it is clear that in addition to its role in the degradation of abnormal cellular components, a failure in the function of autophagy in energy metabolism may promote disease.

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DISCLOSURES
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REFERENCES


