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Autophagy, Myocardial Protection and the Metabolic Syndrome

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Abstract

Autophagy is a housekeeping process which helps to maintain cellular energy homeostasis and remove damaged organelles. In the heart, autophagy is an adaptive process that is activated in response to stress including acute and chronic ischemia. Given the evidence that autophagy is suppressed in energy-rich conditions, the objective of this review is to examine autophagy and cardioprotection in the setting of the metabolic syndrome. Clinical approaches that involve the induction of cardiac autophagy pharmacologically to enhance the heart's tolerance to ischemia are also discussed.

Introduction

Autophagy is a process whereby double-membrane vesicles (autophagosomes) form in the cytoplasm in response to cellular stress and ultimately sequester damaged cytoplasmic components including ubiquitinated protein aggregates, dysfunctional mitochondria, and endoplasmic reticulum. The machinery is activated by a number of stimuli including caloric restriction, oxidative stress, and brief episodes of ischemia and reperfusion.¹ It begins with nucleation of autophagy proteins that give rise to a cup-shaped phagophore which surrounds the damaged cytoplasmic material and closes to form the autophagosome. This is regulated by the autophagy proteins Atg4, Atg7, LC3, and the complex of Atg12-Atg5-Atg16L. The outer membrane of the autophagosome fuses with a lysosome to form an autophagolysosome, where the cargo is degraded by lysosomal hydrolases yielding amino acids, simple sugars, and fatty acids which are exported back into the cytosol.

With respect to the heart, there is increasing evidence that autophagy is an adaptive response to ischemic stress. Studies in other tissues indicate that autophagy is deficient in the setting of nutritional excess. This could account for the heart's increased susceptibility to ischemic injury in humans with metabolic syndrome (MetS). MetS is characterized by a constellation of factors which include obesity, insulin resistance, and dyslipidemia and is a known risk factor for cardiovascular disease, type 2 diabetes mellitus and all-cause mortality.² MetS is a growing pandemic affecting 20% – 30% of the population in the United States.^{2, 3} The presence of MetS is associated with worse outcomes after myocardial infarction, more severe post-infarction remodeling, and more rapid progression to heart failure. Because

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MetS affects autophagy, which is an adaptive pathway in the heart, it is important to develop a better understanding of the relationship between cardiac autophagy, ischemia/reperfusion, myocardial protection and MetS.

Energy Status

The dynamic process of autophagy is tightly regulated by the availability of nutrients and the metabolic balance of the cell.¹ A circadian rhythm of autophagy has been described in liver^{4, 5} and heart⁵ that show a peak in autophagic activity towards the end of the resting phase. Calorie intake quickly suppresses autophagy,⁶ whereas fasting dramatically enhances it.⁷⁻⁹ Short-term (4 weeks) and long-term (6 months) caloric restriction (CR) have been shown to improve the heart's tolerance to ischemia.¹⁰⁻¹² In contrast, autophagy is suppressed with obesity¹³ and cardioprotection is blunted.¹⁴ Thus, in conditions of sustained nutrient availability such as MetS, attenuation of autophagy may have profound consequences on the heart's adaptability and cardioprotection.

Ischemic Stress

Myocardial infarction is one of the leading causes of death worldwide. It results in acute necrotic and apoptotic cell death and may lead to cardiac dysfunction and acute heart failure. Likewise, cardiac remodeling that occurs after an acute MI may also lead to heart failure and death. In an effort to increase the heart's tolerance to ischemia a number of therapeutic strategies are under investigation. One approach that holds promise is the induction of cardiac autophagy. The rationale for this is based on the growing body of evidence that shows autophagy is an adaptive response to ischemia/reperfusion (I/R) injury.^{9, 15-27}

While there is compelling evidence that autophagy is an adaptive process during ischemia and postinfarction remodeling there are reports that autophagy may be deleterious during reperfusion^{11, 12} and contributes to load induced heart failure²⁸. With respect to the former, Matsui et al. used Beclin1 (+/-) mice and found that they had decreased I/R injury.¹¹ However, recent work from Diwan's group showed that Beclin1 interferes with autophagy. Beclin1 (+/-) mice actually have higher flux (thus fewer autophagosomes at steady state), and the improved flux is responsible for their resistance to I/R injury. Zhai et al. showed that rapamycin increased I/R injury in mice overexpressing constitutively active GSK-3 β .¹² However, Khan et al. showed that rapamycin decreased infarct size in isolated perfused mouse hearts and cardiomyocytes²⁹ and Das et al. showed a similar effect in db/db mice³⁰ raising the possibility that Sadoshima's genetically modified mice developed compensatory pathways that confounded interpretation of results. More recently, Sadoshima's group observed that rapamycin enhanced autophagy abolished the effects of GSK-3 β inhibition and protects against ischemia/reperfusion injury by modulating mTOR and autophagy.

With respect to pathological remodeling secondary to pressure overload, Zhu et al. reported that overexpression of Beclin 1 in mice subjected to aortic banding, accentuated pathological remodeling, whereas in *Beclin 1*^{+/-} hearts, the pressure overload-induced decline in systolic function was attenuated.³¹ This may be explained by the dual role of Beclin 1 in the initiation of autophagy and interference with autophagosome-lysosome fusion.³² In other studies, cardiac deletion of *mTOR* resulted in a smaller increase in left ventricle (LV) wall thickness and a more dilated LV chamber 2 weeks after aortic constriction.²¹ Ablation of Raptor, a positive regulator of mTOR, impaired adaptive hypertrophy, altered metabolic gene expression, and caused heart failure in C57BL/6J mice. It also resulted in the activation of Caspase 3, loss of mitochondria, and a robust increase in autophagy.²³ These contradictory findings may be explained by the fact that the mTOR pathway affects not only autophagy, but several other mechanisms including protein synthesis, cell proliferation and metabolism.³³ The complexity of interpreting studies of mTOR are partially addressed by

Sciarretta's work, in which mTOR inhibition with rapamycin fails to reverse deleterious effects of a high fat diet unless autophagy is restored by upregulation of Atg7. Overall, however, the findings to date suggest that optimal activation of autophagy results in effective cardioprotection and may lead to decreased long-term detrimental effects of post-infarction remodeling.

Cardioprotection

One of the most potent and well characterized cardioprotective mechanisms is the phenomenon of ischemic preconditioning (IPC). Recently, it has been shown that autophagy plays an important role in mediating the phenomenon in variety of animal models. Gurusamy *et al.* demonstrated upregulation of LC3 and Beclin 1 after IPC followed by 30 min global ischemia in isolated perfused rat hearts.³⁴ In our laboratory, we have demonstrated induction of autophagy by IPC and that autophagy is required for the cardioprotective effects of IPC in a number of models.³⁵ We have shown that Parkin, a protein necessary for selective removal of mitochondria by autophagy, translocates to mitochondria in response to IPC and that it is required for cardioprotection.³⁶ Yan et al. showed that autophagy plays a role in the late phase of IPC in the hibernating myocardium, although they could not detect the induction of autophagy in the early phase of IPC.³⁷ In this model of preconditioning pigs were subjected to IPC by 2×10 min coronary occlusion or 90 min 30% coronary stenosis every 12 hours for 3 days. An increase in autophagy was reflected by elevated expression of Beclin 1 and higher LC3-II/I ratio.³⁷ Indirect evidence that autophagy plays an important role in IPC also comes from studies performed in senescent animals. It is generally accepted that cardioprotection by IPC is impaired in these animals.^{38, 39} However, after two weeks of CR, a strong inducer of autophagy was found to restore the cardioprotective effects of IPC in aged rats.¹² CR also improved left ventricular function after acute MI in young and old animals. CR also increased phosphorylation of AMPK, which regulates autophagy as well as other potentially cardioprotective pathways.¹²

There are only limited data regarding the role of autophagy in preconditioning by pharmacological agents. We showed that autophagy is necessary for sulfaphenazole-induced cardioprotection.⁴⁰ Rapamycin limited acute necrotic cell death and decreased infarct size in isolated mouse heart, although autophagy was not assessed.²⁹ In swine, chloramphenicol administration before ischemia or at reperfusion afforded profound cardioprotection and was accompanied by elevated LC3 and Beclin 1 levels.⁴¹ Other agents such as biguanides induce cardioprotection and may involve induction of autophagy through activation of AMPK. The AMPK pathway can induce autophagy through direct phosphorylation of Ulk1.⁴² AMPK deficiency increased myocardial I/R injury as shown in mice with cardiomyocyte specific overexpression of a mutant alpha2 subunit of AMPK.⁴³ Maintaining AMPK phosphorylation by the expression of constitutively active protein C also reduced infarct size.⁴⁴ In C57BL/6J mice subjected to I/R, Matsui *et al.* found that AMPK was phosphorylated during ischemia and dephosphorylated at reperfusion in parallel with activation and suppression of autophagy. Autophagy during reperfusion, however, was accompanied by upregulation of Beclin 1. In mice expressing a dominant negative form of the AMPK protein they observed that ischemia-induced autophagy was attenuated.⁴⁵ This suggests that autophagy may be mediated through AMPK-dependent mechanisms during ischemia, but through AMPK-independent mechanisms upon reperfusion. The importance of AMPK has been further elucidated in experiments where AMPK was modulated by metformin, AICAR and rosiglitazone. In Langendorff-perfused rat hearts metformin pretreatment reduced infarct size and improved heart function.⁴⁶ In the same model metformin and AICAR given at reperfusion decreased infarct size.⁴⁷ Mice receiving the AMPK-activator rosiglitazone at reperfusion also showed reduced infarct size.⁴⁸ These findings suggest that activation of AMPK pathway before or after an acute ischemic insult

plays a significant role in cardioprotection and is related to the induction of autophagy. It is important to note, however, that the AMPK system modulates many metabolic pathways⁴⁹ which may also contribute to its cardioprotective effects. Other autophagy-related protective pathways include sequestration of damaged mitochondria via mitophagy, production of amino acids and other metabolites for energy production, and proton pumping into lysosomes to limit cytosolic acidification.

Remodeling

Cardiac autophagy may also play an important role in the setting of chronic ischemia and adverse remodeling. Metformin and AICAR have been reported to have a salutary effect in heart failure models as evidenced by an improvement in cardiac function and a reduction in pathological remodeling.^{50, 51} Rats treated with rapamycin for 4 weeks after MI exhibited less cardiac dysfunction, lower levels of atrial natriuretic factor, a marker of remodeling processes, and smaller infarct size. In the border zone of the infarction autophagy was induced and apoptosis was diminished.⁵² Interestingly, rapamycin treatment initiated 28 days after the induction of MI also attenuated pathologic remodeling and improved heart function.⁵² In mice, rapamycin significantly mitigated post-infarction left ventricular dilatation and both systolic and diastolic dysfunction. When bafilomycin A1 was used to arrest autophagy these beneficial effects were lost.⁵³ It has also been reported that disruption of autophagy by cardiac specific deletion of *atg5* leads to enhanced ER stress, contractile dysfunction, heart failure and cardiac hypertrophy.⁵⁴

Metabolic Syndrome

There are substantial differences between genetic and diet-induced models of MetS.^{55, 56} This explains, in part, the seemingly contradictory findings that have been reported regarding the relationship between autophagy and myocardial protection in this setting. For example, *ob/ob* diabetic mice, which bear a mutation in their leptin gene, do not develop dyslipidemia, while *db/db* mice with a leptin receptor deficiency do. Neither strain develops hypertension. Similarly, since leptin supplementation has been shown to induce myocardial autophagy and confer protection against ischemic injury,^{57, 58} it is possible that the results obtained in models with a genetically disturbed leptin system (*e.g. ob/ob, db/db* mice, Zucker diabetic fatty rats) are at times at variance with the findings obtained using diet-induced obesity models. In regards to diet-induced models, a wide spectrum of "high-fat diets" are used in experimental studies and there is considerable variation in duration of the dietary intervention and carbohydrate and lipid content. Thus, it is important to establish whether a diet is isocaloric or hypercaloric and be aware that some, but not all high fat diets result in cardiac dysfunction.^{59, 60} In general, however, it now appears that rat and swine models based on a high carbohydrate, high fat diets are the preferable models for studying MetS since they develop many of the features of MetS that characterize the syndrome in humans.⁶¹ This is not always possible however given the complexity of MetS and the need to elucidate the effects of each of its components on autophagy.

For example, to assess the role of insulin, wild type C57BL/6 mice were placed on a high fat diet (HFD) for 8 weeks to develop obesity. This resulted in increased plasma insulin levels, and decreased insulin sensitivity without changes in fasting blood glucose levels. In these animals, hepatic autophagy was decreased as reflected by lower LC3-II/I ratios and increased Akt phosphorylation levels.¹³ When these animals were rendered hyperglycemic by streptozotocin, however, they exhibited increased hepatic autophagy manifested by an elevation in the LC3-II/I ratio and a decrease in p62 and polyubiquitinated protein levels.¹³ In contrast, 16 week old *ob/ob* mice and C57BL/6 mice placed on a HFD for 5 months had lower levels of hepatic LC3, Beclin 1, Atg5 and Atg7 proteins. These findings are consistent

with the report that Atg7 protein expression is diminished in the liver of 15 week old *db/db* mice⁶² albeit autophagy protein expression levels in C57BL/6 mice rendered diabetic by a HFD were reportedly comparable to the levels measured in non-diabetic mice fed a normal diet.⁶³ C57BL/6 mice on a HFD for 12 weeks that caused hepatic steatosis, however, was associated with increase in hepatic autophagy.⁶⁴ In white adipose tissue of diabetic *db/db* mice, insulin stimulation resulted in a decrease in the phosphorylation of Akt compared to lean animals; although LC3 expression was unchanged.⁶⁵ In omental adipose tissue obtained from obese patients, expression of LC3, p62 and Atg5 proteins was elevated. This increase in LC3 and Atg5 was even greater in patients with insulin resistance.⁶⁶ C57BL/6 mice fed a HFD for 5 months exhibited a marked decrease in Atg5, Atg7 and LC3 protein in the hypothalamus.⁶⁷ Indirect evidence of decreased autophagy has also observed in pancreatic, kidney, liver and hippocampal tissue samples obtained from 19 week-old Zucker diabetic fatty rats manifested by the accumulation of large polyubiquitinated protein aggregates, but not in muscle specimens.⁶⁸ These results highlight the importance of glucose, insulin and lipid homeostasis in the regulation of autophagy in different tissues. Taken together, it would appear that insulin and the duration of hyperglycemia and obesity play an interactive role in modulating autophagy. The challenge is to dissect their various contributions in order to better understand the conditions in which autophagic activation can be optimized.

Much less is known about cardiac autophagy in the setting of MetS. Autophagy was reduced in the hearts of OVE26 diabetic mice as shown by a reduction in LC3-II. This reduction was abrogated by chronic treatment with the oral hypoglycemic agent metformin.¹⁷ In contrast, autophagy was upregulated in a C57BL/6J mouse model of pressure overload with transverse aortic constriction for 4 weeks. Whether this represents an adaptive or maladaptive process due to an accumulation of LC3-II as a result of impaired flux or an increase in autophagy per se is not known.⁶⁹ It does appear however, that the mammalian target of rapamycin (mTOR)-pathway plays an important role in the regulation of autophagy in MetS (Figure 1). Insulin-induced phosphorylation of Akt, an upstream regulator of the mTOR pathway, is blunted in obese, hyperlipidemic and hypertensive rats fed a high cholesterol-high fructose diet for 15 weeks.⁷⁰ Hyperactive cardiac mTOR signaling has been reported in Yucatan minipigs fed a hypercholesterolemic diet for 4 weeks that were not yet obese but had elevated total plasma cholesterol levels and LDL/HDL ratios. In these animals, mTOR signaling was assessed by measuring Akt and mTOR phosphorylation and upregulation of raptor.⁷¹ Similarly, Glazer *et al.* linked dyslipidemia to the dephosphorylation of AMP-activated protein kinase (AMPK), another known activator of autophagy,⁷¹ a finding consistent with a study reported in mice fed a HFD for 6 weeks.²⁷ Although most of these upstream pathways show changes that suggest cardiac autophagy is suppressed in MetS and is related to mTOR hyperactivity, direct measurements of mTOR activation and the inducibility of autophagy and autophagic flux have yet to be performed. Nevertheless, just as MetS may suppress autophagy through direct signaling and downregulation of Atg7 through excessive mTOR signaling, advanced age is also associated with downregulation of rate-limiting autophagy proteins in aged animals and results in impaired autophagy/mitophagy. Thus, both aging and MetS may contribute to ischemia/reperfusion injury and post-infarction remodeling as a result of dysregulation of autophagy machinery.⁷²

Consequences of Impaired Autophagy

Insufficient autophagy as seen in many MetS-like conditions may also result in impaired quality control of organelles such as mitochondria and endoplasmic reticulum (ER) and exacerbate their dysfunction. Mitophagy, the selective removal of damaged or dysfunctional mitochondria, is required for normal metabolism and heart function.^{73, 74} Dysfunctional mitophagy may explain why mitochondrial dysfunction is associated with insulin resistance

in various tissues including skeletal muscle, liver, fat, heart, and pancreas.^{75–81} Similarly markers of ER stress in the liver are increased in *ob/ob* mice.⁶² These observations are consistent with the notion that mitochondrial impairment leads to increased oxidative stress,⁸² higher levels of ROS,⁸³ reduced antioxidant capacity⁸⁴ and decreased fatty acid oxidation⁸⁵ and that ER stress results in unfolded protein response⁸⁶ and disturbed lipid metabolism.⁸⁷ In summary, various conditions associated with MetS appear to interfere with autophagy and conversely its dysregulation may contribute to the adverse energy household that exists in MetS. The relative contributions of the individual components of the MetS and/or their interactions as it relates to the modulation of autophagy however remain to be determined.

Cardioprotection, Autophagy, and Metabolic Syndrome

The degree to which MetS or its components contribute to the injury after an acute and chronic ischemic insult is unknown. For the most part, however, the efficacy of cardioprotective interventions appears to be diminished in the presence of pathological conditions associated with MetS such as obesity, diabetes or dyslipidemia.^{14, 19, 25, 88} A majority of the studies indicate that the cardioprotection conferred by IPC is lost or diminished in diabetic dogs,²² sheep,⁸⁹ rabbits,⁹⁰ rats^{20, 91} and humans.^{92, 93} There is also evidence that IPC protection in diabetes can be restored by increasing the intensity of the IPC stimulus.⁹⁴ Similarly, dyslipidemia has been reported to abrogate the cardioprotection afforded by IPC. In rats fed a HFD⁹⁵ or high cholesterol diet,⁹⁶ IPC failed to reduce infarct size. The effectiveness of late phase IPC also appears to be lost in cholesterol-fed rabbits.⁹⁷ With respect to ischemic postconditioning (IPoC), the findings are less clear: in some cases dyslipidemia is associated with a blunting of IPoC,²⁴ in others it has been reported to be effective.²⁵ In *ob/ob* mice²⁶ and in WOKW rats, cardioprotection by IPC is lost, possibly due to decreased phosphorylation of GSK3 β ⁹⁸ or AMPK.²⁶ In rats, cholesterol feeding interfered with cardioprotection afforded by pharmacologic conditioning by a cGMP analogue or by BNP.⁹⁹ Taken together, these reports suggest a correlation between the loss of various cardioprotective interventions and the presence of various components of MetS. A cause and effect relationship however has yet to be demonstrated.

With respect to insulin resistance and diabetes, it was recently reported there was no change in basal cardiac autophagy 4 weeks after an MI induced by permanent coronary occlusion in diabetic mice.⁶³ This suggests that modulation of autophagy might not be an effective method for salvaging diabetic myocardium. This is at variance however, with other reports that autophagic activation is beneficial. In OVE26 diabetic mice, which display reduced basal cardiac LC3-II expression, chronic metformin treatment attenuated AMPK dephosphorylation and alleviated cardiac dysfunction and severe cardiomyopathy. The beneficial effects paralleled the inhibition of mTOR signaling and suggest that activation of autophagy played an important role.¹⁷ In the same study, treatment with metformin improved cardiac function and reduced mortality in streptozotocin-induced diabetic mice. The salutary effects of metformin on cardiac function were abrogated in mice expressing dominant negative AMPK α 2.¹⁷ Likewise, the administration of rosiglitazone, which augments Akt phosphorylation, reduced infarct size in Zucker diabetic fatty rats.¹⁰⁰ Treatment with metformin, an AMPK activator, reduced infarct size in diabetic Goto-Kakizaki rats which were dependent upon Akt activity as well.¹⁰¹ Metformin as an acute pretreatment or given at reperfusion to diabetic *db/db* mice also has been shown to protect the myocardium against I/R injury.¹⁰² Other inducers of AMPK such as A-769662¹⁰³ and the globular domain of adiponectin¹⁹ also proved to be cardioprotective. Resveratrol, a natural phenolic compound, has been reported to be cardioprotective in a number of animal models.¹⁰⁴ Reportedly, it activates autophagy,¹⁰⁵ via activation of AMPK.¹⁰⁶ In Yorkshire miniswine on high cholesterol diet for 11 weeks, which induced glucose tolerance,

dyslipidemia and hyperglycemia, resveratrol reversed the detrimental effects of 7 weeks ameroid constriction of the left circumflex coronary artery on myocardial function, while restoring glucose transporter-1 expression, decreasing PPAR gamma levels and increasing AMPK phosphorylation.¹⁰⁷ These findings suggest that polyphenols may render the heart resistant against ischemic injuries in MetS disease-like models. Additional studies, however, are needed to confirm that resveratrol is cardioprotective and its effects are mediated by autophagy.

Therapy for MetS

Since MetS is a risk factor for heart disease and numerous studies show worse prognosis of and I/R injury in MetS-related conditions, a therapeutic goal could be to normalize preexisting metabolic abnormalities. Physical exercise and moderation of food intake, (calorie restriction or fasting) are potent inducers of autophagy in animal models.¹⁰⁸ Should this be the case in humans, these lifestyle changes could help in moderating both the causes and consequences of MetS and render patients less susceptible to I/R injury.

Pharmacological approaches could include the administration of hypoglycemic agents, such as metformin, which have been shown to enhance to enhance autophagy. The majority of the existing data imply that activation of AMPK and its downstream targets by these biguanides renders the heart more resistant against I/R injury. Their cardioprotective effects may be related to the induction of autophagy.^{17, 46, 50, 101, 102} The thiazolidinedione rosiglitazone and pioglitazone which bind to PPAR gamma and improve insulin resistance have also been shown to activate autophagy, which may be mediated by the induction of the AMPK pathway. They have been shown to induce cardioprotection in non-diabetic and MetS animal models.^{48, 100}

It is well known that the use of statins in diabetic patients with ischemic heart disease has been reported to decrease the risk of death.¹⁰⁹ Interestingly, simvastatin was found to limit infarct size in diabetic-hypercholesterolemic rats subjected to I/R injury.¹¹⁰ A possible mechanism of action may be related to the activation of autophagy that has been observed in various cell types.^{111–113} However, evidence is lacking in intact animals or humans that cardioprotection conferred by statins is related to their effect on autophagy in the setting of MetS.

Another agent that has been shown to mimic both acute (early) and delayed (late phase) preconditioning is the nucleoside adenosine.¹¹⁴ It has been shown to have multiple beneficial effects during myocardial ischemia.¹¹⁵ We have shown that autophagy is required for the cardioprotection exhibited by a selective adenosine A1 receptor agonist,¹¹⁶ although its efficacy is yet to be tested in conditions mimicking MetS. Since adenosine receptor signaling mechanisms may interact with the AMPK pathway, there may be a role for the use of A1 agonists in patients with MetS, similar to that of the biguanides.

The mTOR pathway is a target for treatment of a variety of malignancies. Rapamycin analogues have been used with success in the prevention of in-stent coronary artery neointimal hyperplasia.¹¹⁷ However, its use in the setting of acute I/R injury has not been extensively studied, although there are a few reports that the inhibition of the mTOR pathway results in diminution of ventricular remodeling after I/R injury.^{21, 52, 53} Given the potential involvement of the mTOR pathway in the attenuation of cardioprotection in dyslipidemic animals,^{70, 71} the use of mTOR inhibitors may be useful for the treatment of adverse post-myocardial infarction remodeling in humans with MetS.

Conclusion

In summary there is persuasive evidence that some or all of the components of the metabolic syndrome adversely affect cardiac autophagy, its upstream signaling pathways, and the heart's endogenous response to ischemia-reperfusion injury. While the mechanisms have yet to be identified, the elucidation of the role of autophagy in ischemia-reperfusion injury and MetS could lead to the development of more effective strategies to treat myocardial infarction, adverse post-infarction remodeling and heart failure in patients with dyslipidemia, insulin resistance, and obesity.

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Figure 1. Autophagy and signaling pathways

Insulin signaling activates the mTOR pathway via Akt and thus suppresses autophagy. The mTOR inhibitor rapamycin and its derivatives enhance autophagy. The AMPK pathway modulates the mTOR pathway via multiple mechanisms including interaction with Akt, TSC1, mTOR and Ulk1. Biguanides such as metformin and phenformin, and thiazolidinediones (TZDs), rosiglitazone and pioglitazone, enhance AMPK signaling.