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Myocardial autophagic energy stress responses—macroautophagy, mitophagy, and glycophagy

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Delbridge LM, Mellor KM, Taylor DJ, Gottlieb RA. Myocardial autophagic energy stress responses—macroautophagy, mitophagy, and glycophagy. Am J Physiol Heart Circ Physiol 308: H1194–H1204, 2015. First published March 2, 2015; doi:10.1152/ajpheart.00002.2015. —An understanding of the role of autophagic processes in the management of cardiac metabolic stress responses is advancing rapidly and progressing beyond a conceptualization of the autophagosome as a simple cell recycling depot. The importance of autophagy dysregulation in diabetic cardiomyopathy and in ischemic heart disease—both conditions comprising the majority of cardiac disease burden—has now become apparent. New findings have revealed that specific autophagic processes may operate in the cardiomyocyte, specialized for selective recognition and management of mitochondria and glycogen particles in addition to protein macromolecular structures. Thus mitophagy, glycophagy, and macroautophagy regulatory pathways have become the focus of intensive experimental effort, and delineating the signaling pathways involved in these processes offers potential for targeted therapeutic intervention. Chronically elevated macroautophagic activity in the diabetic myocardium is generally observed in association with structural and functional cardiomyopathy; yet there are also numerous reports of detrimental effect of autophagy suppression in diabetes. Autophagy induction has been identified as a key component of protective mechanisms that can be recruited to support the ischemic heart, but in this setting benefit may be mitigated by adverse downstream autophagic consequences. Recent report of glycophagy upregulation in diabetic cardiomyopathy opens up a novel area of investigation. Similarly, a role for glycogen management in ischemia protection through glycophagy initiation is an exciting prospect under investigation.

autophagy; heart; cardiac; diabetes; ischemia; cardiomyocyte

SUGAR ISN’T ALWAYS SWEET,
When your heart is worn and skips a beat
Membranes keep it in or out
And insulin gives a special route
Glut transporters form a pore
Bringing sugar in the open door
Too much is bad-too little, too:
Cells need the proper fuel.
Inside the cell sugar’s stored
Glycogen the sweetest hoard.
Two enzymes live to break it down:
A neutral enzyme can be found
In cytosol where granules roam
But in the acidic lysosome
Another waits on bended knee
To play its role in glycophagy.
Excess carbs are bad: this much is clear.
So consider maltose when quaffing beer!

Roberta A. Gottlieb

Energy Stress and Autophagy Induction Overview

Autophagy is an intracellular degradation and recycling function to support energy homeostasis and/or to eliminate defective cell components. Autophagy is upregulated with cellular stress and in the heart was first identified four decades ago to be induced by ischemia (37) and starvation (8), and later determined to be critical for neonatal cardiac tissues during the period of temporary nutrient deprivation accompanying post-natal transition (45). Although upregulated expression of autophagy mediators can be associated with acute performance improvement post-ischemia (24, 35), a sustained elevation of autophagy has been linked with transition to cardiac failure in vivo (112). The general consensus is that autophagy can be beneficial and pro-survival as a short-term strategy to deal with acute stress, but when chronically elevated or constitutive, excess autophagic activity is linked to cell death, stimulating cytokine-mediated collagen infill responses (31, 66, 81). The signaling complexity regulating autophagy in acute and chronic conditions under various trophic and endocrine settings is yet to be fully elucidated. Involvement from PI3K/Akt/mTOR (60, 77) and AMPK (100) pathways in regulating...
autophagy has been identified in diabetic cardiomyopathy and may play a role in other cardiac pathological settings.

The term autophagy has been considered synonymous with the term macroautophagy (implying a single macromolecular degradative pathway). Macro-autophagy machinery has been relatively well characterized and involves assembly of a membrane structure and parallel enzymatic conjugations analogous to ubiquitin ligation, resulting in conjugation of Atg12 onto Atg5, and subsequent conjugation of Atg8 proteins (e.g. LC3, GABARAPL1, GABARAP) onto phosphatidylethanolamine in the phagophore membrane (additional details provided in Fig. 1). Engulfment of selective cargo requires adaptor proteins, which bridge Atg8 proteins and the target. More recently, a new understanding of selective autophagy processing has emerged, with specific protein machinery recognized for autophagic degradation of mitochondria [mitophagy (22, 33)], endoplasmic reticulum [reticulophagy or ERphagy (26)], peroxisomes [pexophagy (89)], lipid droplets [lipophagy (52)], and glycogen [glycophagy (39, 76)]. In this context, the term macroautophagy is best used to denote the process of general autophagic degradation of heterogeneous macromolecules. A case can now be made that the autophagic processes delineated by the commonly used markers LC3B and p62 (and possibly Beclin-1) are predominantly those involved in macroautophagic breakdown of protein aggregates and some organelles (including mitochondria). Identification of the machinery specific to each autophagic pathway has enabled new tools for investigation of selective autophagy, and new insights are emerging in relation to their differential roles in cardiac pathology.

This review examines the role of autophagy in myocardial energy stress settings, most particularly in diabetic/insulin resistant and ischemic states. We focus on current questions relating to macroautophagy/glycophagy signaling pathways in these contrasting settings, and consider the interpretation of disparate findings of autophagy occurrence in similar disease models. Finally, prospects for targeted manipulation of specific autophagic processes of potential therapeutic value are highlighted.

Diabetes: An Energy Access Denial in the Myocardium

In diabetes, cardiac complications are evident even in the absence of vascular abnormalities and represent a primary manifestation of the disease. Diastolic abnormality is an early sign of diabetic cardiomyopathy, linked with altered metabolism and energetics (25, 51, 74, 91). At a molecular level, suppression of the PI3K/Akt insulin signaling pathway, either due to insulin ligand deficiency (type 1 diabetes, or T1D) or due to tissue insulin resistance (type 2 diabetes, or T2D), by 10.220.33.6 on May 8, 2017 http://ajpheart.physiology.org/ Downloaded from
results in reduced GLUT4 glucose transporters in the plasma membrane and limited glucose uptake (10). These glucose handling abnormalities may underlie the observed metabolic shift decreasing carbohydrate oxidation with associated increase in fatty acid oxidation for energy supply (95). Paradoxically, diabetic cardiomyocytes exhibit an accumulation of glycogen, despite suppressed glucose uptake. In contrast with other tissues (i.e., skeletal muscle, liver), the heart uniquely increases glycogen content in periods of energy deprivation (e.g., fasting) (76), perhaps as a protective mechanism to mobilize glucose from nonessential tissues and elevate cardiac stores. In diabetes, the intracellular glucose deprivation conferred by impaired glucose transport (albeit in the context of extracellular fuel overabundance) may trigger a pseudo fasted heart phenotype. Energy stress is also a potent activator of cardiac autophagy in vivo and in isolated cardiomyocytes (3, 42, 76). The role of autophagy in the diabetic heart is emerging as an important research question. Reports to date provide interesting and discrepant findings, and these are evaluated in detail below.

**New insights into the role of autophagy in the diabetic heart.** Since our first report of increased cardiac autophagy in the type 2 diabetic fructose-fed mouse (59), the experimental literature in this field has expanded considerably, but it is not yet possible to synthesize a comprehensive understanding. Relying on a variable selection of molecular tools, states of increased (4, 12, 49, 50, 59, 61, 78, 93, 102), unchanged (47, 48, 57, 62), and decreased (6, 23, 28, 29, 73, 82, 101, 103, 104, 110) basal cardiac autophagy activity have all been reported in diabetic/insulin resistant contexts (see Table 1). These discrepancies are not necessarily attributable to the different models of diabetes, since contrasting findings have been observed within the same diabetic model [e.g., STZ-mouse: increased LC3BII (12) vs. decreased LC3BII (103)]. Duration/severity of diabetes is also unable to explain the variable findings [e.g., high fat-fed mouse 18–20 wk duration: increased LC3BII (102) vs. decreased LC3BII (82)]. New work involving evaluation of autophagic flux will be important in gaining mechanistic understanding to resolve these variable findings, and we have recently highlighted the importance of flux measurement in relation to interpreting data from a dietary intervention model (21). Suppressed insulin signaling through the PI3K/Akt/mTOR pathway is a common observation in most diabetic models due to either lower insulin ligand stimulation of the receptor (T1D) or tissue insulin resistance (T2D). Downregulation of this pathway would be expected to be linked to upregulated autophagy as inhibition via mTOR would be relieved (40). Conversely, downregulation of AMPK signaling, also commonly observed in the diabetic heart, would be expected to suppress autophagy activity (11, 27, 108). Thus the autophagy outcome may reflect the net effect of these opposing autophagy regulators and depend on the extent of downregulation of Akt versus AMPK signaling (as shown in Fig. 2). As summarized in Table 1, suppressed cardiac autophagy in diabetes is often observed coincident with decreased AMPK activity, despite variable findings relating to Akt (29, 82, 103, 110). The absence of both Akt and AMPK data in many of these diabetes-autophagy studies limits these conclusions, and new studies are required to elucidate the role of autophagy in the diabetic heart.

**A role for glycophagy in the heart.** Recently we have demonstrated that the autophagic/lysosomal glycogen degradation pathway (glycogen-specific autophagy, ‘glycophagy’) is operational in the adult heart and is upregulated by diabetes in vivo (61, 76). Glycophagy is well characterized in the newborn liver and heart where a transient upregulation of lysosomal acid α-glucosidase mediates an increase in autophagic glycogen degradation (43). In this setting autophagic glycogen breakdown is thought to provide glycolytic substrate support during the placental-lactational transition. This bulk glycogen degradation pathway is understood to operate in parallel with the well-described conventional kinase-regulated process of glycogen breakdown by glycogen phosphorylase. In that pathway, Akt via GSK3β mediates glycogen synthase activation, whereas PKA activation via glycogen phosphorylase kinase mediates glycogen phosphorylase activation (as shown in Fig. 3). Thus glycogenolysis may take place in the cytosol at neutral pH or in the lysosome at acidic pH, mediated by distinct enzymes. Lysosomal glycogen degradation is mediated by the acid α-glucosidase, which possesses glycogen, maltose, and isomaltose hydrolyzing activities (43). Glucose liberated from glycogen degradation exits lysosomes via the lysosomal membrane glucose carrier (55). The glycogen hydrolyzing activity of acid α-glucosidase is enhanced by Ca²⁺, which in the setting of increased cytosolic Ca²⁺ in the ischemic myocardium, in concert with AMP-activated augmented lysosomal Ca²⁺ uptake, may prime lysosomes for accelerated glycogen degradation (41, 79). There is strong evidence from glycogen storage diseases that autophagic/lysosomal glycogen breakdown is important for maintaining normal cardiac glycogen levels and does not simply constitute a redundant alternative breakdown route for glycogen (68). Deficiency of functional lysosomal acid α-glucosidase as observed in Pompe disease results in the accumulation of glycogen-filled lysosomes in multiple tissues including the heart (75), indicating that lysosomal degradation of glycogen is essential in normal physiology. Glycophagy may provide a quantitatively different route of glycogen breakdown in comparison with phosphorylase-mediated degradation, facilitating large glucose release in settings of high energy demand. Glycogen destined for autophagic/lysosomal degradation may also be qualitatively different to that degraded in the cytosol, thus necessitating a different mode of degradation. For example, abnormally branched, insoluble, and/or hyperphosphorylated glycogen may impede phosphorylase action and favor recruitment to the glycophagosome.

Starch binding domain protein 1 (STBD1) is a likely candidate for facilitating glycogen sequestration into the glycophagosome. STBD1 contains both a carbohydrate binding domain and an Atg8 interacting motif (39). Proteomics analysis has demonstrated that STBD1 is localized to the glyogen particle (86). In cell lines overexpressing HA-tagged STBD1, colocalization (via immunostaining) and interaction (via co-immunoprecipitation) of STBD1 and the autophagy (Atg8) protein, GABARAPL1, has been observed (38, 39). STBD1 colocalization with the lysosomal protein, LAMP1, has also been reported (38). Thus STBD1 may play a role in intracellular trafficking of glycogen to the autophagosome by binding to GABARAPL1 on the autophagosome membrane, constituting specific autophagy machinery for glycophagy. We have demonstrated that STBD1 does not colocalize with the macroautophagy Atg8 protein, LC3B, in cultured cardiomyocytes, suggesting that the glycophagy process is distinct from conventional macroautophagy (Fig. 4) (61). Visualization of...
STBD1 and GABARAPL1 colocalization has been demonstrated in a noncardiac cell line, and further information is required regarding the direct/indirect interaction of these two proteins in vitro in cardiomyocytes. It is likely that, in pathological settings, selective autophagy pathways play differential roles and identification/characterization of these specific pathways may lead to more targeted intervention opportunities.

Glycogen flux in the diabetic myocardium. Glycogen pathology in human diabetic myocardium was first reported over 80 years ago (96). This is a replicated finding in many diabetic experimental settings (2, 46, 84) and occurs in parallel to a depletion of glycogen stores in the liver and skeletal muscle (30, 85, 87). The fundamental bases for differential glycogen storage diseases have provided considerable new insight into the role of disrupted glycogen handling in the etiology of cardiac pathology. In these diseases, glycogen disturbance results in contractile dysfunction, derangement of myofibrillar and cytoskeletal proteins, arrhythmia, and hypertrophy (63). Presentation of vacuole-filled glycogen granules in cardiomyocytes

Table 1. Literature reports of autophagy in the insulin resistant or diabetic heart

<table>
<thead>
<tr>
<th>Autophagy</th>
<th>Model</th>
<th>Autophagy Measurement</th>
<th>Duration of Diabetes</th>
<th>Akt Activity</th>
<th>AMPK Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ Fructose-fed mouse</td>
<td>T2D</td>
<td>LC3B/I ratio, Beclin1, p62 protein</td>
<td>12 wk</td>
<td>↓</td>
<td>↑</td>
<td>59</td>
</tr>
<tr>
<td>↑ Goto-Kakizaki rat</td>
<td>T2D</td>
<td>LC3B/I ratio, Beclin1, p62 protein</td>
<td>18 wk old</td>
<td>nr</td>
<td>↑</td>
<td>50</td>
</tr>
<tr>
<td>↑ STZ-mouse (5 inj)</td>
<td>T1D</td>
<td>LC3I/I ratio, p62, Beclin1 protein</td>
<td>16 wk</td>
<td>nr</td>
<td>nr</td>
<td>93</td>
</tr>
<tr>
<td>↑ STZ rat (1 inj)</td>
<td>T1D</td>
<td>LC3* puncta</td>
<td>4 wk</td>
<td>nr</td>
<td>nr</td>
<td>49</td>
</tr>
<tr>
<td>↑ High-fat fed mouse (ins res)</td>
<td>LC3B/I ratio</td>
<td></td>
<td>8 wk</td>
<td>↓</td>
<td>nr</td>
<td>61</td>
</tr>
<tr>
<td>↑ High-fat fed mouse (ins res)</td>
<td>LC3* puncta</td>
<td></td>
<td>4 wk</td>
<td>nr</td>
<td>nr</td>
<td>49</td>
</tr>
<tr>
<td>↑ High-fat fed mouse (ins res)</td>
<td>LC3I/I ratio, Beclin1 protein</td>
<td></td>
<td>8 wk</td>
<td>↑</td>
<td>nr</td>
<td>78</td>
</tr>
<tr>
<td>↑ STZ mouse (x1 inj)</td>
<td>T1D</td>
<td>LC3B/I ratio</td>
<td>7 wk</td>
<td>nr</td>
<td>nr</td>
<td>12</td>
</tr>
<tr>
<td>↑ T2D humans</td>
<td>T2D</td>
<td>LC3I/I ratio</td>
<td>7–20 yr</td>
<td>nr</td>
<td>nr</td>
<td>62</td>
</tr>
<tr>
<td>↑ High-fat fed rat</td>
<td>ins res</td>
<td>LC3I/I ratio, Beclin1, LAMP1 protein</td>
<td>10 wk</td>
<td>nr</td>
<td>nr</td>
<td>57</td>
</tr>
<tr>
<td>↑ High fat-STZ rat</td>
<td>T2D</td>
<td>LC3I/I ratio, Beclin1, LAMP1 protein</td>
<td>8 wk</td>
<td>nr</td>
<td>nr</td>
<td>57</td>
</tr>
<tr>
<td>↑ High-fat fed mouse (ins res)</td>
<td>LC3I/I ratio, p62 mRNA</td>
<td></td>
<td>10–12 wk</td>
<td>nr</td>
<td>nr</td>
<td>47</td>
</tr>
<tr>
<td>↔ pancreatocentomy rats</td>
<td>T1D</td>
<td>LC3I/I ratio, p62 protein</td>
<td>11 wk</td>
<td>↑</td>
<td>nr</td>
<td>48</td>
</tr>
<tr>
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<td>LC3I/I ratio, Beclin1 protein</td>
<td></td>
<td>20 wk</td>
<td>↑</td>
<td>nr</td>
<td>104</td>
</tr>
<tr>
<td>↓ High-fat fed mouse (ins res)</td>
<td>LC3B/I ratio, Beclin1 protein</td>
<td></td>
<td>22 wk</td>
<td>nr</td>
<td>↓</td>
<td>23</td>
</tr>
<tr>
<td>↓ High-fat fed mouse (ins res)</td>
<td>LC3B/I ratio</td>
<td></td>
<td>8 wk</td>
<td>nr</td>
<td>↔</td>
<td>6</td>
</tr>
<tr>
<td>↓ db/db mouse</td>
<td>T2D</td>
<td>LC3I/I ratio (ns), p62 protein</td>
<td>12 wk</td>
<td>nr</td>
<td>nr</td>
<td>28</td>
</tr>
<tr>
<td>↓ High-fat fed mouse (ins res)</td>
<td>LC3I/I ratio, Beclin1, Atg12-Atg5 protein</td>
<td></td>
<td>14–18 wk old</td>
<td>↓</td>
<td>↔</td>
<td>73</td>
</tr>
<tr>
<td>↓ STZ mouse (x1 inj)</td>
<td>T1D</td>
<td>LC3I/I protein, Beclin1, Atg12-Atg5 protein</td>
<td>18–20 wk</td>
<td>↑</td>
<td>↓</td>
<td>82</td>
</tr>
<tr>
<td>↓ STZ mouse (x5 inj)</td>
<td>T1D</td>
<td>LC3I/I protein</td>
<td>6, 9, 12 wk</td>
<td>↑</td>
<td>↓</td>
<td>103</td>
</tr>
<tr>
<td>↓ STZ mouse (x5 inj)</td>
<td>T1D</td>
<td>LC3I/I protein</td>
<td>8 wk</td>
<td>↓</td>
<td>↓</td>
<td>110</td>
</tr>
<tr>
<td>↓ STZ mouse (x5 inj)</td>
<td>T1D</td>
<td>LC3I protein</td>
<td>16 wk</td>
<td>↓</td>
<td>↓</td>
<td>29</td>
</tr>
<tr>
<td>↓ OVE26 mouse</td>
<td>T1D</td>
<td>LC3I/I protein</td>
<td>16 wk</td>
<td>nr</td>
<td>↓</td>
<td>101</td>
</tr>
<tr>
<td>↓ Autophagic vacuoles (EM)</td>
<td>Beclin1 staining (IHC)</td>
<td></td>
<td></td>
<td>↑</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>↓ db/db mouse</td>
<td>T2D</td>
<td>LC3I/I protein (+ Baf, sample blot)</td>
<td>10–14 wk old</td>
<td>nr</td>
<td>nr</td>
<td>57</td>
</tr>
<tr>
<td>Ambiguous</td>
<td>High-fat fed mouse (ins res)</td>
<td>LC3B/I, Beclin1 protein</td>
<td>20 wk</td>
<td>↑ tAkt2</td>
<td>↓</td>
<td>102</td>
</tr>
</tbody>
</table>

nr, not reported; STZ, streptozotocin; T2D, type 2 diabetes; T1D, type 1 diabetes; Atg, autophagy; ns, not significant; ins res, insulin resistance; LAMP, lysosome associated membrane protein 1; Baf-Con, bafilomycin-control; EM, electron microscopy; CQ, chloroquine. ↑, Diabetes-induced increase; ↓, diabetes-induced decrease; ↔, no effect of diabetes. *Personal communication; *LC3 isofom nonselective antibody used. All data presented are obtained in the absence of lysosomal inhibition unless otherwise noted.
supports a role for glycophagy in these settings (32). Glycogen handling dysregulation in diabetes may similarly reflect a storage anomaly.

In diabetes, disturbances in insulin signaling and glucose handling may underlie cardiac glycogen pathology. In cultured neonatal rat cardiomyocytes, protein expression of the glycophagy marker STBD1 was increased by extracellular insulin concentration linked with activation of the insulin-regulated PI3K/Akt signaling pathway (61). Similarly, fasting-induced upregulation of STBD1 and GABARAPL1 protein content was associated with activation of Akt in vivo (76). In cultured cardiomyocytes, it has been demonstrated using chromatin immunoprecipitation that FoxO1 and FoxO3, which are inhibited by Akt, directly bind to the GABARAPL1 promoter in the nucleus and regulate transcription of this glycophagy marker (83). Thus Akt activation may lead to inhibition of FoxO-mediated GABARAP1 transcription, but such a response is difficult to reconcile with the experimental observations that there is parallel upregulation of glycophagy and Akt activity with fasting (76). A more detailed dissection of the signaling nexus of PI3K/Akt and AMPK pathways in mediating glycophagy induction is required to resolve these contrasting observations, in both physiological and pathophysiological settings.

Ischemia-Reperfusion: An Energy Supply Crisis

Role of autophagy in the ischemic and reperfused heart. Autophagy is powerfully upregulated as part of the response to ischemic stress, and it has been suggested that macroautophagy induction is beneficial during ischemia but deleterious during reperfusion (58). We have shown that autophagy is essential for cardioprotection observed with ischemic preconditioning (35), and others have shown its importance in postconditioning (98). In a Langendorff model of ischemia/reperfusion, inhibition of autophagy with the cell-permeable inhibitor (TAT-Ag5K130R) neither increased nor reduced infarct size, suggesting that in the absence of pre- or post-conditioning, the beneficial and deleterious effects of autophagy may be balanced (34). Inhibition of autophagic flux increases hypoxia/reoxygenation injury (109) and blocks the beneficial effects of sevoflurane postconditioning (107). Ischemia/reperfusion injury disrupts autophagosome-lysosome fusion (54), accompanied by a significant increase in Beclin-1. Beclin-1 effects are complex. Beclin-1 plays an essential role in autophagosome initiation for both canonical and noncanonical macroautophagy pathways, but it also partners with Rubicon to interfere with autophagosome-lysosome fusion (111), and binds to Bcl-2, thereby fostering mitochondria-dependent apoptosis (36). The preponderance of evidence supports the notion that autophagy is a protective response to acute ischemia/reperfusion injury, and new studies are required to elucidate the mechanism by which autophagy confers benefit in this setting.

Mitochondrial clearance (mitophagy) and mitochondrial sequestration (lysosomal regulation). Mitochondria represent both a target of injury during ischemia, and a source of tissue damage, via production of reactive oxygen species (ROS), during reperfusion. Clearance of damaged mitochondria by autophagy (mitophagy) may play a beneficial role in the setting of myocardial ischemia/reperfusion. We have demonstrated that ischemic preconditioning ameliorates reperfusion injury by triggering Parkin-dependent mitophagy (33). Given the role of autophagy in liberating metabolic substrates (amino acids, carbohydrates, lipids) during nutrient deprivation, it has been generally assumed that both autophagic flux and lysosomal function are essential for autophagy-mediated cardioprotection. We have provided evidence that sequestration of mitochondria in autophagosomes may mediate the cardioprotective effects of chloramphenicol in the absence of lysosomal function. Chloramphenicol-induced infarct size reduction was preserved when the lysosomal inhibitor chloroquine was administered in isolated ischemia/reperfused rat hearts (19). Although autophagic sequestration of nutrient sources without lysosomal degradation during an episode of metabolic stress seems counterintuitive, it is also a process by which regulated lysosomal function participates in protective energy management.

In contrast, lysosomal function appears to play an important role in cardioprotection in other contexts. Enhanced lysosome biogenesis was shown to attenuate apoptosis mediated by overexpression of BNIP3 in neonatal rat cardiomyocytes (53). Increased lysosome biogenesis (via cobalt protoporphyrin treatment) contributes to autophagy-mediated cardioprotection in sepsis (90). It is noteworthy that mTOR is associated with the lysosome, and during starvation, mTOR is inactivated until amino acids derived from the lysosome restore mTORC1 signaling (106), enabling new protein synthesis required for cellular repair processes. It may be that sequestration of mitochondria (or other cellular components) into autophagosomes is protective as a short-term strategy to minimize cellular injury in ischemia-reperfusion, and delayed/inhibited lysosomal degradation slows the negative feedback on autophagy via mTOR.
The serine/threonine kinase GSK3β has been extensively investigated in the past decade with respect to its role in cardioprotection. Ser9 phosphorylation of GSK3β is considered to exert its cardioprotective effects through inhibition of mitochondrial permeability transition pore (mPTP) opening. The precise nature of the GSK3β-mPTP interaction is unclear; however, several mechanisms have been proposed including preservation of hexokinase II in the mPTP complex (72), suppression of the interaction between adenine nucleotide translocase and cyclophilin D (67), and inhibition of p53 (97). GSK3β inhibition has been implicated in the cardioprotection mediated by a multitude of agents and therapeutic strategies (13, 20, 70, 92); however, little attention has been given to the original process from which it derives its name - in regulating glycogen turnover. GSK3β inhibition during ischemia should favor glycogen synthesis and decreased degradation, which in turn may reduce the availability of glycolytic substrate, the oxidation of which promotes ischemic acidosis and worsened reperfusion injury. Although a role for GSK3β in regulating glycophagy is speculative at this time, two separate reports have recently demonstrated that GSK3β-inhibition promotes lysosomal biogenesis (56, 71). If GSK3β inhibition in preconditioned hearts promotes lysosomal biogenesis, this may hypothetically prime the cell for enhanced autophagic glycogen disposal. Similarly GSK3β has been shown to regulate autophagic activity in both tumor and nontumor cell lines (18).
Thus it should be noted that pharmacologic agents that target GSK3β (some of which have been proposed as cardioprotective drugs) may at least partially act through a mechanism involving glycogen action.

**Glycogen turnover in the preconditioned heart.** Glycogenolysis contributes significantly to glycolytic substrate supply during ischemia, and a number of studies have sought to determine a relationship between ischemic glycogen consumption and infarct severity (5, 15, 80). Ischemic glycogenolysis may have beneficial effect through increased glycolytic ATP supply or detrimental effect due to enhanced cellular lactate and proton production. Interventions that increase pre-ischemic glycogen content have been shown to be both beneficial and detrimental to the ischemic heart (80). One study demonstrated that separate cardioprotective interventions, which deplete or enhance pre-ischemic glycogen, can confer a similar level of cardioprotection (7). Because any intervention intended to manipulate glycogen levels before ischemia will likely involve parallel activation of signaling pathways related to cardioprotection, delineating the precise contribution of glycogen depletion to ischemic injury is problematic. Indeed, it has recently been reported that ischemic preconditioning and insulin administration independently protected against ischemia/reperfusion injury while having differential impacts on glycogen turnover (17). When both ischemic preconditioning and insulin administration were combined, cardioprotection was abolished.

The role of myocardial glycogen breakdown in the cardioprotection observed with preconditioning is controversial: one study reported that ischemic preconditioning attenuated glycogenolysis in rat hearts (99), whereas another study showed that 24-h in vivo fasting resulted in increased glycogen content and greater glycogen utilization during ischemia/reperfusion in a Langendorff model (80), attributed to activation of phosphorylase a (94). Interestingly, it was also observed that insulin pretreatment increased glycogen content to a similar extent but suppressed glycogen utilization during ischemia/reperfusion, resulting in increased injury and function (80). Fasting upregulates autophagy, whereas insulin treatment suppresses both autophagy and glycogen utilization, which might explain the differential effects. The adenosine A1 agonist CCPA (2-chloro-N6-cyclopentyladenosine) has been shown to increase pre-ischemic glycogen content and suppress glycogen utilization in the first 2–5 min of ischemia, but total glycogen utilization over the ischemic period was increased relative to the ischemic preconditioning group (7). These findings must also be considered in the context of studies showing that adenosine or adenosine A1 agonists inhibited glycolysis and glycolytic proton production yet had no effect on glucose oxidation, resulting in tighter coupling of glycolysis to glucose oxidation (14). In a related study it was observed that A1 agonists promoted glycogen synthesis without affecting glycogen degradation; yet glycogen turnover was accelerated (16). Little information is available to address the question of whether stimulated glycogenolysis occurred in the cytosol or the lysosome. We have demonstrated that cardioprotection by CCPA is dependent on autophagy (105), which would be consistent with lysosomal glycogen breakdown. Although no clear answers have emerged, it may be speculated that autophagic engulfment of glycogen particles (i.e., glycophagy) might sequester glycogen and prevent its utilization during acute ischemic stress, thereby limiting lactic acidosis and risk of Ca²⁺ overload.

**Glycophagy in the ischemic heart.** An issue of major importance relating to ischemic glycogen turnover that has been largely overlooked is the contribution of autophagic (i.e., glycophagic) sequestration of glycogen. Cytosolic glycogen degradation is normally regulated via the actions of the cytosolic phosphorylase and debranching enzymes, and it could be also assumed that some glycogen is constitutively degraded via lysosomes. The advantage of sequestering glycogen into autophagosomes for subsequent delivery to the lysosomes for degradation is not yet understood. Cytosolic glycogen is localized within distinct myocyte subcellular regions, including intermyofibrillar, intramyofibrillar, and subsarcolemmal compartments (65). In stimulated skeletal muscle fibers, rates of glycogen depletion differ depending upon subcellular localization (64). It is conceivable, therefore, that in the setting of local high rates of glycogenolysis, glycophagy may function to supplement the cytosolic machinery and that localized glycophagic hotspots provide important metabolic support under

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**Fig. 5.** Electron micrograph of glycogen and a glycophagosome in an adult rat ventricular cardiomyocyte. Free glycogen particles (short arrowhead) are aligned between myofibrils. Some glycogen particles are seen sequestered in a glycophagosome (arrow). m, Mitochondria. Bar indicates 500 nm.
ischemic stress. In cardiomyocytes we have been able to observe sequestered glycogen particles positioned adjacent to free glycogen particles between myofibrillar bundles (Fig. 5). This suggests the possibility of a differential functional role for glycogen stored intra- and extraphagosomally.

A second possibility is that glycogen destined for autophagic degradation is qualitatively different from normal glycogen and therefore requires a separate means of degradation. Glycogen molecules are heterogeneous in size; an ordered distribution of branch points contributes to the formation of a sphere whereby the nonreducing chain ends are exposed to the cytosol, rendering the molecule soluble (69). In Lafora disease, loss of function of the phosphatase laforin or the E3 ubiquitin ligase malin results in disordered glycogen structure. It is characterized by the accumulation of abnormally branched, hyperphosphorylated glycogen, leading to the formation of insoluble aggregates within the cell (88). Glycogen phosphorylation may compromise glycogen solubility indirectly by interfering with intermolecular bonds between adjacent branches, or directly by preventing branching due to phosphorylation at C6 of the glucose residues. Understanding of how excess glycogen phosphorylation confers macromolecular solubility is limited; however, recent evidence suggests that this phosphorylation is a regulated process (9, 69). Overexpression of laforin has been shown to augment autophagy (1). Whether glycosylation serves to deliver abnormally branched glycogen to the more amenable lysosomal acidic environment to achieve more complete mitochondrial breakdown is a pressing question, and whether this route of degradation assumes more importance in an ischemic context is not yet established.

Conclusions and Directions

An understanding of the role of autophagic processes in the management of cardiac metabolic stress responses is advancing rapidly and progressing beyond a conceptualization of the autophagosome as a simple cell recycling depot. The importance of autophagy dysregulation in diabetic cardiomyopathy and in ischemic heart disease - both conditions comprising the majority of cardiac disease burden - has now become apparent. New findings have revealed that specific autophagic processes may operate in the cardiomyocyte, specialized for selective recognition and management of mitochondria and glycogen particles in addition to protein macromolecular structures. Thus mitophagy, glycosylation, and macroautophagy regulatory pathways have become the focus of intensive experimental effort, and delineating the signaling pathways involved in these processes offers potential for targeted therapeutic intervention. A body of evidence is emerging that suggests that cytosolic autophagosomal sequestering of cellular structures has a role in acute energy management and cell viability preservation. Subsequent metabolic access to sequestered stores appears to rely on more complex lysosomal-autophagosomal regulatory mechanisms yet to be delineated. Chronically elevated macroautophagic activity in the diabetic myocardium is generally observed in association with structural and functional cardiomyopathy; yet there are also numerous reports of detrimental effect of autophagy suppression in diabetes. Autophagy induction has been identified as a key component of protective mechanisms that can be recruited to support the ischemic heart, but in this setting benefit may be mitigated by adverse downstream autophagic consequences. New studies are required in both these disease contexts to undertake detailed mapping of convergent AMPK and PI3K/Akt signaling pathways to identify metabolic leverage points to optimize macroautophagic responses. Recent report of glycophagy upregulation in diabetic cardiomyopathy opens up a new area of investigation. Similarly, a role for glycogen management in ischemia protection through glycophagy initiation is an exciting prospect under investigation.

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DISCLOSURES

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