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Adaptive mechanisms to compensate for overnutrition-induced cardiovascular abnormalities

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Pulakat L, DeMarco VG, Ardhanari S, Chockalingam A, Gul R, Whaley-Connell A, Sowers JR. Adaptive mechanisms to compensate for overnutrition-induced cardiovascular abnormalities. Am J Physiol Regul Integr Comp Physiol 301: R885–R895, 2011. First published August 3, 2011; doi:10.1152/ajpregu.00316.2011.—In conditions of overnutrition, cardiac cells must cope with a multitude of extracellular signals generated by changes in nutrient load (glucose, amino acids, and lipids) and the hormonal milieu [increased insulin (INS), ANG II, and adverse cytokine/adipokine profile]. Herein, we review the diverse compensatory/adaptive mechanisms that counter the deleterious effects of excess nutrients and growth factors. We largely focus the discussion on evidence obtained from Zucker obese (ZO) and Zucker diabetic fatty (ZDF) rats, which are useful models to evaluate adaptive and maladaptive metabolic, structural, and functional cardiac remodeling. One adaptive mechanism present in the INS-resistant ZO, but absent in the diabetic ZDF heart, involves an interaction between the nutrient sensor kinase mammalian target of rapamycin complex 1 (mTORC1) and ANG II-type 2 receptor (AT2R). Recent evidence supports a cardioprotective role for the AT2R; for example, suppression of AT2R activation interferes with antihypertrophic/antifibrotic effects of AT1R blockade, and AT2R agonism improves cardiac structure and function. We propose a scenario, whereby mTORC1-signaling-mediated increase in AT2R expression in the INS-resistant ZO heart is a cardioprotective adaptation to overnutrition. In contrast to the ZO rat, heart tissues of ZDF rats do not show activation of mTORC1. We posit that such a lack of activation of the mTORC1-AT2R integrative pathway in cardiac tissue under conditions of obesity-induced diabetes may be a metabolic switch associated with INS deficiency and clinical diabetes.

obesity; mammalian target of rapamycin complex; angiotensin II-type 2 receptor; Zucker obese

THE DYNAMIC ABILITY TO RESPOND to extracellular and intracellular stress is fundamental to cell function and survival in the myocardium and coronary vasculature. Understanding how the heart reacts to the stress of chronic overconsumption of highly processed foods and sugar-sweetened beverages (Western diet) is a major area of research interest in response to the alarming increases in the incidences of diet-induced obesity, type 2 diabetes mellitus (T2DM), and cardiovascular (CV) disease (CVD) (19, 26, 32, 42, 58, 77, 89, 105). Chronic exposure to excessive circulating nutrients, glucose and insulin (INS), promote INS resistance, and the underlying chronic conditions (i.e., obesity, diabetes, and hypertension) exacerbates CV risk. Attenuations of INS-mediated vascular relaxation and glucose transport in CV and skeletal muscle tissue in conditions of INS resistance are major contributing factors responsible for development of these comorbidities (1, 31, 48, 70, 76, 80, 109).

Diet-induced obesity (DIO) also induces activation of systemic and tissue renin-angiotensin system (RAS) that results in chronic exposure of CV tissue to excess ANG II, a vasoconstrictive, progrowth/inflammatory hormone (25, 36, 50). ANG II promotes both INS resistance and CVD via ANG II-mediated activation of ANG II type 1 receptor (AT1R) (3, 5, 13, 34, 43, 53, 56, 81, 100, 104). Thus, in DIO, cardiac and vascular cells must cope with a plethora of extracellular signals generated by changes in the hormonal milieu [increased INS and ANG II, an adverse cytokine/adipokine profile], and excessive nutrients (glucose, amino acids, lipids). This scenario evokes...
evolutionarily conserved signaling mechanisms to protect these differentiated cells from overstimulation by excess growth-stimulating extracellular signals. One such signaling pathway involves the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) that serves as a converging point for signals from nutrients, INS, and ANG II, and is frequently activated in CV tissues in conditions of overnutrition. Increased mTORC1-mediated signaling is implicated in left ventricular (LV) remodeling, myocardial infarction, hypertrophic cardiomyopathy, and atherosclerosis (27, 30, 45, 46, 66, 72, 78, 82, 90, 101, 103, 114). One of the substrates of mTORC1, p70S6 kinase (S6K1) is a serine (Ser)/threonine kinase that phosphorylates INS receptor substrate 1 (IRS-1), a critical INS signaling/docking molecule, on specific Ser residues. This site-specific Ser phosphorylation results in diminished association of these IRS-1 with phosphoinositide 3-kinase (PI3K) and attenuates INS metabolic signaling. Therefore, mTORC1 activation is a contributor to heart and skeletal muscle INS resistance. From a physiological point of view, it can be argued that “development of INS resistance in chronic overnutrition” is an evolutionarily conserved metabolic switch to protect differentiated cells (such as those of CV tissues) from responding excessively to chronic growth stimulation by nutrients, INS, ANG II, or cytokines. In this context, activation of mTORC1 serves as an adaptive metabolic switch that promotes INS resistance to protect cells from excess growth-stimulating signals. This concept gains support from recent reports in the literature that suggest ablation of mTORC1 signaling is detrimental to cardiac tissue (27, 37, 52, 67, 86, 87, 90, 113).

Rodent models of genetic or acquired obesity-related cardiac dysfunction have utility for understanding cardiomyopathies in the setting of INS resistance or diabetes. It is well established that animals fed high-fat diets consistently develop INS resistance, impaired glucose tolerance, decreased energy expenditure, and obesity (35, 49, 93). Despite differences in heart rate, as well as contractile and ion channel protein isoforms, rodent models manifest similar cardiomyopathies as humans in conditions such as obesity, INS resistance, and diabetes. For example, rodent models of genetic or DIO with INS resistance (early stage) or T2DM (more advanced stage) develop an array of metabolic, structural, and functional cardiac abnormalities that include LV hypertrophy, increased cardiac fatty acid (FA) uptake and utilization, increased myocardial lipid storage (steatosis), decreased mitochondrial energetics and cardiac efficiency, impaired Ca\textsuperscript{2+} handling, and diastolic dysfunction (17). Despite the aforementioned impairments that accrue in rodent hearts during transition from normal body weight to overweight/obesity, the myocardium exhibits various adaptive morphological, biochemical, and functional responses that permit maintenance of function, allowing the heart to exist in a state of compensated heart failure. Nonetheless, persistent exposure to overnutrition and the associated comorbidities results in a transition from adaptation to maladaptation or decompensated heart failure. The focus of this review will be to discuss some of the recently recognized cardiac adaptive/compensatory mechanisms that permit the heart to maintain cardiac function in the setting of overnutrition/obesity and associated comorbidities. We will focus most of the remaining discussion on the Zucker Obese (ZO) rat model that develops a variant of the metabolic syndrome, characterized by obesity, INS resistance, hypertriglyceridemia, and mild hypertension in the absence of progression to diabetes or decompensated heart failure. Further, this genetic model manifests biochemical and functional cardiac abnormalities that are seen in obese humans. This contrasts with the Zucker Diabetic Fatty (ZDF) rat that develops diabetes at an early age. Finally, we will discuss the cardioprotective role of a novel signaling loop that mediates the cross talk between the nutrient sensor kinase mTOR and the ANG II receptor (AT2R) in DIO-induced INS resistance and the metabolic switches that may differentially regulate the mTOR/AT2R signaling loop in diabetic cardiac tissue.

**Metabolic Switches/Metabolic Flexibility**

The postnatal mammalian heart is an energy-intensive organ that generates ATP needed for contractile/relaxation function largely via a balance between FA and glucose oxidation. However, the heart normally maintains metabolic flexibility, and other substrates, such as amino acids, lactate, and ketone bodies, are also metabolized by the heart. Although the heart normally derives a greater proportion of its energy needs through β-oxidation of FA (normally 50–75%), the ratio of FA to glucose utilization is regulated by a number of factors, including the concentration of these energy substrates in the circulation, the hormonal milieu, energy demands, and oxygen availability. These regulatory factors enable the normal heart, sometimes referred to as a “metabolic omnivore,” to use the most efficient/abundant substrate available depending on physiological conditions (94, 95). Thus, metabolic flexibility is a critical adaptation that permits the heart to respond to changing physiological/environmental stimuli. This is in contrast to organs like the brain, which derives almost all of its energy needs through oxidation of glucose. Unlike the metabolism of the normal heart that adjusts readily to varying energy substrate availability, metabolic flexibility is typically diminished in the failing heart. Thus, the heart undergoes a form of “metabolic remodeling” that contributes to the structural and functional remodeling, which are the hallmarks of the failing heart (95). It has been suggested that the loss of metabolic flexibility, at least in the initial stages that precede the onset of contractile dysfunction, represents an adaptive process that helps to maintain cardiac function (95).

**Obesity Alters Cardiac Metabolism: Burn and Store More Fat**

Both FA and glucose are efficiently taken up by cardiomyocytes from the circulation via membrane transport proteins to meet the high-energy needs of the heart (68). Recent evidence suggests that during pathological states, such as occurs in obese and/or INS-resistant individuals, cardiomyocytes exhibit increases in the uptake, oxidation, and storage of FA (18, 59). The INS-resistant heart initially adapts to increases in circulating INS, glucose, and lipids by upregulating genes involved in FA oxidation (FAO), such as peroxisome proliferator-activated receptor-α (PPAR-α), PGC-1α, carnitine palmitoyltransferase (CPT-1), acyl-CoA oxidase, and uncoupling protein 3 (UCP3), which results in an increase in myocardial expression of FA-metabolizing proteins (75). It is postulated that the heart compensates to an increase in available FA by increasing FAO, thereby limiting ectopic lipid accumulation. Moreover, switching to preferential reliance on FA as an energy substrate...
temporarily preserves contractile function (94). The hearts of obese mice have been shown to counter the increased uptake and accumulation of FA by increasing cardiac expression of microsomal triglyceride (TG) transfer protein, which mediates the formation of apolipoprotein B-containing lipoproteins that are secreted by cardiomyocytes (8). Despite these varied compensatory responses, metabolic maladaptation may develop in the event that additional stresses, such as increased hemodynamic load or hyperglycemia, are placed on the INS-resistant heart. Hypertension and hyperglycemia are associated with decreased PPAR-α expression and reduced FAO in the heart. The increased FA availability in combination with reduced capacity for FAO promotes intramyocardial FA accumulation, lipotoxicity, and contractile dysfunction (21, 110).

Cardiomyocytes, like most nonadipocytes, do not normally store lipids; therefore, the abnormal accumulation of lipids in myocytes suggests a failure of homeostasis that results in steatosis and lipotoxicity (14, 54, 112). It has been postulated that leptin plays an important role in confining the storage of excess lipids to adipocytes, while simultaneously limiting the storage of intracellular lipids in myocytes and other nonadipocytes (97). The implication is that leptin sufficiency represents an adaptation that protects nonadipocytes from lipotoxicity, while permitting the organism to store excess fat at times when nutrients are seasonally abundant. While this may, in part, be so, recent evidence suggests that cardiac steatosis may be a compensatory mechanism that limits lipotoxic cardiomyopathy via sequestration of neutralized FA and harmful lipid metabolites [e.g., ceramide, long-chain fatty acyl-coenzyme A (CoA) esters, and diacylglycerol] (14). Although it is likely that myocardial lipid sequestration confers some degree of tolerance to excess lipids, an alternative explanation suggests that local storage of harmful lipid intermediates facilitates lipid-mediated cardiomyopathy. Regardless, there is growing evidence that the inappropriate deposition of fats in the heart promotes the development of INS resistance, cardiac hypertrophy, impaired cardiac function, FA-induced programmed cell death, and interstitial fibrosis. This scenario of metabolic derangement-induced cardiomyopathy is of great concern, given the emerging epidemic of obesity among young people and has been documented in both human and animal models of genetic or acquired heart disease (21, 85, 115, 116).

Emerging evidence from experimental animal models of genetic and DIO suggest that obesity is an independent risk factor for development of diastolic and systolic function. Terms, such as lipotoxic cardiomyopathy (21, 116) or obesity cardiomyopathy (4, 106, 112), imply that chronic overnutrition, resulting in impaired cardiac metabolism with consequent ectopic deposition of TG induces cardiac dysfunction. Although it is axiomatic that obesity is associated with structural and functional changes in the heart, isolating the direct effects of obesity from any number of comorbid conditions, such as ventricular hypertrophy, hypertension, INS resistance, diabetes, and sleep apnea is problematic (74). Thus, most investigations of obesity-associated cardiomyopathy utilize animal models of the cardio-renal metabolic syndrome.

**Myocardial FA Uptake and Utilization in Zucker Obese Rats**

We and others have characterized obesity-associated cardiomyopathy in the ZO rat, which is polyphagic due to a mutation in the leptin receptor (115). The INS-resistant ZO rat differs from the hyperglycemic ZDF rat in that the ZO rat has sufficient pancreatic β-cell function. Myocardial FA uptake and utilization have been examined in some detail in ZO and ZDF rats and support the notion that myocardial INS resistance and the associated cardiac dysfunction develops, in part, as a consequence of increased reliance on FAO, impaired FAO, and accumulation of FA in the myocardium. Consistent with this notion, we have observed elevated lipid accumulation in ZO heart compared with Zucker Lean (ZL) heart (Fig. 1) (110, 115). The isolated perfused ZO rat heart exhibits decreased reliance on carbohydrates and increased dependence on FAs as a fuel source (110). Despite the increased substrate preference for FA in the ZO myocardium, the availability of FA exceeds the capacity of the myocardium for FAO. The myocardium of ZL, but not ZO rats, increases FAO in response to fasting-induced increase in circulating FA, suggesting impaired myocardial FAO in ZO rats. Despite the increased substrate preference for FA in the ZO myocardium, impairment in FAO combined with increased uptake and availability of FA promotes the accumulation and storage of lipids in the ZO heart observed with immunostaining and biochemical analyses (Fig. 1) (110, 115).

Long-chain FA (LCFA) uptake into cardiomyocytes is, in part, mediated by membrane transporters, fatty acid translocase (FAT/CD36) (62, 63) and plasma membrane-associated FA binding protein, which can be induced to translocate from the cytoplasm to the plasma membrane by muscle contraction or INS. Cardiomyocyte contraction directs LCFAAs to mitochondria to undergo β-oxidation, while INS preferentially promotes the esterification of LCFAAs. Contraction-induced FAT/CD36 translocation and LCFA uptake are mediated by activation of AMPK (61), while INS-induced FAT/CD36 translocation and LCFA transport is mediated by P3K activation (62), findings that support the existence of functionally separate intracellular pools of FAT/CD36. Once LCFAAs are transported into the cytoplasm of myocytes, they can be directed to different intracellular sites to undergo mitochondrial β-oxidation, esterification to TG and phospholipids, and activation of signaling pathways that affect gene expression (28, 98). Under basal conditions, cardiac myocytes from ZO rats have a greater proportion of the FAT/CD36 pool localized at the plasma membrane compared with ZL (60). INS induces translocation of FAT/CD36 in ZL, but not in ZO rats, which supports the notion that a substantial portion of FAT/CD36 pool is permanently relocated to the sarcolemma in ZO cardiomyocytes and that this condition facilitates TG accumulation (23).

Very recent studies demonstrate that myocardial FA uptake occurs by two separate pathways; a FAT/CD36 process that transports FA derived from very low-density lipoproteins (VLDL) and passive process for chylomicron-derived FA uptake (9). FA generated by lipoprotein lipase (LpL)-mediated hydrolysis of VLDLs tends to occur in the fasting state, whereas FAs generated from LpL-mediated hydrolysis of TG-rich chylomicrons occurs largely in a postprandial state. Thus, it is apparent that the type of FA transport depends upon the lipoprotein source of FAs and, therefore, on nutritional state. Both FA uptake processes involve the activity of LpL and the importance of LpL-mediated hydrolysis of lipoproteins to lipoprotein metabolism, and FA uptake in the heart is now well established (6, 7). Moreover, long-term reduction of FA uptake
in cardiac specific LpL knockout mice results in impaired cardiac function (6).

Like FAT/CD36, the normal pattern of expression, translocation, and activity of the major cardiac glucose transporter, GLUT4, is altered by excessive nutrient intake. In healthy cardiac tissue, such as that of the ZL rat, INS induces mobilization of GLUT4 from intracellular stores to the sarcolemma. However, since overnutrition induces INS resistance, INS signaling via the IRS-1→PI3K→Akt pathway is down-regulated in the heart of the ZO rat; consequently, there is a decrease in INS-induced GLUT4 translocation to the sarcolemma (47). These observations have led to the suggestion that in the setting of excessive nutrient intake, the patterns of intracellular-to-membrane levels of FAT/CD36 and GLUT4 could be “diametrically opposed” and, in part, explain the increased reliance on FAO (60).

Myocardial Lipid Accumulation Promotes Cardiac Dysfunction

Cardiac-specific expression of transgenes involved in cardiac metabolism has also been helpful in illustrating a more direct contribution of obesity to contractile dysfunction and heart failure (8, 20, 21, 107). One such example in a transgenic mouse model utilizes cardiac-specific expression of long-chain acyl-CoA synthetase (MHC-ACS), a protein that is highly expressed in plasma and intracellular membranes of cardiomyocytes and catalyzes the initial step in FA metabolism, specifically, esterification of LCFA with CoA (21). At 4 wk of age MHC-ACS mice, fed a normal diet, exhibit elevations in cardiomyocyte TG accumulation, lipo-apoptosis, LV mass, as well as reduction in LV systolic function and survival. Another example illustrating the contribution of abnormal cardiac metabolism to cardiac dysfunction is the transgenic mouse with cardiac-specific overexpression of FA transport protein 1 (FATP1) (20). TG FATP1 mice exhibit increases in FA uptake, accumulation, and metabolism in concert with LV hypertrophy (LVH) and echocardiographic evidence of diastolic dysfunction. Mice with cardiac-specific ablation of mitochondrial TG transfer protein, a protein that attenuates cardiac TG accumulation, exhibit impaired cardiac function. Data from these transgenic and knockout rodent models support the hypothesis that accumulation of ectopic FA as a result of abnormalities in cardiac lipid metabolism represents an important risk factor promoting cardiac dysfunction.

Structural Adaptation

One particularly well-recognized adaptive response that occurs in response to both physiological and pathophysiological conditions is cardiac hypertrophy, a process that involves an increase in cardiac mass (64, 69). Because postnatal myocytes rarely undergo cell proliferation (91), physiological and pathophysiological cardiac hypertrophy is largely the result of in-
Progression to More Advanced Cardiac Dysfunction but not Severe Heart Failure?

To determine whether diastolic heart failure with preserved systolic function, such as that observed in normotensive 9-wk-old male ZO rats, progresses to more advanced cardiac dysfunction with development of clinically relevant hypertension, we examined cardiac function in 12-wk-old male ZO rats using pressure volume (PV) loop analyses via direct catheterization of the left ventricle (Fig. 2). ZO rats had elevated SBP, diastolic blood pressure, and MAP, as well as elevations in LV end-diastolic pressure and LV end-systolic pressure as determined by (PV) loop analysis. LV end-diastolic and systolic volumes tended to be higher in ZO (but neither trend was significant). Markers of systolic dysfunction, including cardiac index (cardiac output/body wt) and the load-independent indices, end-systolic elastance (Ees) and maximal elastance (Emax), were decreased in the ZO heart compared with its lean counterpart. The load-independent indices of diastolic function, tau (τ, the time constant of isovolumic relaxation), and the slope of the end-diastolic pressure volume relationship were both significantly increased, demonstrating impairments in both active and passive properties of LV relaxation. These cardiac hemodynamic and functional abnormalities in the ZO were associated with LVH. These findings demonstrate that between 9 and 12 wk of age, the INS-resistant ZO rat becomes hypertensive and progresses to a more advanced stage of heart failure characterized by LVH, impaired relaxation, as well as early contractile impairment.

The transition to clinical diabetes promotes the development of diabetic cardiomyopathy and increases the risk of more severe heart failure (5). In its early stages, diabetic cardiomyopathy manifests as asymptomatic diastolic dysfunction with preserved ejection fraction in the absence of coronary artery disease and hypertension. With strict metabolic control, early diabetic cardiomyopathy may be reversible. At this stage, the LV stiffens mildly due to accumulation of connective tissue and collagen; however, contractility remains normal or is enhanced. Over time this compensated stage progresses to symptomatic or decompensated heart failure. The compliance of the LV chamber wall decreases further, active relaxation becomes impaired, and systolic contractility decreases. Thus, further metabolic complications may occur as a consequence of hyperinsulinemia and hyperglycemia.

We tested this notion by comparing echocardiographic measures of cardiac function among normal, obese INS resistant, and obese diabetic Zucker rats. Interestingly, 19-wk-old ZDF rats demonstrate impaired glucose uptake via PET analysis, in concert with decreased GLUT4 expression, hyperglycemia, and elevated circulating free FA in the absence of evidence of LVH or dilated cardiomyopathy upon M mode echocardiographic analysis (88). Thus, it seems reasonable to predict that abnormalities in cardiac function during the progression to diabetes in this model largely involve impaired cardiac LCFA and glucose metabolism. For this purpose, we studied 13-wk-old ZL and ZO rats and 22-wk-old ZDF rats (unpublished data). Pulsed-wave Doppler mode determination of the myocardial performance index (MPI) is the sum of the isovolumic relaxation and contraction times divided by ejection time. MPI is an index of global cardiac function, combining diastolic and systolic function estimation in one measurement. MPI confirmed cardiac dysfunction in the ZO rat compared with the ZL...
There was a trend suggesting increased MPI in ZDF rats compared with ZL rats (ZL vs. ZDF; \( P < 0.067 \)), suggesting diabetic cardiomyopathy, although echocardiographic measure of myocardial performance appeared no more impaired in ZDF rats than in ZO rats. The significantly lower heart rate in ZDF rats compared with ZL and ZO rats (301 ± 12 vs. 348 ± 5 and 355 ± 15 for ZDF, ZL, and ZO, respectively; \( P < 0.05 \)) represents one possible limitation of eff.
mTORC1 activation in the heart represents a pathological function is essential for the heart to cope with stress induced by response (2, 87, 90, 111). Thus, a delicately balanced mTORC1 pathological hypertrophy associated with inflammatory relation of cardiac Raptor results in impairment of adaptive mTOR protects against cardiac dysfunction following LV pressure overload. Raptor is the scaffolding protein that mediates signaling via AT2R. We posit that mTORC1 activation can initiate an mTOR++AT2R signaling loop that can serve as a protective feedback mechanism that regulates mTORC1 signaling via the AT2R. We posit that mTORC1 signaling was activated and AT2R expression was elevated in mouse cardiomyocytes in response to chronic exposure to INS or ANG II. Furthermore, rapamycin-inhibition of mTORC1 in ANG II- or INS-exposed mouse cardiomyocytes attenuated the increase in AT2R, suggesting that mTORC1 activation is, in part, responsible for the increase in AT2R protein levels. Moreover, in 12-wk-old ZO rats subjected to a 9-day infusion (200 μg·kg\(^{-1}\)·day\(^{-1}\)) of novokinin, an AT2R agonist, attenuated the increase in mTORC1 signaling and improved myocardial performance, suggesting that AT2R activation regulates mTORC1 in the LV of hyperinsulinemic ZO rats. This is not surprising since INS activates mTORC1 and increases AT2R expression (30, 44, 79, 82). What is paradoxical is that AT2R signaling inhibits cell growth, and accumulating evidence suggests that AT2R activation is cardioprotective, as it mediates the beneficial effects of AT1R blockade and PPAR-γ activation, reduces fibroblast growth and myocardial hypertrophy, and mediates the antihypertrophic and antifibrotic effects of AT1R blockade (10 –12, 29, 55, 71, 99, 108). The AT2R is known to activate phosphatases and increased expression and activation of the AT2R could conceivably regulate mTORC1 kinase and downstream signaling and regulate cell growth. Indeed, we recently reported that mTORC1 signaling was activated and AT2R expression was elevated in mouse cardiomyocytes in response to chronic exposure to INS or ANG II. Furthermore, rapamycin-inhibition of mTORC1 in ANG II- or INS-exposed mouse cardiomyocytes attenuated the increase in AT2R, suggesting that mTORC1 activation is, in part, responsible for the increase in AT2R protein levels. Moreover, in 12-wk-old ZO rats subjected to a 9-day infusion (200 μg·kg\(^{-1}\)·day\(^{-1}\)) of novokinin, an AT2R agonist, attenuated the increase in mTORC1 signaling and improved myocardial performance, suggesting that AT2R activation regulates mTORC1 in ZDF rats.

**Is mTORC1 Activation a Metabolic Switch to Protect the INS-Resistant Heart from Progressing to the Diabetic Heart?**

Hyperinsulinemia with excessive nutrient intake is an ideal metabolic milieu for activation of the nutrient sensor kinase mTOR. Accumulating evidence suggests that signaling mediated by the mTOR/S6K1 pathway is central to diseases such as the cardio-renal metabolic syndrome, diabetes, atherosclerosis, hypertension, and hypertrophic heart disease. Overnutrition also induces activation of the RAS, and this is supported by evidence demonstrating that inhibition of the AT1R by oral administration of the AT1R antagonist irbesartan reduces INS resistance in ZO rats (39, 79). ANG II acting through the AT1R also activates mTORC1 in CV tissues (27, 37, 82). Activation of mTORC1 leads to increased translation and cell growth by two mechanisms: first, the ribosomal protein S6 (RPS6) that increases translation of 5'TOPmRNAs is activated by S6K1 via phosphorylation of five evolutionarily conserved residues of RPS6, Ser235, Ser236, Ser240, Ser244, and Ser247 factor for protein synthesis in various cell types. Thus, activation of mTOR/S6K1 increases translation of different proteins via the S6K1-RPS6 pathway. The second substrate of mTOR is 4E-BP, which, in its hypophosphorylated form, functions as a translation repressor by binding to translation initiation factor eIF4E. mTORC1-mediated inhibitory phosphorylation of 4E-BP on Thr37 and Thr46 relieves this repression and enhances translation (2, 24, 38, 46, 65, 111). Increased mTORC1-mediated translation underlies cardiac hypertrophy and is implicated in CV dysfunction. However, cardiac overexpression of mTOR protects against cardiac dysfunction following LV pressure overload. Raptor is the scaffolding protein that mediates mTORC1 formation and mTOR substrate activation. Ablation of cardiac Raptor results in impairment of adaptive cardiac hypertrophy and causes heart failure in mice (87). Moreover, mTOR is required for protection of the heart from pathological hypertrophy associated with inflammatory response (2, 87, 90, 111). Thus, a delicately balanced mTORC1 function is essential for the heart to cope with stress induced by exercise, pressure overload, and inflammation. Thus, in the setting of overnutrition/INS resistance, the notion that mTORC1 activation in the heart represents a pathological

**Nutrient-Induced mTOR Signaling in the INS-Resistant Heart**

In this context, our observation that an mTOR++AT2R signaling loop is activated in 12- to 15-wk-old ZO rat hearts that exhibit hypertrophy and diastolic and systolic dysfunction is particularly relevant. We recently reported elevated cardiac expression of the AT2R and a concomitant activation of mTORC1 in the LV of hyperinsulinemic ZO rats. This is not surprising since INS activates mTORC1 and increases AT2R expression (30, 44, 79, 82). What is paradoxical is that AT2R signaling inhibits cell growth, and accumulating evidence suggests that AT2R activation is cardioprotective, as it mediates the beneficial effects of AT1R blockade and PPAR-γ activation, reduces fibroblast growth and myocardial hypertrophy, and mediates the antihypertrophic and antifibrotic effects of AT1R blockade (10 –12, 29, 55, 71, 99, 108). The AT2R is known to activate phosphatases and increased expression and activation of the AT2R could conceivably regulate mTORC1 kinase and downstream signaling and regulate cell growth. Indeed, we recently reported that mTORC1 signaling was activated and AT2R expression was elevated in mouse cardiomyocytes in response to chronic exposure to INS or ANG II. Furthermore, rapamycin-inhibition of mTORC1 in ANG II- or INS-exposed mouse cardiomyocytes attenuated the increase in AT2R, suggesting that mTORC1 activation is, in part, responsible for the increase in AT2R protein levels. Moreover, in 12-wk-old ZO rats subjected to a 9-day infusion (200 μg·kg\(^{-1}\)·day\(^{-1}\)) of novokinin, an AT2R agonist, attenuated the increase in mTORC1 signaling and improved myocardial performance, suggesting that AT2R activation regulates mTORC1 in ZDF rats (unpublished data). Thus mTORC1 activation can initiate an mTOR++AT2R signaling loop that can serve as a protective feedback mechanism that regulates mTORC1 signaling via the AT2R. We posit that mTOR++AT2R signaling loop is a compensatory mechanism to protect the heart in conditions of excessive nutrient-induced INS resistance and RAS activation.
mTOR→AT2R signaling loop, and INS deficiency attenuates this process, it is therefore, conceivable that the lack of mTORC1 activation in diabetes reflects this transition from a hyperinsulinemic, prediabetic state to an INS-deficient state (Fig. 5). It is known that INS inhibits AMPK (51). Consistent with this notion, we observed a significant reduction in phos-
pho Thr172 AMPK in hyperinsulinemic ZO LV tissues, compared with ZL LV tissues. This lack of AMPK activation resulted in a significant reduction in phosphorylation of Raptor at Ser722/792 that would have contributed to increased mTORC1 activation in ZO LV tissues (Pulakat L, DeMarco VG, Gul R, Ma L, Arnold S, Mugerfeld I, Johnson M, Whaley-Connell A, Sowers JR, unpublished data). Under INS-deficient conditions, AMPK is activated, and this can negatively mod-
ulate mTORC1 (Fig. 6). Thus, in overnutrition-induced advanced diabetes, multiple signaling mechanisms drive toward attenuating mTORC1 signaling, which can be viewed as a maladaptive mechanism.

Perspectives and Significance

INS resistance is an underlying abnormality associated with cardiac and vascular maladaptive changes observed in conditions of obesity, T2DM, and hypertension. Overconsumption of diets rich in fat and carbohydrates results in activation of the RAS and chronic exposure of cardiovascular tissues to increased circulating FA, glucose, ANG II, and INS. The heart is an omnivoruous organ due to an innate metabolic flexibility that enables the myocardium to metabolize multiple substrates to

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\text{Fig. 5. Immunoblots of left ventricle protein extracts show representative bands of phospho-mTOR and β-actin from ZL, ZO, and ZDF rats. Anti-phospho mTOR(Ser2448) antibody was used to detect Ser2448 phosphorylation of mTOR in lysates. Ser2448 phosphorylation of mTOR results in mTOR activation. *P < 0.05. Bars represent means ± SE; n = 3–5.}
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\text{Fig. 6. The mTOR AT2R feedback loop is cardio-protective in the setting of overnutrition-induced prediabetes (A) while this pathway is suppressed in the heart during late-stage diabetes (B). See text for explanation. Rap: Rapamycin, the mTORC1 inhibitor; Nov: Novokinin, the AT2R agonist; p-Tyr, phospho-tyrosine; IRS-1, INS receptor substrate 1; GßL, G protein beta protein subunit-like; RPS6, ribosomal protein S6; 4E-BP1, elongation factor 4E binding protein 1; S6K1, p70S6 kinase; mTORC1, mammalian target of rapamycin complex 1; AT2R, ANG II-type 2 receptor; T2DM, type 2 diabetes mellitus.}
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meet demanding energy needs under different physiological and pathophysiological conditions. In this context, activation of the evolutionarily conserved nutrient sensor mTOR-S6K1 signaling pathway that serves as a critical converging point for signaling from nutrients, INS and ANG II in cardiovascular tissue could serve as a focal point for investigation of potential novel metabolic compensatory mechanisms. Amino acids, INS, and ANG II, the latter acting through the ANG II type 1 receptor AT1R, induce activation of mTOR/S6K1 signaling in cardiovascular tissues. Mechanisms to counterbalance the growth-promoting effects of multiple simultaneous activators of the mTOR signaling cascade have yet to be elucidated. We propose that the enigmatic nature of mTORC1-signaling in the heart is, in part, due to the likely existence of protective and integrative feedback mechanisms such as the mTOR++-AT2R signaling loop that are activated downstream of mTOR complex 1 stimulation. Ablation of this pathway in the heart under diabetic conditions supports the concept of a metabolic switch associated with increased lipotoxicity and advanced pathology.

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