Overnutrition, mTOR signaling, and cardiovascular diseases

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THE CARDIORENAL METABOLIC syndrome (CRS) consists of a constellation of cardiac, renal, and metabolic disorders, including insulin resistance, obesity, metabolic dyslipidemia, high blood pressure, and evidence of early cardiac and renal disease (28). Epidemiological studies showed that 36.1% of adult men and 32.4% of women in the United States in 2010 had metabolic disorders, often termed the metabolic syndrome (66). Many persons with the metabolic syndrome will also have evidence of insipid renal and cardiovascular disease, and, thus, the CRS constellation. Overweight or obesity, especially abdominal obesity, caused by consumption of excess nutrients, especially high fructose and fat, has been increasingly implicated as an independent risk factor for development of diabetes, cardiovascular disease (CVD), and chronic kidney disease (CKD) (29). Indeed, obesity is a proinflammatory, prooxidative, and maladaptive immune state associated with the accumulation of dysfunctional adipose tissue, altered vascular homeostasis, and endothelial dysfunction, which predisposes individuals to CVD and CKD (30). The vascular pathologies associated with the CRS, namely, endothelial dysfunction and increased cardiovascular stiffness increase the risk of myocardial infarction, stroke, limb ischemia, and associated mortality.

In this context, our research group and others have established translational rodent models of obesity, which when fed Western diets (WD) high in fructose and fat, consistently develop insulin resistance, impaired glucose tolerance, decreased energy expenditure, maladaptive immune function, inflammation, as well as cardiovascular stiffness and associated functional abnormalities (48). For example, mice consuming a WD manifest activation of immune cellular components, including granulocytes, mast cells, monocytes, macrophages, dendritic cells, and natural killer cells (81), and display increased heart and vascular fibrosis leading to left ventricular hypertrophy (LVH), myocardial diastolic dysfunction, and vascular stiffness (87). Indeed, impaired insulin metabolic signaling in cardiovascular tissues may explain the link between obesity, hypertension, LVH, cardiac and vascular stiffness, and associated increases in CVD risk (48, 61, 70).

The metabolic signaling pathways involved in obesity-related insulin resistance and associated medical abnormalities include signaling through the mammalian target of rapamycin (mTOR), a serine-threonine kinase that serves as a converging point for signals mediating cellular growth, energy metabolism, nutrient availability, and stress. The mTOR’s signaling pathway is frequently activated in various tissues during conditions of excessive nutrient intake (8) (Fig. 1). Thus, activa-
tion of mTOR and downstream signaling molecules, such as ribosomal S6 kinase (S6K)1, functions as an adaptive metabolic response that promotes insulin resistance and ultimately protects cells from continued stimulating signals, such as excess nutrients and associated activation of the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (5, 18, 34).

Research from our laboratory and others has confirmed that mTOR/S6K1 activation is enhanced under conditions of excess intake of carbohydrates, fats, and branched-chain amino acids; hypoxia; or/and increased levels of ANG II (88) (Fig. 1). Enhanced activation of mTOR/S6K is associated with cardiac hypertrophy and impaired endothelial function, and rapamycin treatment to reduce this activation corrects some of these abnormalities (30). Treatment with rapamycin has also been shown to prevent renal fibrosis and proteinuria in a rodent model of obesity and diabetic nephropathy (86). In this review, we will highlight how excess activation of the mTOR/S6K promotes metabolic and cardiovascular abnormalities and discuss potential therapeutic strategies to mitigate these abnormalities.

Overnutrition, Obesity, and Cardiovascular Diseases

Excessive nutrient consumption and the resultant overweight/obesity state promote increases in CVD via a number of metabolic abnormalities. Consumption of excess carbohydrates and fat may promote an atherogenic dyslipidemia consisting of hypertriglyceridemia, small low-density lipoprotein cholesterol (LDL-C) particles, and suboptimal high-density lipoprotein cholesterol (HDL-C) levels (57). In a large series of 26,000 overweight children, lipid concentrations were abnormal in 32% of the children. Total cholesterol was abnormal in 14.1%, LDL-C in 15.8%, HDL-C in 11.1%, and triglycerides (TG) in 14.3% of those children from whom data were available (62). Indeed, obesity has been associated with increased coagulability, endothelial dysfunction, increased systemic inflammation, and other clinical consequences, such as hypertension, coronary artery diseases, insulin resistance, Type 2 diabetes, and CRS (72). Adipocytes can produce over 50 active substances, such as monocyte chemotactic protein-1 (MCP-1), TNF-α, IL-6, C-reactive protein, ANG II, and aldosterone, all of which may contribute to insulin resistance, abnormal lipid metabolism, maladaptive immune response, systemic inflammation, hypercoagulability, and hypertension (97). A number of activated signaling pathways/molecules may contribute to these CVD risk factors. For example, MCP-1 plays a key role in the infiltration of macrophages into adipose, cardiovascular, hepatic, and renal tissues, as well as in the development of insulin resistance, CVD, and CKD (7). Also, plasma levels of plasminogen activator inhibitor 1 (PAI-1), an inhibitor of fibrinolysis, are generally increased in obese patients and in those with Type 2 diabetes (49). Increased amounts of PAI-1 contribute to reduced plasmin activation and a prothrombotic state, which may promote atherogenesis and increase the risk of CVD (49, 72). Conversely, a reduction in plasmin activation is also associated with reduced activation of transforming growth factor-β (TGF-β), a molecule important for suppressing the proliferation and migration of smooth muscle cells that contributes to atherosclerotic lesion formation (71). This highlights the association of excess nutrient ingestion and obesity in driving CVD and CKD with various components of the CRS (29, 70).

Role of the mTOR/S6K1 Signaling Pathway in Linking Overnutrition with the CRS

The mTOR/S6K1 signaling pathway mediates various biological effects of nutrients, insulin, energy, and various envi-
Environmental stressors. This pathway is closely associated with the activities of two different protein complexes, mTOR Complex 1 (mTORC1) (14) and mTORC2 (24). Sensing mitogen, energy, and nutrient signals, mTORC1 functions to regulate protein synthesis, autophagy, and ribosome biogenesis (98). mTORC1 is rapamycin-sensitive and consists of the mTOR catalytic subunit, raptor, mLST8, and PRAS40; mTORC2 has Rictor rather than Raptor and works closely with the mammalian stress-activated protein kinase interacting protein and Protor (14) (Fig. 2). It was previously believed that mTORC2 was rapamycin-insensitive, but recent studies have demonstrated rapamycin’s ability to inhibit the assembly and activity of mTORC2, including regulation of survival, metabolism, and proliferation by mediating the protein kinase B (Akt), PKC, and serum- and glucocorticoid-regulated kinase (SGK) in response to growth factor stimulation (98) (Fig. 2).

Growth factors, such as insulin and insulin-like growth factor 1 (IGF-1), activate mTORC1 predominantly via Akt signaling (2). Akt phosphorylates tuberous sclerosis complex 2 (TSC2) and causes it to dissociate from TSC1. Dissociated TSC2 is captured by 14-3-3 and is prevented to form the complex (77). The TSC1/2 plays a key role in the activation of mTOR. TSC1/2 together with a third component, TBC1D7, functions as a GTPase-activating protein for the small G protein Ras homology enriched in brain (Rheb). GTP-bound Rheb, the active form, directly binds to mTORC1 and activates its kinase activity (77). Proteins unique to mTORC1 are regulatory associated proteins of mTOR (Raptor) and proline-rich Akt substrates of 40 kDa (PRAS40). Raptor is necessary for assembly of mTORC1 and for interaction of mTORC1 with upstream regulators and downstream substrates (43). Inhibition by rapamycin on mTORC1 depends, in part, on preventing the association between mTOR and Raptor, thereby impairing the interaction of mTORC1 with its downstream targets. PRAS40, when unphosphorylated, binds to and inhibits mTORC1 (43). Growth factors also activate mTORC1 through RAAS signaling pathway effectors, such as ERK1/2 and p90 RSK (2). Recent research has also shown that the Wnt signaling pathway activates mTORC1 through inhibition of glycogen synthase kinase-3β (GSK3β) and TSC2 (80).

Amino acids, especially branched-chain amino acids, such as leucine, may enhance mTORC1 signaling. In response to amino acid sufficiency, the Rag complex is recruited to the lysosomal membrane by the trimeric Ragulator complex, which consists of MP1, p14, and p18, thereby allowing Rheb to activate mTORC1 (67). Amino acids also induce an extracellular calcium influx that activates calmodulin, which, in turn, binds and activates Vps34, a member of class III phosphatidylinositol 3-kinase (PI3Ks), resulting in increased production of phosphatidylinositol 3-phosphate (PI3P) and, consequently, activation of mTOR (27, 40).

Cells ‘sense’ energy status by relying on the activity of AMPK, which is activated by a decreased adenosine triphosphate (ATP)/AMP ratio under conditions of glucose deprivation (41). Activated AMPK inhibits mTORC1 via GSK3β and AMPK-mediated phosphorylation, and consequently, activation of TSC2, as well as via phosphorylating Raptor. AMPK phosphorylation of Raptor causes Raptor’s sequestration by 14-3-3 proteins, inactivating Raptor as a scaffolding molecule for mTORC1 and its downstream signaling pathways (43). There are several upstream kinases that can activate AMPK by phosphorylating a threonine residue on its catalytic α-subunit, such as liver kinase B1, calcium/calmodulin kinase, and TGF-β-activated kinase-1 (63, 84). Thus, by coupling AMPK activity with energy status, AMPK tightly regulates cellular function in various human physiological and pathological processes.

In states of excessive nutrient intake and obesity, microvascular endothelial dysfunction can result in impaired blood perfusion and associated tissue hypoxia (6). Hypoxia is associated with low ATP levels, which, in turn, reduce mTORC1 signaling via AMPK activation of tuberous sclerosis protein.

Fig. 2. Proposed model for the regulation of mTORC1 signaling in conditions of overnutrition and the cardiorenal syndrome (CRS). Accordingly, mTORC1 relies upon Raptor to enable mTORC1 to bind to its substrates and regulate autophagy, ribosome biogenesis, and protein synthesis. Factors affecting mTORC1 activation include growth factors, amino acids, energy availability, and hypoxia. For example, the mTORC2 pathway can be activated by both insulin and insulin-like growth factor one (IGF1). In turn, mTORC2 regulates cell survival, metabolism, and proliferation by mediating the Akt, PKC, and glucocorticoid-regulated kinase (SGK) signaling.
(TSC) and inhibition of Raptor (44). Inhibition of mTORC1 activity is also promoted by increases in hypoxia-inducible transcription factor-1 (HIF1) α and β, which enhance the expression of regulated in development and DNA damage response-1 (REDD1) and REDD2 (2). One study also showed that a regulatory associated protein of mTOR (Raptor) interacts with HIF1α and requires an mTOR signaling (TOS) motif located in the NH₂ terminus of HIF1α. Furthermore, a mutant of HIF1α lacking this TOS motif dominantly impaired HIF activity during hypoxia and was unable to bind to the coactivator CBP/p300 (37). Thus, the inhibition of mTOR activity by REDD1 activation may occur through AMPK-independent and AMPK-dependent mechanisms and its downstream targets 4E-BP1, S6K1, CBP/p300, and cyclin D under hypoxic stress (98) (Fig. 2). TNF-α is the only known factor to activate mTORC1 via TSC1 rather than TSC2 and activates 1kB kinase-β (1IKKβ), which interacts and inactivates TSC1 physically, to activate mTORC1 (43, 56).

Signaling through the mTOR2 pathway is also activated by increased insulin and IGF-1 exposure. Insulin and/or IGF-1 receptor binding leads to recruitment and phosphorylation of tyrosine residues on insulin receptor substrate (IRS)1/2, which serve as a docking motif for the regulatory subunit of class I PI3K, p85, and enable the production of PI3P (19, 23). Activated Akt phosphorylates and inactivates TSC2, likely by increasing TSC1 and TSC2 degradation rate (40). TSC1/2 disrupts the small guanosine triphosphate (GTPase) molecule Rheb by maintaining it in the inactive guanosine diphosphate (GDP)-bound form (40, 93). Indeed, Rheb has been shown to be an important mTORC1 upstream activator and indirectly has negative effects on mTORC2 regulation (98). Rheb activates mTORC1 and S6K1 to suppress the PI3K-Akt signaling pathway via IRS inhibition in many cell types (98). These data suggest that Rheb directly activates mTORC1, while Rheb inhibition toward mTORC2 may be indirect via negative feedback.

Role of Enhanced mTOR/S6K1 Signaling in the Development of Insulin Resistance

Signaling through the mTOR/S6K1 pathway may act as a double-edged sword in the maintenance of β-cell function and glucose metabolism in response to overnutrition. Initially, mTORC1/S6K1 signaling positively regulates β-cell function and insulin secretion. However, chronic activation of mTORC1/S6K1 signaling increases insulin resistance in islets through the feedback inhibition of IRS-1 and IRS-2, which lowers β-cell survival and increases apoptosis (38). Thus, overnutrition activates mTOR/S6K1 signaling, which turns on insulin secretion and induces hyperinsulinemia and associated peripheral insulin resistance. The negative effects of enhanced mTOR/S6K1 signaling include that of marked increases in the phosphorylation of JNKs and an associated increase in β-cell apoptosis in islet β-cells (40). While a moderate increase of JNK activity in overnutrition states probably allows partial protection to β-cells from oxidative stress, this stress eventually outweighs the self-protection capacity resulting in further activation of JNK (40). This continued activation of JNK is involved in the secretion of inflammatory cytokines, including TNF-α, IL-1β, and IL-6, leading to phosphorylation of IRS-1 at serine residues and decreasing insulin metabolic signaling (5). Therefore, mTOR/S6K1 plays an important role in the regulation of β-cell function and survival, but excessive sustained activation of this pathway may lead to insulin resistance, glucose intolerance, and eventually diabetes.

Studies have shown that mTORC1/S6K1 also activates a negative feedback loop to suppress insulin signaling, as suggested by findings that amino acids stimulate mTORC1 and inhibit insulin-induced PI3K activity in a rapamycin-sensitive manner (96). Activated S6K1 increases serine phosphorylation of IRS-1, resulting in downstream PI3K inhibition (46) (Fig. 2 and Fig. 3). Consistent with these studies, our research has shown that excess fat and carbohydrates promote insulin resistance in the heart, muscle, fat, and liver (Fig. 3). In addition to overnutrition, enhanced activation of RAAS and the mTOR/S6K1 signaling pathway increases inflammation, and oxidative stress to further inhibit insulin metabolic signaling in cardiovascular tissues (47) (Fig. 3). This action leads to decreased PKB/Akt activation, reduced glucose uptake, impaired endothelial nitric oxide (NO) production, and inhibited glycogen synthase activity and ATP production in cardiovascular tissue (4). Insulin resistance and malfunctioned mitochondrial fatty acid oxidation lead to reduced glucose and fatty acid utilization (47). Thus, mTORC1/S6K1 generates a feedback loop that decreases IRS-1 levels and downstream metabolic signaling (Figs. 1 and 3).

Effects of the RAAS on mTOR/S6K1 and Insulin Metabolic Signaling

ANG II and aldosterone are potent vasoconstrictors that can cause hypertension, coronary artery disease, insulin resistance, and diabetes (3). In the course of RAAS-induced CVD, ANG II and aldosterone bind to ANG II receptor-1 (AT₁R) and the mineralocorticoid receptor (MR), respectively, to induce oxidative stress, which is mainly mediated by NADPH oxidase activation in cardiovascular tissues (70). ANG II activates mTOR/p70S6K1, phosphorylating IRS-1 at Ser²³⁶⁻²⁶⁹ and inactivating insulin-stimulated endothelial NO synthase (eNOS) phosphorylation that reduces the production of NO and impairs insulin-induced vasodilation (34).

Recently, overactivity of the mTOR/S6K1 signaling pathway was observed in patients with primary aldosteronism (74). An impact of MR signaling related to its activation of the mTOR/S6K pathway is highlighted by the finding that MR antagonism targets mTOR/S6K1 signaling to reduce fibrosis, enhance proximal tubule integrity, and lessen proteinuria (88). In this context, the amelioration of renal fibrosis following mTOR inhibition with rapamycin has been shown to be related to reductions in phosphorylation/activation of S6K1 (86). Further, hypertensive rats overexpressing the renin gene display increased renal tissue Ser²⁴⁴₈ phosphorylation of mTOR and downstream S6K1 in concert with tubulointerstitial fibrosis. Blockade of the AT₁R reduces mTOR/S6K activation and attenuates the tubulointerstitial structural abnormalities and proteinuria observed in the kidneys of these rats (86).

Interestingly, hyperinsulinemia-induced cardiac hypertrophy is accompanied by a reduction in AT₁R, an increase in AT₂R, and activation of S6K1 via the PI3K/Akt signaling pathway (61). The association between increases in AT₂R protein and mTOR/S6K1 activation in insulin resistance-induced cardiac hypertrophy prompts us to posit that mTOR/S6K1-induced
enhanced AT1R and MR-mTORC1 signaling in CVD in contrast to corticoid receptor. IRS, insulin receptor substrate; MR, mineralocorticoid receptor.

increases in translation could, in part, contribute to increases in AT1R protein levels (60). Thus, mTORC1 activation leads to the formation of an AT1R-mTOR-signaling loop to balance enhanced AT1R and MR-mTORC1 signaling in CVD in conditions of increased hemodynamics load associated with obesity (Fig. 3). Thus, inappropriate activation of the RAAS may promote the development of hypertension, insulin resistance, and CRS through activation of the downstream mTOR/S6K1 signaling pathway (70). Consequently, rapamycin, an mTOR inhibitor, may represent a viable therapeutic strategy for the treatment of RAAS-associated hypertension, insulin resistance, and CVD in CRS (Figs. 1 and 3).

Leptin and mTOR/S6K Signaling

Leptin is an adipokine that stimulates the sympathetic nervous system (68). Expression of the leptin receptor in cardiovascular tissues also suggests that there are direct cardiovascular effects of leptin (1). Although leptin has been shown to have cardiovascular protective effects in young mice, this protection is lost in aged mice. Moreover, high concentrations of leptin have been shown to promote impaired myocardial contractility (64). Leptin also enhances vascular oxidative stress and inflammation, which may contribute to CVD complications associated with obesity (85). Recent studies have demonstrated that leptin-induced cardiac fibrosis was associated with increased oxidative stress and activation of the mTOR pathway in cardiac myofibroblasts (50). Leptin also activates mTOR/S6K1 signaling in immune cells and thus promotes an inflammatory immune phenotype. Leptin increases the expression of TNF-α in macrophages, a process that is mitigated by S6K1 inhibition (39). Given the role of mTOR/S6K1 regulation of innate and adaptive immunity in obesity, leptin modulation of mTOR/S6K1 signaling may be an important link between metabolism and immunity in regulating cardiovascular function in obesity (69).

Enhanced mTOR/S6K1 Signaling and CVD

The mTOR/S6K1 signaling pathway is involved in the regulation of cardiovascular metabolism. Inappropriate activation of this signaling pathway can lead to impaired insulin metabolic signaling and the associated dysfunction of endothelial cells (ECs), vascular smooth muscle cells (VSMCs), and cardiomyocytes (13).

Role of mTOR/S6K1 signaling in EC function in normal and disease states. Activation of mTOR mediates Akt signaling in ECs. Phosphorylated Akt subsequently activates several GSK3 signaling cascades (55). Rapamycin, an mTOR inhibitor, decreases Akt phosphorylation, and thus inhibits the downstream activation of GSK3 in ECs. The major effect of mTOR inhibition in ECs is to suppress Akt-inducible prosurvival signals (20). Interestingly, increased mTOR/S6K1 signaling has been observed in aortic tissue from mice fed a high-fat diet, and increases in this signaling pathway are associated with vascular senescence and vascular dysfunction. The mechanisms responsible for the latter are likely to include oxidative stress, as a persistent hyperactive mTOR/S6K1 signaling system was associated with increased O₂⁻ production derived from eNOS uncoupling in cultured senescent ECs and in aortas of old rats (95). In the same study, it was also confirmed that mTORC1/S6K1 signaling is involved in the expression of endothelial tissue factor expression and adhesion molecules, such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1, which contribute to vascular inflammatory responses and thrombosis formation (95). Thus, mTOR/S6K1 inhibition may represent a pharmacological strategy to treat overnutrition-related EC dysfunction in the CRS. To this point, we have
observed that ANG II-induced inhibition of NO production can be reversed by inhibition of mTOR/S6K1 in ECs (34).

The role of mTORS6K1 in insulin metabolic signaling in VSMCs. Myosin binding protein (MBP) activation attenuates Ca\(^{2+}\) myosin light-chain kinase (MLCK) activity and Ca\(^{2+}\) sensitivity, a mechanism involved in insulin-induced VSMC relaxation. MBP, when activated, dephosphorylates and inactivates MLCK (17). Insulin/Akt signaling, via elevations in NO/cyclic guanosine monophosphate (cGMP), increases MBP activity by decreasing phosphorylation of MBP (75). Overnutrition and increased RAAS activity promote mTOR/S6K1 activation. This is associated with a decrease in MBP activity that results from interference with insulin-induced NO production in ECs. A reduced bioavailability of NO then leads to increases in phosphorylation of MLCK, Ca\(^{2+}\) MLCK sensitization and decreased VSMC relaxation (26). In this regard, mTOR/S6K1 inhibition, as well AT1R and MR blockade, may improve MBP activation in response to insulin via reductions in IRS-1 and consequent increases in P38/Akt signaling (26, 34).

VSMCs can differentiate from a quiescent, contractile phenotype to a proliferative/synthetic phenotype in atherosclerotic lesions related to overnutrition and the CRS (73). For example, diabetes increases VSMC PKC activity, nuclear factor-κB (NF-κB) production, and generation of oxygen-derived free radicals, all of which heighten VSMC migration and the formation of atherosclerotic lesions (16). These pathophysiological changes in VSMCs are associated with activation of mTOR, p70S6K, ribosomal protein S6, and 4E-binding protein 1 to promote protein translation and cell growth (42). Thus, inhibitors of mTOR, such as rapamycin have potent antiproliferative effects capable of hindering neointimal formation and intimal hyperplasia, and may be used as drug-eluting stents in coronary artery disease to prevent restenosis. In this regard, treatment with rapamycin increases the levels of the cyclin-dependent kinase inhibitors p21\(^{cip1}\) and p27\(^{kip1}\) and induces cell cycle arrest, resulting in inhibition of VSMC proliferation and atherosclerosis (89).

mTORS6K1 signaling pathway in the heart. The mTOR/S6K1 signaling pathway could represent a common convergence point for the various signals, leading to cardiac hypertrophy. Among the effectors of the hypertrophic response triggered by mTOR, S6K1 is very important in the ribosome biogenesis, translation, cell cycle progression, and hypertrophy (98). In this regard, endogenous cardiac mTOR/S6K1 activation has been reported to be significantly elevated in heart failure patients with preserved ejection fraction and in mice with pathological hypertrophy and left ventricular diastolic dysfunction (25). Overnutrition, neurohormones, and growth factors contribute to cardiac hypertrophy in an mTOR/S6K1-dependent manner (78). Overnutrition promotes mTOR/S6K1 activation, and this activation is associated with upregulation of RAAS in the heart (61) (Fig. 3). Our research supports the notion that inhibition of the AT1R by oral administration of an AT1R antagonist reduces mTOR/S6K1 signaling and associated insulin resistance in the Zucker obese (ZO) rat heart (61). However, the mTOR/S6K1 signaling pathway in the heart is associated with a Janus effect. Increased mTORC1-mediated translation underlies cardiac hypertrophy and is implicated in cardiovascular dysfunction. Meanwhile, cardiac overexpression of mTOR protects against cardiac dysfunction following left ventricular pressure overload through activation of AT1R, which provides a downstream cardioprotective compensatory servoregulatory mechanism (61).

The mTOR signaling pathway is involved in autophagy, a primary normal cellular-integrity degradative pathway that regulates cellular homeostasis via degradation of aggregated proteins, damaged organelles, and intracellular pathogens (83). Dysfunction of autophagy may contribute to the development of overnutrition-related cardiac dysfunction in CRS via its association with mTOR. Autophagy is tightly controlled by mTOR-dependent signaling, which phosphorylates and inhibits the UNC51-like kinase 1 (ULK1) complex and prevents autophagy induction (90). Under nutrient-rich conditions, mTOR is mainly activated through a signaling cascade involving activation of class III PI3K, phosphorylation of TSC2, and activation of the GTP-binding protein Rheb, which, in turn, activates mTOR (31). During starvation, the class III PI3K pathway is switched off; therefore, mTOR is inactive (52). In mammals, compromised cellular energy production inhibits mTOR through activation of AMPK (92). Investigations in our laboratory and others have demonstrated that overnutrition and resultant obesity activate mTOR signaling, which inhibits ULK1 activation by phosphorylating ULK1 Ser\(^{757}\) and disrupts the interaction between ULK1 and AMPK, preventing the initiation of autophagy and resulting in CRS (33, 70) (Fig. 3). Indeed, increased levels of free fatty acids (FFAs) usually increase autophagic activity through inhibition of mTORC1, promoting activation of eukaryotic initiation factor 2 (eIF-2α), and enhancing activation of PKC (84). However, dyslipidemia, a major characteristic of the CRS and diabetes, has been reported to impair the autophagic process. A Western diet high in fructose and fat also increases serum levels of triglycerides and cholesterol, which may lead to impairment of autophagy. The impairment of autophagy due to prolonged lipid exposure can be attributed to defects in autophagosome and lysosome fusion (35). Therefore, it is likely that the beneficial effects of mTORC1 inhibition contribute to increasing autophagy in patients with cardiac dysfunction.

Role of mTOR in Immune Regulation

Dysfunctional immunity and cardiovascular insulin resistance. Accumulating evidence suggests that dysfunctional innate and adaptive immunity contributes to cardiovascular dysfunction in overnutrition conditions and obesity (5, 18, 65). Systemic and cardiovascular insulin resistance is associated with infiltration of macrophages into adipose tissue, liver, and cardiovascular tissues (65, 76). Macrophage polarization toward enhanced M1 proinflammatory response and suppression of M2 anti-inflammatory responses (66, 76) occurs in obesity. The proinflammatory M1 macrophages secrete inflammatory cytokines, such as TNF-α that cause insulin resistance. In contrast, M2 macrophages secrete IL-10, which can improve the insulin signaling impaired by proinflammatory cytokines (5, 18). IL-10 also suppresses NADPH-mediated oxidative stress in the vasculature (32). Studies employing deletion of MR from specific cell types in mice suggest macrophage MRs play a key role in modulating macrophage polarization and promoting cardiac hypertrophy, inflammation, and fibrosis (10, 11, 51, 79). Loss of MR in macrophage-MR knockout (KO) mice results in reduction in M1 phenotype and mRNA levels for markers of vascular inflammation (10).
Role of mTOR/S6K1 signaling and maladaptive immune modulation. Recently, the mTOR/S6K1 signaling pathway has been reported to regulate innate and adaptive immune responses, including the differentiation, activation, and function of monocytes and macrophages, as well as of β-cells and CD4 and CD8 T lymphocytes (15). Activated dendritic cells (DC) are critical for the modulation of innate and adaptive immunity. Using rapamycin as an inhibitor of mTOR, Yang et al. (94) demonstrated an important role for mTOR in Toll-like receptor 4-mediated DC immune response, differentiation, antigen uptake, maturation, and migration. These studies have shown that mTOR signaling promotes proinflammatory M1 macrophage polarization (12, 53). Further, mTORC1 signaling promotes differentiation of naive CD4+ T cells into T helper (Th) 1 lymphocytes and Th17 subsets, which is an event dependent on the small GTPase Rheb (58). mTOR also modulates T-cell immune responses by promoting T-cell activation. IL-2, as well as other growth-promoting cytokines, activates mTOR. In this scenario, rapamycin functions to inhibit lymphocyte proliferation, costimulator molecule expression, and cytokine production (82). mTOR inhibition also promotes the generation of CD4+FoxP3+ regulatory T (Treg) cells both in vitro and in vivo (45). Meanwhile, rapamycin promotes Treg differentiation and the generation of memory in CD8+ T cells (59). Thus, the mTOR/S6K1 signaling pathway represents a novel target to correct abnormal metabolic regulation of immunological functions and associated systemic insulin resistance and cardiovascular dysfunction.

Targeting mTOR/S6K1 Signaling in Cardiovascular Disease

As discussed, mTOR1/S6K1 signaling is involved in CVD due to multiple interactions between metabolic signaling and immune and inflammatory responses. Therefore, modulation of mTOR1/S6K1 signaling is an attractive strategy for treatment of cardiovascular complications of obesity and diabetes. Rapamycin is an FDA-approved drug for coating coronary stents, treating renal cancer, and suppression of immune response following transplant surgery (36). Although several derivatives of rapamycin (rapalogs) are now available, these drugs have not shown promising results in human studies when used for the treatment of cancer (36). In this regard, it is noteworthy that prolonged inhibition of mTOR1 may result in either enhanced PI3K and Akt signaling (36), and persistent activation of Akt is often associated with cardiac dysfunction (91). Therefore, specific targeting of mTOR1/S6K (95) appears to be more beneficial in improving cardiovascular function in obesity and diabetes. Several FDA-approved drugs targeting glycemic control and inappropriate RAAS activation, as well as reducing CVD risk in obesity and diabetes, also inhibit mTOR/S6K1 signaling. For example, aspirin decreases TNF-α-induced activation of S6 phosphorylation and activates AMPK (22). Metformin, one of the first-line diabetic drugs, activates AMPK and inhibits mTOR1 (9). There is evidence that this drug may reduce the development and progression of various cancers and may also decrease mortality from all causes, including diabetes and CVD (21). Another example is resveratrol, a phytonutrient that may have cardiovascular protective effects through inhibition of mTOR1/S6K signaling in animal models of aging and diabetes (54). Inhibition of RAAS signaling and the associated attenuation of mTOR/S6K has also been reported to restore insulin sensitivity and ameliorate CVD (60, 70). Thus, there is evidence that strategies that interrupt excessive mTOR/S6K signaling can reduce insulin resistance and associated medical disorders.

Conclusion

The mTOR/S6K1 signaling pathway plays an important role in insulin signaling, autophagy, cardiovascular function, and immune regulation. Thus, it is important to better understand the upstream and downstream mTOR signaling pathways and their roles in the regulation of metabolic and cardiovascular physiology. Finally, development of more refined and effective therapeutic strategies for the regulation of the mTOR/S6K1 signaling pathway remains an important goal for future research.

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DISCLOSURES

James R. Sowers is on the advisory board for Merck Pharmaceuticals.

AUTHOR CONTRIBUTIONS


REFERENCES

Review


