

Review

The Impact of Overnutrition on Insulin Metabolic Signaling in the Heart and the Kidney

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Key Words

Overnutrition • Hypertension • Insulin metabolic signaling • Progressive kidney disease

Abstract

Overnutrition characterized by overconsumption of food rich in fat and carbohydrates is a significant contributor to hypertension, type 2 diabetes, and the cardiorenal syndrome. Overnutrition activates the renin-angiotensin-aldosterone system (RAAS) and causes chronic exposure of cardiovascular and renal tissue to increased circulating nutrients, insulin (INS), and angiotensin II (ANG II). Emerging evidence suggests that overnutrition, aldosterone, and ANG II promote INS resistance, a chronic condition that underlies these co-morbidities, through activation of the mammalian target of the rapamycin (mTOR)/S6 kinase 1 (S6K1) signaling pathway in cardiovascular tissue and the kidney. However, a novel ANG II type 2 receptor (AT₂R)-mediated cross talk between the RAAS and mTOR pathways ameliorates overnutrition-induced activation of mTOR/S6K1 signaling in cardiovascular tissue of rats, mice, and humans and confers cardioprotection.

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Introduction

Diminished insulin (INS) metabolic signaling is a common feature of disease states such as obesity, hypertension, diabetes, and the cardiorenal metabolic syndrome (CRS). Overnutrition (especially that characterized by excess intake of fat and carbohydrates) is a major

factor in the increased prevalence of hypertension, CRS, and diabetes. These co-morbidities may be driven by decreases in INS-mediated vascular relaxation, and glucose transport in cardiovascular (CV) and skeletal muscle tissue [1–7]. In addition to overnutrition, several other mechanisms, such as enhanced activation of the renin-angiotensin-aldosterone system (RAAS), inflammation, and associated abnormalities in INS metabolic signaling, may help explain the linkage between INS resistance, hypertension and the CRS [8–15].

There is emerging evidence that overnutrition, aldosterone and angiotensin (ANG) II may promote INS resistance through the mammalian target of rapamycin (mTOR)/S6 kinase 1 (S6K1) signaling pathway. The mTOR molecule, a highly conserved nutrient sensor, modulates INS metabolic signaling through its phosphorylation [(P)] of S6K1, an evolutionarily conserved serine (Ser) kinase [16–21]. Evidence is mounting that chronic activation of S6K1, by excessive nutrients, promotes INS resistance in fat, liver, heart, skeletal muscle, and renal tissue through increased Ser(P) of the critical INS signaling/docking molecule, INS receptor substrate protein-1 (IRS-1), leading to impaired phosphoinositol 3-kinase (PI3-K) engagement and protein kinase B (Akt) stimulation [21–23]. Our recent work indicates that S6K1 is activated in CV tissue of an overnutrition rodent model that exhibits diminished INS metabolic signaling and biological consequences, such as impaired nitric oxide (NO)-mediated vascular relaxation, cardiac diastolic dysfunction, and promotion of kidney tubulointerstitial fibrosis.

Research conducted in classically INS-sensitive tissue, such as muscle and liver, indicates that S6K1 is activated in conditions of INS resistance due to excessive intake of fat, sucrose, and protein [17–20]. Evidence for the importance of S6K1 signaling in INS resistance is based on findings that a murine knockout model of S6K1 mice maintained on a high-fat diet still remains INS sensitive (fig. 1) and siRNA knockdown of S6K1 protein in cells potentiates INS metabolic signaling [16]. Further, Ser(P) of IRS-1 was reduced in S6K1^{-/-} mice and siRNA-treated cells [16]. Studies conducted in fibroblasts exhibiting constitutive S6K1 activation revealed that certain IRS-1 Ser moieties (Ser²⁶⁵, Ser³⁰², Ser⁶³², and Ser¹⁰⁹⁷ of mouse IRS-1) are substrates for S6K1 [22]. In man, overnutrition-related reductions in glucose disposal are associated with overactivation of S6K1 and IRS-1 Ser(P) [19]. Thus, S6K1 is a convergence point that has evolved to suppress INS metabolic signaling under conditions of nutrient overload.

Over the past several years, we have explored the mechanisms by which ANG II and aldosterone contribute to CV and skeletal muscle INS resistance [2, 10]. Recent research from our laboratory suggests that ANG II may inhibit INS metabolic signaling in CV tissue, in part by promoting S6K1-mediated IRS-1 Ser(P). This work in cultured endothelial cells and cardiomyocytes, and in ex vivo CV and skeletal muscle tissue, suggests that excessive Ser(P) of IRS-1 interferes with IRS-1/phosphoinositol 3-kinase (PI3-K) docking and the consequent activation of Akt. In skeletal muscle, cardiomyocytes and vascular smooth muscle cells (VSMCs), the PI3-K/Akt pathway stimulates glucose transporter-4 (GLUT4) recruitment to the plasma membrane resulting in glucose uptake in all three tissues, as well as relaxation of the vasculature [24, 25] and diastolic relaxation of the heart [26–28]. INS-mediated signaling via this pathway promotes INS-induced vasorelaxation through increased endothelial NO synthase (eNOS) activity and reductions in myosin light chain (MLC) activation in VSMCs and decreases in calcium (Ca²⁺) in cardiac tissue [29].

Utilizing rodent models of RAAS activation (ANG II-infused C57BL/6 mice and transgenic Ren2 rats) and overnutrition [db/db mice and Zucker Obese (ZO) rats], we have recently made the novel observation that cardiac and skeletal muscle S6K1 activation and Ser(P) of IRS-1 is increased in concert with reduced tissue INS metabolic signaling [2, 6, 10]. In vivo treatment with a low dose of ANG II type 1 receptor (AT₁R) blocker substantially reduced S6K1 activation/IRS-1 Ser(P) and improved INS metabolic signaling. Emerging data suggest that AT₁R-mediated activation of mTOR/S6K1 signaling is involved in cardiac hy-

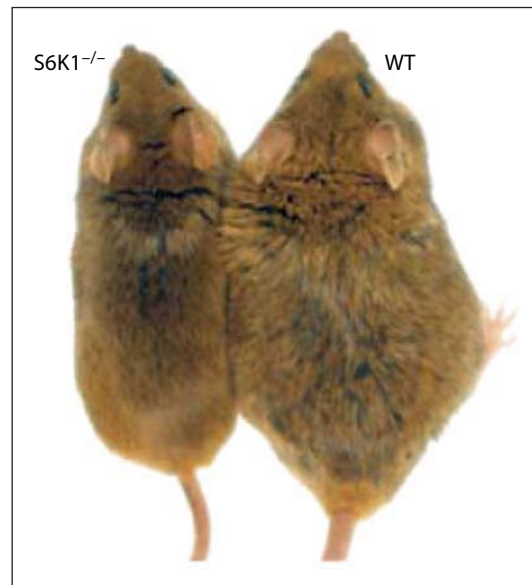


Fig. 1. Wild-type (WT) and S6K1^{-/-} mice after 6 months of high-fat diet. Reprinted with permission from Macmillan Publishers Ltd. (*Nature* [16], Copyright 2004).

peritrophy and increases protein synthesis in VSMCs [30–32]. Thus, AT₁R-induced mTOR/S6K1 activation interferes with INS metabolic signaling and biological responses in CV as well as other tissues.

CV Effects of Overnutrition and RAAS (mTOR/S6K1)-Mediated INS Resistance

INS induces vasodilation by enhancing Akt stimulation of eNOS(P) activation and NO production. The role of INS-stimulated IRS-1 tyrosine (Tyr) (P) in mediating eNOS activation is underscored by the observation that IRS-1 overexpression in aortic endothelial cells increases NO production [33], and introduction of a mutant IRS-1 unable to bind to the p85 subunit of PI3-K [33] reduces INS-stimulated NO production. In VSMC, ANG II increases intracellular calcium ([Ca²⁺]_i) and promotes MLC kinase activation [34]. INS decreases ANG II-induced increases in VSMC [Ca²⁺]_i and MLC kinase activity [1]. INS normally promotes myocardial glucose uptake and utilization, mechanical-electrical coupling and diastolic relaxation via signaling through the IRS-1/PI3-K/Akt pathway [35–37] (fig. 2). INS metabolic signaling increases coronary vessel NO production which, in turn, contributes to the beneficial effects of INS on glucose uptake, coronary blood flow, and diastolic relaxation [36–41]. These observations suggest coupling between the metabolic and coronary vascular actions of INS metabolic signaling in the heart. These beneficial cardiac effects of INS metabolic signaling are decreased in states of INS resistance [37–44]. We have observed ANG II-induced inhibition of NO production can be reversed by inhibition of mTOR/S6K1 in endothelial cells. Cardiomyocytes and cardiac fibroblasts express high-affinity AT₁R [10] and many of the adverse effects of ANG II are due to AT₁R-mediated signaling.

To evaluate the cardiac functional effects of INS metabolic signaling we utilize positron emission tomography (PET). In the INS-resistant state, myocardial glucose uptake and metabolism is impaired, leading to diastolic dysfunction, attenuated myocardial blood flow, and impaired ischemic reconditioning. PET imaging, using ¹⁸F-deoxyglucose (¹⁸FDG), is used to evaluate INS-stimulated glucose uptake (fig. 3) [41, 45]. We have utilized magnetic resonance imaging in conditions of overnutrition and excessive tissue RAAS activation, and

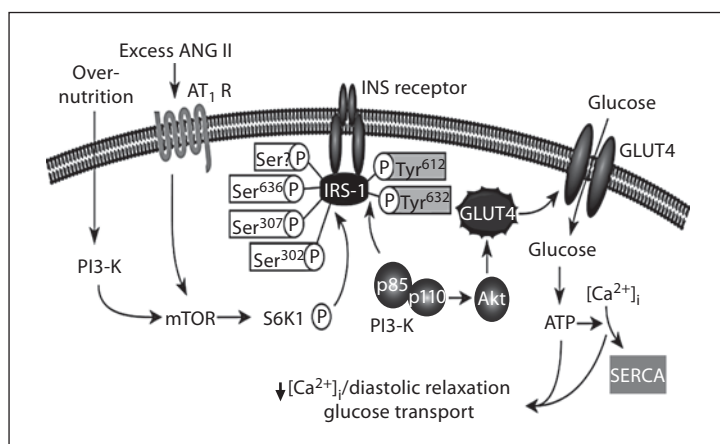


Fig. 2. Function/metabolic effects of INS in cardiac muscle cells.

have demonstrated impaired/prolonged diastolic relaxation [7]. We have further demonstrated that both impaired INS-stimulated glucose uptake and diastolic dysfunction are related to impaired systemic and myocardial INS metabolic signaling in models of obesity and increased tissue RAAS expression [2, 7]. Interestingly, we have also observed that drug treatments that improved INS resistance in rodent models of overnutrition attenuated mTOR/S6K1 signaling. Collectively, these observations indicate that a combination of enhanced tissue RAAS activation and a westernized high-sucrose/high-fat diet will reduce INS metabolic signaling and enhance mTOR/S6K1 activation to a greater extent than either intervention alone.

AT₂R↔mTOR Signaling Loop in Overnutrition, Hyperinsulinemia, and Excess RAAS Activation

Accumulating evidence suggests that AT₂R is a modulator of cardiac pathology [46–51]. AT₂R is up-regulated in failing human hearts [50], the vasculature of diabetic patients [47], and animal models of INS resistance, myocardial infarction, senescence, and hyperinsulinemia [49, 52]. AT₂R signaling reduces fibroblast growth and myocardial hypertrophy [53]. The suppression of AT₂R activation interferes with anti-hypertrophic/anti-fibrotic effects of AT₁R blockade in experimental myocardial infarction [54–58]. In cardiomyocyte-specific AT₂R transgenic mice, moderate overexpression of AT₂R in ventricular myocytes is cardioprotective under conditions of pressure overload induced by aortic banding [54]. On the other hand, excessive AT₂R overexpression in ventricular myocytes leads to dilated cardiomyopathy [56].

Signaling pathways that increase AT₂R protein in cardiac pathology are not well understood. Interestingly, hyperinsulinemia-induced cardiac hypertrophy is accompanied by a reduction in AT₁R, an increase in AT₂R, and activation of S6K1 via the PI3-K/Akt signaling pathway [57]. The association between increases in AT₂R protein and S6K1 activation in INS-induced cardiac hypertrophy prompts us to posit that mTOR/S6K1-induced increases in translation could, in part, contribute to increases in AT₂R protein levels. Thus, conditions that activate mTOR-mediated signaling in cardiac tissue could also elevate AT₂R protein levels [58–60]. Activated mTOR nucleates two large protein complexes, mTOR-Raptor complex 1

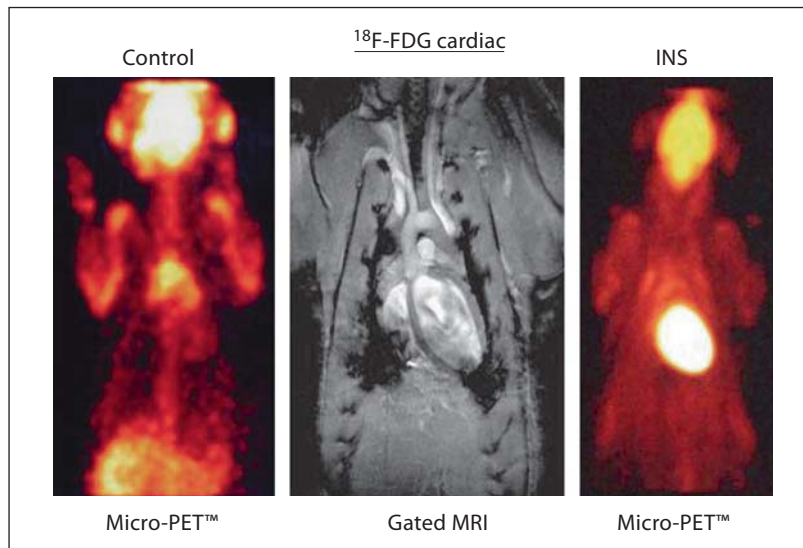


Fig. 3. Micro-PET determination of INS/glucose uptake [Cooper, Am J Physiol Heart Circ Physiol, 2007; American Physiological Society, used with permission].

(mTORC1) and mTOR-Rictor complex 2 (mTORC2) that mediate mTOR signaling. When associated with Raptor, mTOR functions as the physiological Thr³⁸⁹ kinase for its substrate S6K1 [61, 62]. Ribosomal protein S6 (RPS6) is a substrate of S6K1 and a key factor for protein synthesis in various cell types [62, 63]. 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 translational repressor by binding to translation initiation factor eIF4E. In this context, mTORC1 enhances protein synthesis by inhibitory phosphorylation of 4E-BP on Thr³⁷ and Thr⁴⁶. Interestingly, hyperinsulinemia-induced cardiac hypertrophy is accompanied by a reduction in AT₁R, an increase in AT₂R, and activation of S6K1 [57]. The association between increases in AT₂R protein and S6K1 activation in INS-induced cardiac hypertrophy prompted us to posit the existence of an mTOR-S6K1↔AT₂R signaling loop that may serve to protect the heart during conditions of overnutrition.

We have recently shown that the ZO rat, a genetic model for overnutrition and the CRS, has significant maladaptive changes in cardiac tissue [2, 7, 10]. The ZO rat heart exhibits prolonged diastolic relaxation time and reduced initial diastolic filling rate as well as increased interstitial and pericapillary fibrosis, and elevated 3-nitrotyrosine and NADPH oxidase-dependent superoxide production compared to heart tissue of syngeneic Zucker lean (ZL) controls [7]. Since overnutrition, a predicted activator of mTOR, is the key contributor for cardiac pathology in this model system, we tested for co-existence of mTOR-mediated signaling and elevation of AT₂R in the left ventricle of the ZO rat heart. Our recent data show increased cardiac activation of mTOR signaling, characterized by downstream activation of RPS6 and inhibition of 4E-BP in hypertrophic hearts of 12-week-old ZO rats, which manifest diastolic dysfunction. Moreover, in the ZO rat heart, AMP kinase, normally a compensatory negative modulator of mTOR, remained unactivated in ZO compared to ZL cardiac tissue. Second, as in the case of failing human hearts, we observed that dysfunctional, hypertrophied ZO rat hearts exhibited an increase in AT₂R protein levels. Since AT₂R functions as a cell growth inhibitor and has cardioprotective effects, this observation suggests that the increase in the AT₂R protein can be a downstream event of mTOR-S6K1 signaling and the subsequent increase in translation (fig. 4). Additionally, we observed that chronic exposure

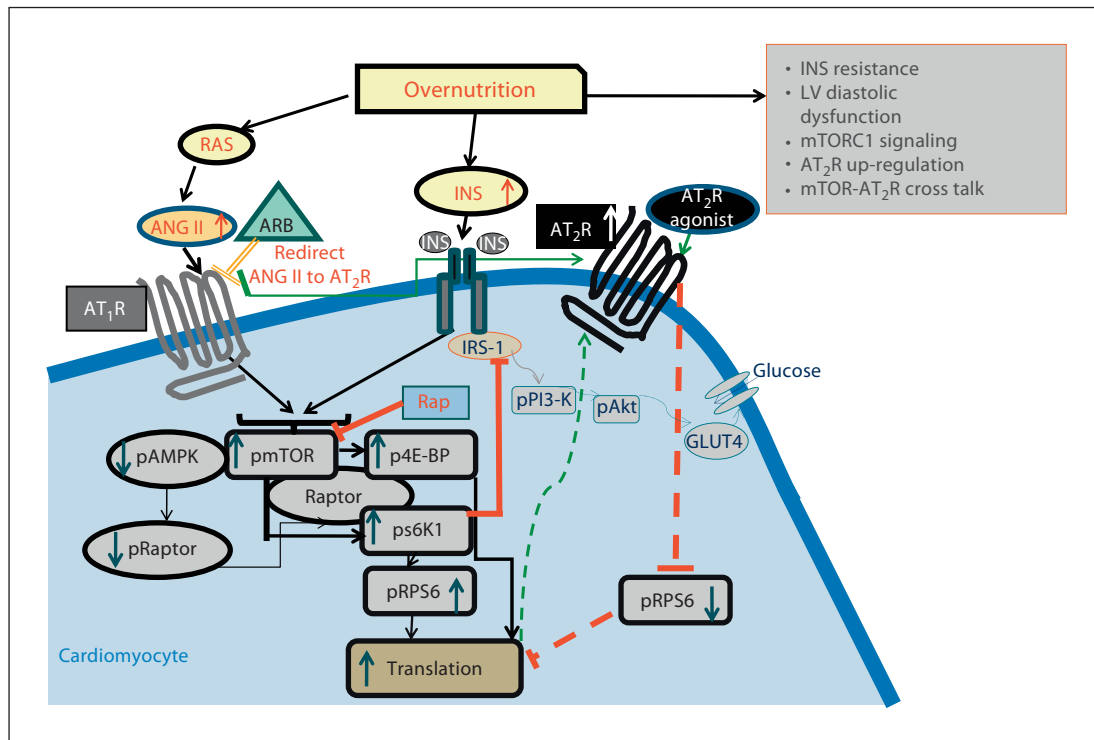


Fig. 4. Up-regulation of the AT_2R may be a protective feedback mechanism to regulate excess mTOR/S6K1 signaling in cardiomyocytes. Excess nutrients (due to overnutrition) or chronic exposure to ANG II/INS can activate mTOR/S6K1 signaling, increase translation, induce myocardial remodeling and left-ventricular hypertrophy, and up-regulate AT_2R protein levels. AT_2R can be activated by redirected ANG II (due to ANG receptor blocker-mediated inhibition of ANG II binding to the AT_1R) or AT_2R agonist. AT_2R signaling inhibits phosphorylation of RPS6 and regulates excess mTOR/S6K1 signaling and subsequent cardiac pathology.

to ANG II or INS induced mTOR/S6K1 signaling and increased AT_2R protein in mouse cardiomyocytes and human VSMCs. Interestingly, drug treatments that attenuated mTOR/S6K1 signaling in CV tissue of the ZO rat, mouse cardiomyocytes or human VSMCs also reduced AT_2R protein. Conversely, agonist activation of AT_2R in cardiomyocytes inhibited stimulatory phosphorylation of RPS6, the downstream effector of mTOR/S6K1 signaling. These findings suggest a novel branch of cross talk between RAAS and mTOR mediated by AT_2R and the existence of a novel signaling loop (mTOR \leftrightarrow AT_2R signaling loop) in CV tissue of rats, mice and humans in conditions of overnutrition.

Impact of Overnutrition and mTOR Signaling on the Kidney

There is emerging evidence that the mTOR/S6K1 pathway is involved in a spectrum of kidney diseases, including that of diabetic kidney disease [64–70]. Diabetic kidney disease is often characterized by progression of albuminuria to proteinuria and a gradual but progressive decline in glomerular filtration rate [71, 72]. Many individuals who are destined to develop diabetic kidney disease manifest the clinical characteristics of the CRS prior to the development of overt diabetes [72]. In evolving through the renal abnormalities character-

izing the CRS to that of diabetic kidney disease, the morphological process is characterized by glomerular hypertrophy and hyperfiltration, podocyte loss and associated glomerulosclerosis, and finally progressive tubulointerstitial fibrosis [72].

The initial hypertrophic process is likely mediated by overnutrition and RAAS stimulation of the mTOR signaling pathway [64, 70, 73]. The mTOR molecule is a large protein with many domains, and when complexed with GβL and Raptor (regulatory-associated protein of mTOR) it forms the rapamycin-sensitive complex referred to as mTORC1 [35, 44, 63]. The activity of mTORC1 appears to be regulated through a dynamic interaction between mTOR and Raptor mediated by GβL. While the initial hypertrophic and proliferative lesions in diabetic kidney disease are now thought to occur through mTOR, the regulatory role of S6K1, the substrate of mTORC1, in the kidney appears to occur through promotion of proximal tubule epithelial cell fibrosis [64–70]. Recent data suggest that targeting reductions in mTOR activity by targeting mTORC1 and S6K1 with rapamycin treatment improves tubulointerstitial fibrosis and proteinuria in rodent models of diabetic nephropathy and polycystic kidney disease [64–70].

Rapamycin is clinically used to suppress rejection of transplanted organs. In addition to its immunosuppressive actions, rapamycin inhibits growth factor-mediated proliferation and promotes survival of many non-immune cells, including renal tubular cells and fibroblasts [64–70, 73–75]. There is increasing interest in using rapamycin to promote prolonged survival in aging as well as expanding its use in chronic disease management.

Recent data suggest that aldosterone stimulates proliferation of mesangial cells [76], in part through downstream mTOR/S6K1 signaling [76]. Tubulointerstitial fibrosis is the critical mechanism by which tubular atrophy and loss of nephron mass occur and thereby promote progressive kidney disease [77, 78]. In young rodent models of kidney disease, ANG II has been shown to induce epithelial-mesenchymal transition (EMT) through actions on the AT₁R in the proximal tubule, a process that leads to tubulointerstitial fibrosis [79–82].

EMT and disruption of adhesion molecules contribute to tubulointerstitial fibrosis [83, 84]. EMT is a phenotypic conversion with loss of epithelial cell-basement membrane adherens junctions and acquisition of a fibroblastic phenotype. Disruption of adhesion molecules is the first phase of EMT that results in tissue fibrosis [85–87]. Recent evidence has established EMT as a critical initial mechanism for tubulointerstitial fibrosis in humans and other species. Tubulointerstitial fibrosis is characterized by loss of differentiated epithelial cells and activation of renal fibroblasts leading to fibrosis. Under unstressed, basal conditions, proximal tubule cells are attached to each other and to the basement membrane through specialized junctional complexes (adherens junctions) that include molecules such as cadherin. During injury, epithelial cells lose polarity and the mechanisms of adhesion. The best-studied cadherin in the promotion of EMT is E-cadherin, which typically resides in epithelial tissue; however, it is not found in human proximal tubule cells. Recent data suggest the cadherin in the kidney and specific to the proximal tubule is N-cadherin [87, 88]. N-cadherin in the proximal tubule has been shown to bind cytoskeletal components that provide a structural foundation for adherens junctions. Of note, cadherins not only function as static structural components of adherens junctions but also play a role in cell signaling pathways [88]. Alterations in cadherin expression have been studied in various carcinogenesis models to understand the mechanism of EMT in the fibrotic process [83–85]. Recent evidence further indicates the mTOR pathway is a critical player in the progression of tubulointerstitial fibrosis to progressive kidney disease [64–70]. Therefore, the collective evidence from rodent and culture models further suggests targeting reductions in RAAS activity may slow progressive kidney disease through potential mechanisms that improve cadherin expression as well as promote survival [10] (fig. 5).

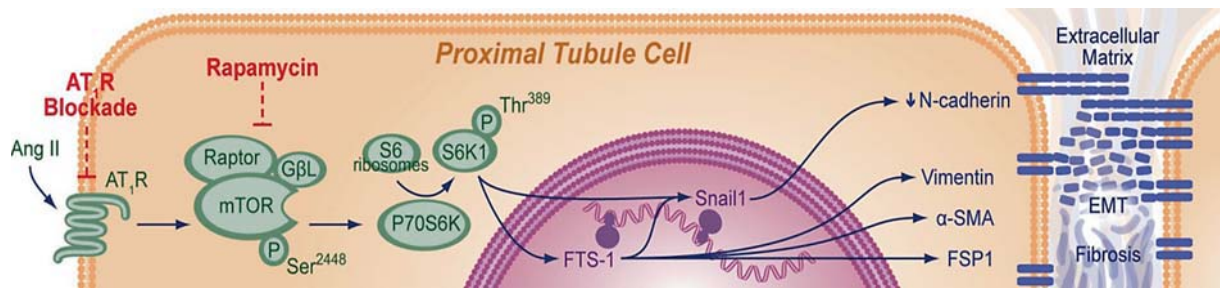


Fig. 5. Proposed mechanism by which ANG II promotes an exaggerated response on mTOR in the kidney with downstream activation of S6K1 and associated tubulointerstitial fibrosis. FTS-1 = Fibroblast transcription site-1; FSP1 = fibroblast secretory protein-1.

Disclosure Statement

The authors have no conflict of interest.

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