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mTORC1 inhibitors rapamycin and metformin affect cardiovascular markers differentially in ZDF rats¹

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Abstract

Mammalian target for rapamycin complex 1 (mTORC1) is a common target for the action of immunosuppressant macrolide rapamycin and glucose-lowering metformin. Inhibition of mTORC1 can exert both beneficial and detrimental effects in different pathologies. Here, we investigated the differential effects of rapamycin (1.2 mg/kg per day delivered subcutaneously for 6 weeks) and metformin (300 mg/kg per day delivered orally for 11 weeks) treatments on male Zucker diabetic fatty (ZDF) rats that mimic the cardiorenal pathology of type 2 diabetic patients and progress to insulin insufficiency. Rapamycin and metformin improved proteinuria, and rapamycin also reduced urinary gamma glutamyl transferase (GGT) indicating improvement of tubular health. Metformin reduced food and water intake, and urinary sodium and potassium, whereas rapamycin increased urinary sodium. Metformin reduced plasma alkaline phosphatase, but induced transaminitis as evidenced by significant increases in plasma AST and ALT. Metformin also induced hyperinsulinemia, but did not suppress fasting plasma glucose after ZDF rats reached 17 weeks of age, and worsened lipid profile. Rapamycin also induced mild transaminitis. Additionally, both rapamycin and metformin increased plasma uric acid and creatinine, biomarkers for cardiovascular and renal disease. These observations define how

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rapamycin and metformin differentially modulate metabolic profiles that regulate cardiorenal pathology in conditions of severe type 2 diabetes.

Abstract

La cible du complexe 1 de la rapamycine chez les mammifères (mTORC1) est une cible courante pour l'action de la rapamycine, un macrolide, et de la metformine, un hypoglycémiant. L'inhibition de la mTORC1 peut exercer des effets bénéfiques comme néfastes dans le cadre de différentes pathologies. Dans les présents travaux, nous avons étudié les effets différentiels de l'exposition à la rapamycine (à 1,2 mg/kg par jour par voie sous-cutanée pendant 6 semaines) et à la metformine (à 300 mg/kg par jour par voie orale pendant 11 semaines) chez des rats Zucker diabétiques obèses (ZDF) qui reproduisent la pathologie cardiorénale des patients atteints de diabète de type 2 ainsi que l'évolution vers le déficit en insuline. La rapamycine et la metformine ont permis d'atténuer la protéinurie, et la rapamycine a aussi permis de diminuer les taux urinaires de gamma glutamyl transférase (GGT), ce qui correspond à une amélioration sur le plan de la santé des tubules. La metformine a entraîné une diminution de l'absorption d'aliments et d'eau, ainsi que de la production de sodium et de potassium urinaire, alors que la rapamycine a entraîné une augmentation des concentrations urinaires de sodium. La metformine a entraîné une diminution des taux de phosphatase alcaline dans le plasma, mais elle a provoqué une hausse des transaminases mise en évidence par une augmentation de l'activité plasmatique de l'AST et de l'ALT. La metformine a aussi provoqué une hyperinsulinémie, mais elle n'a pas entraîné de diminution de la glycémie à jeun après que les rats ZDF aient atteint l'âge de 17 semaines; elle a aussi entraîné une aggravation sur le plan du profil lipidique. La rapamycine a aussi provoqué une légère hausse des transaminases. De plus, tant la rapamycine que la metformine ont entraîné une augmentation des concentrations plasmatiques d'acide urique et de créatinine, des biomarqueurs de la maladie cardiovasculaire et rénale. Ces observations caractérisent la manière dont la rapamycine et la metformine assurent une modulation différentielle des profils métaboliques qui régissent la pathologie cardiorénale en présence de diabète de type 2 grave. [Traduit par la Rédaction]

Keywords

Zucker diabetic fatty rat; rapamycin; metformin; cardiorenal pathologies; rat Zucker diabétique obèse; rapamycine; metformine; pathologies cardio-rénales

Introduction

Type 2 diabetes mellitus (T2DM) is an independent cardiorenal risk factor and the leading cause of chronic kidney disease (CKD) that progresses to end stage renal disease (ESRD) and dialysis (Liyanage et al. 2015). The incidence of T2DM has increased due to the ongoing obesity epidemic (Johnson et al. 2007, 2009). In T2DM patients, factors such as glucotoxicity, dyslipidemia, hyperleptinemia, pro-inflammatory cytokines, and certain diabetes drugs eventually result in “pancreas burnout”, leading to insulin deficiency and worsening cardiovascular and kidney disease (Donath et al. 2005; Lowell and Shulman 2005; Prentki and Nolan 2006). The male Zucker diabetic fatty (ZDF) rat mimics the metabolic, cardiac, and renal phenotype of this large subset of T2DM patients who become

insulin deficient (Arnold et al. 2014; Clark et al. 1983; Fredersdorf et al. 2004; Pulakat et al. 2011; Prentki and Nolan 2006). They exhibit hyperinsulinemia from the age of 6–12 weeks. Then, between 12–24 weeks, their fasting insulin levels decrease and become similar to that seen in healthy Zucker lean (ZL) rats and this decrease in insulin level coincides with disorganization and significant fibrosis of the islets of pancreas (Finegood et al. 2001). When the ZDF rats are older than 24 weeks, they manifest hypoinsulinemia and need insulin supplementation. They also exhibit prolonged severe hyperglycemia, starting at the age of 6 weeks and are reported to have diastolic dysfunction with preserved ejection fraction, reduced left ventricular mass, and kidney injury (Arnold et al. 2014; Clark et al. 1983; Fredersdorf et al. 2004; Pulakat et al. 2011).

Kidney injury manifests initially with hyperfiltration and proteinuria (De Jong and Brenner 2004; Mogensen 2008). Eventually, kidneys develop tubulointerstitial fibrosis, reduction in nephron numbers, and decline in glomerular filtration rate (GFR) (Nistala and Whaley-Connell 2013). The molecular mechanisms underlying this outcome are yet to be elucidated. Increased activation of mammalian target of rapamycin complex 1 (mTORC1) pathway is associated with T2DM, inflammation, nephron hypertrophy, hyperfiltration, and fibrosis (Chen et al. 2005, 2009; Gödel et al. 2011; Inoki et al. 2011; Jiang et al. 2013). mTORC1 consists of mTOR, regulatory-associated protein of mTOR (Raptor); mammalian lethal with Sec13 protein 8 (mLST8, also known as GbL); prolinerich AKT substrate 40 kDa (PRAS40); and DEP-domain-containing mTOR-interacting protein (Deptor). Rapamycin suppresses mTORC1 pathway by inhibiting phosphorylation of p70S6 kinase (p70S6K) at Thr³⁸⁹, which in turn activates ribosomal protein S6 and the protein synthesis machinery (Sengupta et al. 2010). Rapamycin treatment prevents nephron hypertrophy and kidney fibrosis in murine models and in humans with polycystic kidney disease and tuberous sclerosis (Chen et al. 2005, 2009; Jiang et al. 2013; Torres et al. 2010). AMP-activated protein kinase (AMPK) is a positive regulator of tuberous sclerosis complex 1 (TSC1) protein that negatively regulates Rheb/mTORC1 (Declèves et al. 2011; Torres et al. 2010). AMPK activators such as AICAR improves kidney structure and mitigates injury (Chen et al. 2014; Declèves et al. 2011). Metformin is an AMPK activator that suppresses the mTORC1 pathway. Metformin has been shown to have beneficial effects on kidney injury and proteinuria (Kim et al. 2013).

However, inhibition of mTORC1 exacerbates cardiorenal pathophysiology under certain conditions. Genetic ablation of Raptor, the scaffolding protein for mTORC1, impaired adaptive hypertrophy, altered metabolic gene expression, and caused heart failure in mice (Shende et al. 2011). In addition, rapamycin exacerbates pancreatic beta cell dysfunction and hyperglycemia, and aggravates kidney injury in a variety of conditions (Barbari et al. 2015; Constantinescu et al. 2016; Lamming et al. 2012). New onset hyperglycemia by rapamycin results in its withdrawal from 40% of kidney transplant patients (Torres et al. 2010). We showed that rapamycin treatment of male ZDF rats promoted cardiac myofibril injury, and elevated cardiac expression of miR-29 family miRNA that suppress cardioprotective myeloid cell leukemia-1 (MCL-1) (Arnold et al. 2014). In contrast, metformin causes lactic acidosis and is avoided in conditions with increased creatinine, liver failure, and heart failure due to the heightened risk of lactic acidosis (DeFronzo et al. 2016; Haloob and de Zoysa 2016). Because both rapamycin and metformin are mTORC1 inhibitors, we investigated the

differential effects of rapamycin and metformin treatments on metabolic profiles and urinary markers in male ZDF rats that mimic the pathology of T2DM patients who progress to insulin insufficiency.

Methods

Animals

All animal procedures were approved prior to the beginning of these studies by the Harry S. Truman Memorial Veterans Affairs Hospital (HSTMVH) Subcommittee for Animal Safety and University of Missouri Institutional Animal Care and Use Committee (IACUC). All animals were cared for in accordance with the Guidelines for the Care and Use of Laboratory Animals (National Institutes of Health publication 85–23). Male ZL and ZDF rats, age 5 weeks, were obtained from Charles River Laboratories and housed at the HSTMVH animal housing facility as described previously (Arnold et al. 2014). Rats were maintained on ad libitum food and water. Rapamycin treatment started at 9 weeks of age and ended at 15 weeks of age and corresponded to the time period where we previously demonstrated an increase in cardiomyocyte disorganization in ZDF rats (Fig. 1) (Arnold et al. 2014). Pellets designed to deliver rapamycin at a concentration of 1.2 mg/kg per day for 21 days (from Innovative Research of America, Inc., Sarasota, Florida) or placebo (sugar) subcutaneously were implanted in ZDF rats as described previously (ZDF-Rap and ZDF-Pl, respectively) and this procedure was repeated twice to achieve a 6-week rapamycin treatment (Arnold et al. 2014). Drinking water served as the vehicle for metformin treatment (300 mg/kg per day) of ZDF rats from 7 to 18 weeks of age and control rats received just water (ZDF-Met and ZDF-Wa, respectively). To limit the number of groups, drug treatment and their respective controls were done in ZDF rats only. Previous studies have shown that metformin treatment reduces fasting plasma glucose in ZDF rats up to the age of 12 weeks (Sreenan et al. 1996). We wanted to observe whether metformin could reduce fasting plasma glucose after the fasting plasma insulin levels in ZDF rats have reverted to normoinsulinemia (due to early stage of pancreatic islet disorganization), we extended metformin treatment to 18 weeks of age.

Plasma and urine chemistry

Animals were fasted for 6 h before blood collection and blood collection by cardiac puncture was performed as described previously (Arnold et al. 2014). Plasma analysis was performed by Comparative Clinical Pathology Services in Columbia, Missouri, USA. Plasma levels of cholesterol, triglycerides, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT), creatinine, and uric acid were measured using commercially available assays (Beckman-Coulter, Brea, California, USA) on an automated clinical chemistry instrument (AU680; Beckman-Coulter, Brea, California, USA) (Arnold et al. 2014). Glucose and insulin were measured by an automated hexokinase G-6-PDH assay and an ELISA kit specific for rat insulin, respectively, and described previously (Arnold et al. 2014). Urine was collected by placing rats in metabolic chambers for 24 h according to the protocol approved by the University of Missouri-Columbia IACUC and stored frozen at –80 °C until further use. For glucose, enzymatic creatinine (Diazyme, Poway, California, USA), β -N Acetyl glucuronidase (β -NAG), urine protein (Beckman

Coulter), and gamma glutamyl transferase (GGT) measurements, commercially available assays were used on the AU680 automated clinical chemistry analyzer mentioned above. Electrolytes (Na and K) were measured using ion-specific electrodes on the AU680 as well.

Statistical methods

Statistical analysis was performed using SigmaPlot version 12.5. Results are expressed as mean $\pm$ SEM (standard error of mean). Differences among groups were tested by using ANOVA followed by Fisher's LSD post hoc test where appropriate. Fisher's LSD was chosen among other post-hoc tests to highlight some of the pairwise comparisons that had a likelihood to demonstrate significance relatively independent of other means included in the comparisons. A p value less than 0.05 was considered statistically significant.

Results

Differential effects of rapamycin and metformin on proteinuria and kidney injury

Both metformin and rapamycin treatments reduced proteinuria, albeit to different extents. Rapamycin treatment for 6 weeks reduced proteinuria by 39% (16.99 ± 1.83 mg/mg Crt. vs. 23.69 ± 1.95 mg/mg Crt in ZDF-PI, $p < 0.05$) whereas metformin treatment for 11 weeks could reduce it by only 17% (23.34 ± 1.57 mg/mg Crt. vs. 28.14 ± 2.05 mg/mg Crt in ZDF-Wa) (Fig. 2B). Proteinuria in all ZDF groups were significantly higher than the ZL control (Fig. 2B). Urinary GGT-to-creatinine ratio is reported to be an indicator of tubular injury and osteoporosis (Asaba et al. 2006; Yesil et al. 2014). GGT-to-creatinine ratio was significantly lowered by rapamycin treatment (3.48 ± 0.39 U/mg Crt. vs. 5.2 ± 0.11 U/mg Crt in ZDF-PI, $p < 0.05$) (Fig. 2C). In contrast, metformin further tended to increase GGT-to-creatinine ratio (7.73 ± 0.67 U/mg Crt. vs. 6.04 ± 0.82 U/mg Crt. in ZDF-Wa) (Fig. 2C). Urinary β -NAG, a marker of tubular cell dysfunction and a predictor of outcome in primary glomerulonephritis (Bazzi et al. 2002) was not changed by rapamycin or metformin treatment (ZDF-Rap: 1.03 ± 0.08 U/mg Crt. vs. 1.24 ± 0.20 U/mg Crt. in ZDF-PI; ZDF-Met: 1.64 ± 0.62 U/mg Crt. vs. 1.40 ± 0.22 U/mg Crt. in ZDF-Wa) (Fig. 2D).

Rapamycin increases urine glucose and sodium excretion compared with metformin

There was no significant difference in urine glucose excretion between ZDF-PI and ZDF-Rap groups (Fig. 2A). However, urine glucose excretion was significantly higher in ZDF-Rap vs. ZDF-Met groups (50.1 ± 1.49 mmol/mg Crt. Vs. 36.54 ± 1.72 mmol/mg Crt, $p < 0.05$) and ZDF-Met significantly decreased urine glucose excretion compared with ZDF-Wa (36.54 ± 1.72 mmol/mg Crt. vs. 76.52 ± 7.21 mmol/mg Crt.) (Fig. 2A). Both urine sodium and potassium were significantly increased in all ZDF groups compared with ZL group ($p < 0.05$) (Figs. 2E and 2F). There was a significant reduction in sodium excretion in the ZDF-Met vs. ZDF-Wa group (3.25 ± 0.17 mmol/mg Crt. vs. 5.15 ± 0.26 mmol/mg Crt., $p < 0.05$) (Fig. 2E). Conversely, sodium excretion was significantly higher in the ZDF-Rap vs. ZDF-Met group (3.97 ± 0.12 mmol/mg Crt. vs. 3.25 ± 0.17 mmol/mg Crt. $p < 0.05$) (Fig. 2E). Urine potassium excretion was reduced in the ZDF-Met group when compared with ZDF-Wa group (Fig. 2F). However, there were no differences in potassium excretion between the ZDF-Rap and ZDF-Met groups.

Metformin significantly raised insulin levels but worsened the fasting lipid profile

Both metformin and rapamycin treatments did not affect plasma glucose levels towards the end of treatment periods (Figs. 3A and 4A). Metformin treatment significantly increased plasma insulin levels (866.50 ± 68.48 pmol/L in ZDF-Met vs. 413.75 ± 84.06 pmol/L in ZDF-Wa, $p < 0.05$) while rapamycin treatment did not protect from the trend towards insulinopenia in the ZDF rats (242.66 ± 28.73 pmol/L in ZLC vs. 177.69 ± 38.64 pmol/L in ZDF-PI and 157.04 ± 27.87 pmol/L in ZDF-Rap) (Fig. 3B). In addition, rapamycin treatment did not affect fasting lipid profile significantly when compared with the ZDF-PI group (Fig. 3C). However, metformin treatment increased triglycerides (9.0 ± 0.16 mmol/L vs. 4.55 ± 0.93 mmol/L in the ZDF-Wa, $p < 0.05$) (Fig. 3D) and lowered HDL (2.01 ± 0.02 mmol/L vs. 1.71 ± 0.15 mmol/L in the ZDF-Wa group) (Fig. 3E). Compared with metformin, rapamycin treatment reduced triglycerides and increased HDL in ZDF rats (Figs. 3D and 3E). Thus, metformin treatment changed the pathophysiology of T2DM in ZDF rats by inducing hyperinsulinemia and hyperlipidemia.

Differential effects of metformin and rapamycin on the liver

The plasma alkaline phosphatase (ALP) is a marker of liver disease and bone disorders (Camozzi et al. 2007; Seeff 2015). ALP levels were higher both in the ZDF-PI and ZDF-Wa groups when compared with ZLC; however, while metformin treatment significantly suppressed ALP, rapamycin treatment had no effect (19.67 ± 10.19 U/L in ZDF-Met vs. 61.0 ± 15.73 U/L in ZDF-Wa; 51.13 ± 8.04 U/L in ZDF-Rap vs. 45.75 ± 10.77 U/L in ZDF-PI; $p < 0.05$) (Fig. 3F). ALT is a reliable marker for liver injury and disease (Kim et al. 2008). Only metformin significantly elevated ALT levels (634.50 ± 67.47 U/L in ZDF-Met vs. 292.20 U/L in ZDF-Wa and 357.13 ± 99.79 U/L in ZDF-Rap vs. 161.25 ± 28.39 U/L in ZDF-PI, $p < 0.05$), suggesting greater transaminitis from metformin when compared with rapamycin (Fig. 3G). AST, also a marker of liver injury (Seeff 2015), was significantly elevated by both rapamycin and metformin when compared with their respective controls. However, compared with metformin, rapamycin only raised it to much lower levels (751 ± 33.79 U/L in ZDF-Met vs. 321.80 ± 82.21 U/L in ZDF-Wa; 334.50 ± 77.5 U/L in ZDF-Rap vs. 111.25 ± 19.62 U/L in ZDF-PI, $p < 0.05$) (Fig. 3H).

Both metformin and rapamycin increase plasma creatinine and uric acid

Plasma creatinine is a measure of kidney function and used as a reflection of the glomerular filtration rate (GFR) (KDOQI 2007). Plasma creatinine levels trended towards a higher mean in the ZDF rat compared with the ZLC rat (33.59 ± 2.55 μ mol/L in ZDF-Wa vs. 33.81 ± 2.12 μ mol/L in ZDF-PI vs. 28.88 ± 1.34 μ mol/L in ZLC) (Fig. 3I). Importantly, metformin and rapamycin treatments increased plasma creatinine further (38.19 ± 0.89 μ mol/L in ZDF-Met and 42.76 ± 1.90 μ mol/L in ZDF-Rap, $p < 0.05$) suggesting a reduction in GFR in both metformin- and rapamycin-treated groups (Fig. 3I). Uric acid is known to act as a pro-oxidant at higher concentrations and contribute to oxidative stress and cardiovascular diseases associated with T2DM (Koenig and Meisinger 2008; Zhu et al. 2015). While ZDF rats had a significant increase in plasma uric acid levels compared with ZL controls (105.99 ± 27.94 μ mol/L in ZDF-Wa and 83.29 ± 0.00 μ mol/L in ZDF-PI vs. 20.12 ± 4.48 μ mol/L in

ZLC), metformin and rapamycin further increased uric acid levels in ZDF rats (ZDF-Met: 192.77 ± 17.61 $\mu\text{mol/L}$ and ZDF-Rap: 153.90 ± 14.55 $\mu\text{mol/L}$) (Fig. 3J).

Discussion

T2DM and diabetic kidney disease present a challenge to the physician with regards to the ideal combination of drugs to simultaneously ameliorate both pathologies whilst not worsening one or the other. Metformin is generally the first choice oral hypoglycemic in diabetic patients due to its safety profile and important and favorable metabolic effects (Nathan et al. 2009). With accumulating “lack of evidence” for increased lactic acidosis due to metformin in men with creatinine > 1.5 mg/dL and women with creatinine > 1.4 mg/dL, the current recommendations are to start adjusting dose in subjects with eGFR < 45 mL/min and to discontinue in those with eGFR < 30 mL/min (Lipska et al. 2011). However, its effects on kidney function per se remain largely unknown. In contrast, rapamycin is an immunosuppressant drug that is prescribed in the post-kidney-transplant setting and its use in the pre-kidney-transplant setting is limited to polycystic kidney disease and (or) tuberous sclerosis or in “coating” drug-eluting stents in cardiac patients (Torres et al. 2010). However, rapamycin has been studied more extensively as a possible therapy for preventing progression of diabetic kidney disease because activation of mTORC1 occurs in diabetes-associated cardiorenal diseases in both human subjects and murine models (Gödel et al. 2011; Inoki et al. 2011). Because metformin will remain on board in diabetic kidney disease patients till their eGFR < 45 mL/min as per new recommendations, and because rapamycin is known to contribute to T2DM also known as New Onset DM (NOD) in transplant recipients, we examined the differential effects of these 2 drugs on the metabolic profile of ZDF rats. Male ZDF rats were chosen for this study because the metabolic and cardiorenal pathology of ZDF rat mimics the pathology of the subpopulation of T2DM patients who suffer from pancreas burn out. We observed that both rapamycin and metformin improve proteinuria, although the improvement by rapamycin was much more in magnitude. The improvement by rapamycin appears to be related to improvement in tubular integrity as measured by GGT-to-creatinine ratio. Metformin had no beneficial effect on either GGT or β -NAG excretion levels compared with its control, ZDF-Wa.

Urine sodium and potassium excretion was increased in the ZDF rats which is likely a result of hyperfiltration. Rapamycin appears not to affect either sodium or potassium excretion, although the ZDF-Rap group had slightly less food consumption and lost mass, suggesting less intake of sodium (Fig. 4B). Initial body masses at the age of 8 weeks were comparable between these groups (240 ± 8 in ZDF-Rap vs. 252 ± 5 g in ZDF-PI). Taken together, the ZDF-Rap group may have higher sodium excretion when compared with the ZDF-PI group. In contrast, metformin treatment lowered urine sodium and potassium excretion when compared with the ZDF-Wa, which suggests downregulation of tubuloglomerular feedback (TGF). Initial body masses at the age of 7 weeks were comparable between these groups (223 ± 5 in ZDF-Wa vs. 229 ± 4.4 g in ZDF-Met). However, food and water consumption was markedly reduced along with significant mass loss in the metformin-treated group, which suggests restoration of sodium balance (Figs. 4B, 4C, and 4D).

The plasma measurements were more surprising from the point of view of kidney function and uric acid levels. Plasma creatinine values worsened 25%–33% both in the rapamycin- and metformin-treated groups suggesting a reduction in GFR. This could be an effect of both of these drugs to reduce the hyperfiltration via improvements in nephron size and function via mTORC1 inhibition. Another potential mechanism could be the activation of TGF resulting in a reduction of GFR and subsequent rise in creatinine, which is a mechanism involved in the action of SGLT2 inhibitors (Cherney et al. 2014). Also, regulation of vascular tone in the afferent and efferent arterioles may contribute to hyperfiltration and reduction in efferent arteriolar tone may in turn reduce GFR (Schoolwerth et al. 2001). Alternatively, worsening creatinine could be a result of deterioration in heart function and reduction in kidney perfusion that results from a decrease in cardiac output or right heart failure with reduction in forward flow (Arnold et al. 2014). However, we have not measured cardiac function in these rats. Uric acid is now believed to be an oxidant (although it was believed to be an antioxidant) and has been shown to be associated strongly with diabetic kidney injury (Johnson et al. 2009; De Cosmo et al. 2015). Several mechanisms for increase in uric acid levels can be activated in the ZDF rats including activation of the renin–angiotensin–aldosterone system, and severe hyperglycemia. Increase in plasma uric acid levels may reflect the increased conversion of glucose to uric acid in the liver via the pentose phosphate pathway. This is supported by increased liver enzymes measured in the plasma particularly by metformin and to a lesser extent by rapamycin.

Metformin was able to suppress fasting plasma glucose levels in the initial stage of disease in the ZDF rats (Fig. 4A). However, as disease progressed and animals reached 17 weeks of age, metformin was unable to suppress fasting plasma glucose. Metformin caused a greater transaminitis when compared with rapamycin, although both drugs increased transaminase levels when compared with their respective controls. In addition, metformin significantly worsened the lipid profile, suggesting that its use in advanced diabetic disease (eGFR < 45 mL/min) may be limited by these adverse effects on the metabolic profile in addition to the risk of lactic acidosis. However, the incidence of hepatotoxicity with metformin is low to rare in humans (<1% of patients) and is characterized by different combinations of hepatocellular and cholestatic hepatitis in an even lower number of subjects. This may be explained partly by the discontinuation of metformin in advanced cases of diabetes in the past (Crp > 1.5 mg/dL in males and >1.4 mg/dL in females).

Both rapamycin and metformin are mTORC1 inhibitors, albeit in very different ways. While rapamycin inhibits mTOR-mediated phosphorylation of p70S6K, metformin potentiates AMPK mediated suppression of mTORC1/p70S6K activation via TSC1. These differences in the level of drug action may lead to feedback inhibition loop activation, whereby mitogenic (MAPK/ERK, p90RSK), insulin signaling (IRS1, Akt), and survival (Akt, Bcl-2) pathways may be differentially activated and suppressed. Cumulative effects of these and other molecular circuits may manifest in the observed differential regulation of cardiorenal pathophysiology by these drugs in ZDF rats.

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Fig. 1.

Schematic depicting the drug treatment paradigms for Zucker diabetic fatty rats. Rapamycin (Rap) treatment was initiated by subcutaneous implantation of pellets at 9 weeks of age and continued till sacrifice at 15 weeks of age. Metformin (Met) treatment in drinking water was started at 7 weeks of age and continued till animals were sacrificed at 18 weeks of age. Symbols depict the interventions. [Color online.]

Fig. 2.

Graphical representation of urine parameters. (A) Glucose, (B) Protein, (C) GGT, (D) β-NAG, (E) Sodium, (F) Potassium. ZLC, Zucker lean control; ZDF-Wa, Zucker diabetic fatty – water only; ZDF-Met, Zucker diabetic fatty – metformin only; ZDF-Pl, Zucker diabetic fatty – placebo only ($n = 4$); ZDF-Rap, Zucker diabetic fatty – rapamycin only. * $p < 0.05$ vs. ZLC, # $p < 0.05$ ZDF-Met vs. ZDF-Wa, & $p < 0.05$ ZDF-Rap vs. ZDF-Met, and \$ $p < 0.05$ ZDF-Rap vs. ZDF-Pl.

Fig. 3.

Graphical representation of plasma parameters. (A) Fasting glucose, (B) Insulin, (C) Cholesterol, (D) Triglycerides, (E) HDL, (F) ALP, (G) ALT, (H) AST, (I) Creatinine, (J) Uric acid. ZLC, Zucker lean control; ZDF-Wa, Zucker diabetic fatty – water only; ZDF-Met, Zucker diabetic fatty – metformin only; ZDF-Pl, Zucker diabetic fatty – placebo only; ZDF-Rap, Zucker diabetic fatty – rapamycin only. * $p < 0.05$ vs. ZLC, # $p < 0.05$ ZDF-Met vs. ZDF-Wa, & $p < 0.05$ ZDF-Rap vs. ZDF-Met, and \$ $p < 0.05$ ZDF-Rap vs. ZDF-Pl.

Fig. 4.

Graphical representation of metabolic parameters. (A) Fasting glucose, (B) Food Intake per day, (C) Water intake per day, (D) Body mass. ZDF-Wa, Zucker diabetic fatty – water only; ZDF-Met, Zucker diabetic fatty – metformin only; ZDF-Pl, Zucker diabetic fatty – placebo only; ZDF-Rap, Zucker diabetic fatty – rapamycin only. * $p < 0.05$ ZDF-Met vs. rest, # $p < 0.05$ ZDF-Rap vs. ZDF-Pl, and & $p < 0.05$ ZDF-Rap vs. ZDF-Met.