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THE METABOLIC SYNDROME INDUCES EARLY CHANGES IN THE SWINE RENAL MEDULLARY MITOCHONDRIA

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Abstract

The metabolic syndrome (MetS) is associated with nutrient surplus and kidney hyperfiltration, accelerating chronic renal failure. Mitochondria can be overwhelmed by substrate excess, leading to inefficient energy production and thereby tissue hypoxia. Mitochondrial dysfunction is emerging as an important determinant of renal damage, but whether it contributes to MetS-induced renal injury remains unknown. We hypothesized that early MetS induces kidney mitochondrial abnormalities and dysfunction, which would be notable in the vulnerable renal medulla. Pigs were studied after 16 weeks of diet-induced MetS, MetS treated for the last 4 weeks with the mitochondria-targeted peptide elamipretide (ELAM, 0.1mg/kg SC q.d), and Lean controls (n=7 each). Single-kidney renal blood flow (RBF), glomerular-filtration-rate (GFR), and oxygenation were measured in-vivo, while cortical and medullary mitochondrial structure and function and renal injurious pathways were studied ex-vivo. Blood pressure was slightly elevated in MetS pigs, and their RBF and GFR were elevated. Blood oxygen level dependent magnetic resonance imaging demonstrated that this was associated with medullary hypoxia, whereas cortical oxygenation remained intact. MetS decreased renal content of the inner mitochondrial membrane cardiolipin, particularly the tetra-linoleoyl (C18:2) cardiolipin species, and altered mitochondrial morphology and function, particularly in the medullary thick ascending limb (mTAL). MetS also increased renal cytochrome-c-induced apoptosis, oxidative stress, and tubular injury. Chronic mitoprotection restored mitochondrial structure, ATP synthesis and antioxidant defenses, and decreased mitochondrial oxidative stress, medullary hypoxia, and renal injury. These findings implicate medullary mitochondrial damage in renal injury in experimental MetS, and position the mitochondria as a therapeutic target.

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CONFLICT OF INTERESTS

All authors have read the journal's policy on disclosure of potential conflicts of interest. Dr. A. Lerman is a consultant to Stealth BioTherapeutics, Inc.

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Keywords

Metabolic syndrome; Mitochondria; Kidney; Cardiolipin

INTRODUCTION

The metabolic syndrome (MetS), a cluster of major cardiovascular risk factors related to dyslipidemia, obesity, and insulin resistance, affects over 20% of adults in western populations (1). Previous studies have linked MetS with development of chronic kidney disease (2), and their co-existence is associated with increased cardiovascular disease mortality (3, 4). Better understanding of the mechanisms triggering renal injury in MetS may help develop novel strategies to attenuate renal structural and functional deterioration in these patients.

In order to supply energy for active transport of solutes, renal tubular cells possess a high number of mitochondria (5), which also maintain cellular homeostasis by regulating redox state, proliferation, apoptosis, and calcium signaling (6). Emerging evidence suggests that in rodent models diabetic nephropathy is associated with mitochondrial dysfunction. For example, impaired mitochondrial complex-III in the kidney of genetic murine models of diabetes is associated with hyperfiltration and histopathological lesions (7). Increased formation of reactive oxygen species (ROS) is likely a major contributor to mitochondrial dysfunction in diabetes (8), as is hyperglycemia. Glucose overload has been associated with increased renal mitochondrial ROS production, which in turn damages mitochondrial proteins, lipids, and DNA, compromising ATP production, and ultimately impairing renal structure and function (9). Indeed, preservation of the mitochondrial electron transport chain restores mitochondrial function and morphology, and reduces glomerular hyperfiltration and proteinuria in mice with diabetic nephropathy (10). However, whether mitochondrial injury and dysfunction are implicated in renal injury in pre-diabetic normoglycemic MetS remains to be clarified.

Early MetS is characterized by increased systemic markers of lipid oxidation, such as oxidized low-density lipoprotein (Ox-LDL) and isoprostanes, which contribute to development of insulin resistance (11). In the mitochondria, lipid peroxidation particularly afflicts cardiolipin, a phospholipid exclusively expressed on the inner mitochondrial membrane that plays a central role in preserving mitochondrial structure and function (12). The mitochondria-targeted peptide elamipretide (ELAM) is a small compound that specifically binds with high affinity to cardiolipin to preserve or restore mitochondrial energetics (12). Furthermore, this tetrapeptide possesses antioxidant properties and inhibits the activity of cytochrome-c peroxidase, a catalyzer of cardiolipin peroxidation that induced opening of the mitochondrial permeability transition pore (mPTP). We have previously shown that treatment with ELAM restores renal function in swine renovascular disease (13, 14). Furthermore, a recent study shows that ELAM prevents high fat diet-induced glomerulopathy and proximal tubular injury in mice (15), but whether this approach attenuates renal injury in MetS has not been explored in a large preclinical experimental model. This study tested the hypothesis that in normoglycemic pigs, diet-induced MetS

induces mitochondrial and tubular injury, which mitoprotection with ELAM would attenuate.

MATERIALS AND METHODS

Animal characteristics and experimental protocol

Twenty-one juvenile (3-month old) female farm pigs were studied during 16 weeks of observation (Figure 1A). At baseline animals were randomized into two groups: MetS (n=14) and Lean (n=7). MetS pigs started a high-cholesterol/carbohydrate diet (16), while Lean animals were fed with standard pig chow.

Twelve weeks later, 7 MetS pigs started a regimen of daily subcutaneous injections of ELAM (Stealth BioTherapeutics Inc.), 0.1 mg/kg in 1 mL of phosphate-buffered saline (PBS), 5 days/week, consistent with previous experiences with rabbits, pigs, and guinea pigs (17, 18). A PBS vehicle was injected in the remaining seven MetS pigs.

Four weeks later, animals were anesthetized with IM tiletamine hydrochloride/zolazepam hydrochloride and xylazine, and maintained with intravenous ketamine and xylazine. Single-kidney hemodynamics and function were assessed using multi-detector computed tomography (MDCT) and renal oxygenation by blood oxygen level dependent magnetic resonance imaging (BOLD-MRI). Fasting blood samples were collected in anesthetized pigs prior to MDCT studies to assess levels of lipids, isoprostanes, plasma renin activity (PRA), and creatinine (19), and for measurements of glucose and insulin levels for calculation of the homeostasis model assessment of insulin resistance (HOMA-IR) (16, 20, 21). Mean arterial pressure was monitored during MDCT studies using an 8F arterial catheter inserted in the left carotid artery, as previously described (22).

One week after completion of all studies, pigs were euthanized with a lethal intravenous dose of sodium pentobarbital (14). The kidneys were removed using a retroperitoneal incision and immediately dissected, and sections frozen in liquid nitrogen or preserved in formalin or Trump's fixative for ex-vivo studies. For some assays, cortical and medullary tissues were discerned and analyzed separately.

In-vivo studies

BOLD-MRI was performed on a 3 Tesla scanner using fast gradient echo with multiple echo times to measure R2* levels (hypoxic index) in the renal medulla and cortex. Data were analyzed using a histogram-based compartmental method (23), which provides comparable and less variable kidney R2* estimations, and eliminates the need for anatomical cortical and medullary definition. MDCT scanning was performed 3–4 days after BOLD-MRI using a Flash-128 scanner, as previously described (22, 24). A bolus of iopamidol was followed by a flow study, 140 scans, as per routine techniques. Using the AnalyzeTM software, cross-sectional MDCT images were reconstructed, and renal volume, renal vascular resistance (RVR), renal blood flow (RBF), and glomerular filtration rate (GFR) measured, as previously described (22, 24).

Ex-vivo studies

Cardiolipin content, synthesis, and remodeling—Renal cardiolipin content was assessed by mass spectrometry, as described (25). Renal expression of cardiolipin synthase (CRLS)-1 was measured by western blot (26), and mRNA expression of the cardiolipin remodeling enzyme tafazzin (Taz)-1 (27) by real-time quantitative polymerase chain reaction (28).

Mitochondrial morphology and function—Mitochondrial density and morphology was assessed in randomly selected medullary thick ascending limb (mTAL) and proximal tubular (PT) cells, representing cortical and medullary tubular segments, using a digital electron microscopy. The number of mitochondria/cell was averaged to obtain mitochondrial number, and mitochondrial area density measured using Image-J software (29, 30).

Additionally, renal mitochondria were isolated from the cortex and medulla using the MITO-ISO kit (31), and their ATP and ADP levels measured by colorimetric/fluorometric methods. Mitochondrial hydrogen peroxide (H₂O₂) production was measured by colorimetric quantitative methods (32), as well as glutathione activity (GSH/GSSG) and NADPH generation (NADPH/NADP). Cortical and medullary expression of the cytochrome oxidase subunits (COX)-I, II, III, and IV, superoxide dismutase (SOD)-1, SOD-2, and thioredoxin (TRX)-2 were assessed by Western-blotting (33), as were renal mitochondrial levels of peroxisome proliferator-activated receptor (PPAR)- α , optic atrophy (OPA)-1, dynamin-related protein (DRP)-1, and p62.

Renal injury pathways—Renal cortical and medullary apoptosis was assessed in cross-sections of the kidney co-stained with terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) or activated caspase-3 (13, 34). Renal protein expression of the apoptosis regulators B-cell lymphoma (Bcl)-xl and Bcl2-associated X protein (Bax) was assessed by Western-Blotting (14). Renal mitochondrial cytochrome-c release was assessed by the ratio between its mitochondrial and cellular expression, and necrosis by cortical and medullary levels of lactate dehydrogenase (LDH). Renal lipid peroxidation and oxidative stress were evaluated by Ox-LDL staining (35) and in-situ production of superoxide anion by fluorescence microscopy using dihydroethidium (DHE) (19). Intrarenal fat deposition was measured by Oil-red-O staining (21). Renal fibrosis was assessed in renal cross-sections stained with trichrome (14). Tubular injury was scored in a blinded fashion in sections stained with Periodic acid-Schiff (PAS) (13). All quantifications were performed in a blinded manner.

Statistical methods

Results are expressed as mean $\pm$ standard deviation. Comparisons within groups were performed using Student t-test and among groups using analysis-of-variance, followed by Tukey's test as appropriate. Statistical significance was accepted for p 0.05.

For details, please see online supplement.

RESULTS

After 16 weeks of MetS or lean diet, body weight, mean blood pressure, lipid fractions, fasting insulin, and HOMA-IR levels were significantly higher in MetS compared to Lean pigs (Table 1). Fasting glucose, serum creatinine and PRA levels did not differ among the groups.

MetS induces renal hyperfiltration and medullary hypoxia

Cortical and medullary volumes were greater in MetS compared to Lean, and RBF and GFR were elevated (Table 1). Regional perfusion was relatively preserved, yet RVR decreased in MetS groups. Cortical oxygenation was unaltered, whereas medullary R2* (hypoxic index) was elevated in MetS (Table 1).

MetS induces mitochondrial damage and dysfunction

Renal total cardiolipin content decreased in MetS compared to Lean (Figure 1B), particularly the tetra-linoleoyl (C18:2) cardiolipin species (Figure S1). Contrarily, renal cardiolipin C18:1 and C18:0 were higher in MetS compared to Lean, as were renal cardiolipin hydrolysis products (lyso-cardiolipin) (Figure 1C). Renal expression of CRLS-1 remained unaltered (Figure 1D), whereas mRNA expression of Taz-1 was reduced in MetS (Figure 1E).

Electron microscopy revealed in MetS preserved renal tubular mitochondrial number and area (Figure 2A). Contrarily, mitochondrial matrix density decreased in MetS mTAL, but not PT cells. The ratio of ATP/ADP content in isolated renal cortical and medullary mitochondria decreased in MetS compared to Lean, suggesting impaired mitochondrial ATP synthesis (Figure 2B). Despite decreased ATP production, expression of COX subunits I, II, III, and IV was preserved in the cortex and medulla of MetS, suggesting impaired COX activity (Figure S2). Furthermore, mitochondrial oxidative stress increased in the medulla of MetS, reflected in elevated H₂O₂ production (Figure 2C). In addition, medullary mitochondrial glutathione activity and generation of NADPH, as well as SOD-2 and TRX-2 expressions were blunted in MetS (Figure S3), suggesting impaired mitochondrial antioxidant defenses.

Mitochondrial PPAR- α expression decreased in MetS (Figure S4), suggesting decreased biogenesis, but OPA-1 levels were unchanged, indicating preserved fusion. Mitochondrial dynamin-related protein DRP-1 levels were higher in MetS, suggesting increased fission. Yet, expression of p62 was downregulated in MetS, indicating increased mitophagy (Figure S4).

MetS induces early renal changes

The number of TUNEL and caspase-3 positive cells was elevated in the MetS cortex and medulla compared to Lean (Figure 3A), as was renal expression of Bax and Bax/Bcl-xl ratio (Figure 3B). Furthermore, the ratio of mitochondrial/cellular cytochrome-c expression decreased in MetS compared to Lean (Figure 3C), suggesting release from mitochondria to

the cytosol. LDH levels were also elevated in the MetS medulla compared to Lean (Figure 3D), implying increased necrosis.

Circulating levels of isoprostanes (Table 1), renal superoxide anion production (Figure 4A), and renal expression of Ox-LDL (Figure 4B) were notably higher in MetS compared to Lean. Intrarenal fat deposition was considerably greater in PT and mTAL of MetS (Figure S5A–B), as was tubular injury (Figure 4C). Contrarily, tubulointerstitial fibrosis was unchanged (Figure 4D).

Mitoprotection attenuates MetS-induced renal injury

Treatment with ELAM decreased medullary hypoxia (Table 1) and normalized renal cardiolipin content (Figure 1B) and remodeling (Figure 1E). ELAM also restored cardiolipin species profile (Figure S1 and 1C) and improved mitochondrial density in mTAL cells (Figure 2A). Additionally, ELAM normalized cortical and medullary ATP/ADP ratio (Figure 2B), and medullary H₂O₂ production (Figure 2C), glutathione activity, NADPH generation, and SOD-2 abundance (Figure S3). Furthermore, ELAM restored mitochondria-specific PPAR- α and p62 (Figure S4), and decreased the number of TUNEL and caspase-3 positive cells (Figure 3A). Interestingly, renal expression of Bax remained unchanged, but expression of Bcl-xl was exclusively upregulated in ELAM-treated pigs (Figure 3B), thereby normalizing Bax-Bcl-xl ratio, suggesting that ELAM attenuated renal apoptosis primarily by increasing the expression of anti-apoptotic factors rather than downregulating the expression of pro-apoptotic molecules. ELAM also prevented mitochondrial cytochrome-c release (Figure 3C), and attenuated cortical and medullary necrosis (Figure 3D). Furthermore, tubular injury score decreased in MetS+ELAM-treated pigs (Figure 4C), whereas ELAM did not affect renal perfusion, RBF, or GFR.

ELAM also decreased systemic and renal oxidative stress, illustrated by lower circulating levels of isoprostanes (Table 1), renal superoxide anion production (Figure 4A), and expression of OX-LDL (Figure 4B).

DISCUSSION

This study shows that early MetS induces mitochondrial abnormalities and dysfunction, particularly in the mTAL, which is associated with glomerular hyperfiltration and medullary hypoxia. Furthermore, we found that chronic mitoprotection restored medullary mitochondrial structure and function, improved medullary oxygenation, and decreased renal injury, implicating mitochondrial damage in renal injury in experimental MetS and positioning the mitochondria as a therapeutic target.

To explore the mechanism underling MetS-induced renal injury, we took advantage of an early MetS swine model that recapitulates common features of human MetS (16), and cutting-edge techniques that permit studying single-kidney function and structure. After 16 weeks of diet, our MetS pigs developed obesity and dyslipidemia, accompanied by a spontaneous increase in blood pressure and emergence of insulin resistance (HOMA-IR), indicating successful development of MetS. Contrarily, glucose levels remained unaltered, indicating non-diabetic MetS. Renal dysfunction in MetS pigs was characterized by

hyperfiltration, evidenced by increased GFR, associated with medullary hypoxia. Medullary volume increased in MetS compared to Lean, consistent with more extensive oxygen consumption, which leaves this region relatively more hypoxic compared to cortical segments (36). MetS also induced renal apoptosis, which was greater in cortical than medullary regions, yet LDH levels were elevated only in the MetS medulla, implying increased necrosis. Given that under certain conditions renal apoptotic cells are unstable and undergo secondary necrosis, (37, 38), our results suggest that medullary cells might have a higher propensity to switch from apoptosis to adverse necrosis. On the other hand, oxidative stress and tubular injury in MetS were elevated in both the cortex and medulla, underscoring the role of MetS as an important risk factor for kidney damage.

Moreover, the present study shows that renal injury and dysfunction are associated with renal mitochondrial abnormalities and dysfunction. We found that MetS decreased cardiolipin content, although CRLS-1 expression remained unchanged, arguing against MetS-induced modulation of de-novo cardiolipin synthesis. Furthermore, MetS impaired cardiolipin remodeling, reflected in decreased levels of Taz-1. Interestingly, MetS decreased 18:2 species, increased more saturated species like C18:1 and C18:0, as well as renal cardiolipin hydrolysis products (lyso-cardiolipin), implying cardiolipin oxidation. Cardiolipin is essential for cristae membrane formation and organizes the components of the electron transport chain, preserving mitochondrial integrity and structure (39). In our MetS model cardiolipin oxidation was accompanied by decreased mitochondrial density in mTAL, but not in PT cells, suggesting that MetS induces selective destabilization of cristae membranes in vulnerable mTAL cells.

Importantly, mitochondrial bioenergetics were also impaired. Mitochondrial ATP synthesis decreased in the cortex and medulla of MetS, but was not associated with changes in COX subunit expression, implying decreased COX activity. Furthermore, early MetS increased medullary mitochondrial H₂O₂ production and decreased mitochondrial antioxidant defenses, disclosed by reduced glutathione activity, NADPH generation, as well as SOD-2 and TRX-2 expression, consistent with the mitochondrial ROS overproduction and SOD-2 polymorphism observed in diabetes (40, 41). ROS can damage cardiolipin, respiratory complex proteins, and nucleic acids, creating a vicious cycle that aggravates mitochondrial injury. Importantly, our study suggests that these alterations may precede overt diabetes or might be triggered by mechanisms other than elevated glucose levels. Renal adiposity, lipid peroxidation, and elevated levels of isoprostanes observed in MetS likely contribute to renal mitochondrial injury, given that mitochondrial cardiolipin commonly undergoes peroxidation and loss (12, 42). Furthermore, MetS-induced hypoxia in the susceptible renal medulla (43), revealed by BOLD-MRI, may compromise mitochondrial respiration (44), while oxidative stress increases oxygen utilization, aggravating renal hypoxia. Reciprocally, decreased mitochondrial ATP synthesis may have partly contributed to medullary hypoxia in MetS, although we cannot rule out other contributing factors including changes in medullary volume, oxidative stress, apoptosis, necrosis, and tubular injury. Taken together, MetS-induced renal oxidative stress and hypoxia might contribute to mitochondrial abnormalities and dysfunction.

Furthermore, loss of cardiolipin impaired mitochondrial biogenesis, by decreasing the expression of PPAR- α . Although levels of the fusion marker OPA-1 remained unchanged, DRP-1 levels were higher and p62 lower in MetS compared to Lean, indicating increased mitochondrial fragmentation and degradation.

Cardiolipin loss, increased mitochondrial H₂O₂ production, and decreased mitochondrial redox-buffering capacity might have triggered renal oxidative stress, disclosed by increased Ox-LDL expression and in-situ production of superoxide anion. Oxidative stress can in turn amplify renal as well as myocardial dysfunction and injury in MetS (45). Interestingly, tubulointerstitial fibrosis was yet undetectable in our MetS pigs, possibly due to the early stage of MetS.

Furthermore, we found that MetS induced glomerular hyperfiltration, which characterizes both MetS and early diabetes and is associated with elevated metabolic risk (46). We have previously shown that renal hyperfiltration in Ossabaw swine obesity is accompanied by significant intrarenal adiposity, oxidative stress, inflammation, and microvascular remodeling (20, 21). Several factors have been identified as potential mediators of renal hyperfiltration in MetS (47), including the effects of elevated insulin levels. Importantly, increased GFR and avid medullary tubular oxygen-dependent transport without a parallel increase in medullary perfusion might have contributed to medullary hypoxia observed in MetS pigs. Nevertheless, renal perfusion was preserved in MetS pigs, possibly due to vasodilation, implied by decreased RVR.

To establish the contribution of mitochondria to MetS-induced renal injury, we treated MetS pigs with ELAM, a compound that improves renal mitochondrial function in pigs with renovascular disease (13, 14) and in mice fed high-fat diet (15). The current study extends previous observations, demonstrating that ELAM prevents cardiolipin loss, restores mitochondrial remodeling and redox status, and attenuates lipid peroxidation, likely due to a decrease in oxidative stress and an increase in antioxidant systems. Remarkably, mitoprotection in turn ameliorated renal injury (apoptosis, necrosis, oxidative stress, and tubular injury), implying that mitochondrial injury may contribute to development of renal injury and dysfunction in MetS. The capability to prevent cardiolipin peroxidation and loss, restore mitochondrial energetics, and inhibit cytochrome-c peroxidase activity confers ELAM with mitoprotective potency. Furthermore, inhibition of mPTP opening and release of cytochrome-c and ROS to the cytosol might have attenuated renal cell apoptosis and oxidative stress. Indeed, cytochrome-c-induced apoptosis, increased in MetS compared to Lean, normalized in ELAM-treated pigs.

This study is limited by the short duration of the diet, yet our model mimics many features of human disease, providing a solid experimental platform to study the effect of MetS on the kidney. Renal injury in our model is subtle, yet we identified several structural and functional changes in the kidney that place the mitochondria as an early target organelle for MetS. Although 4-week treatment with ELAM sufficed to confer protective effects, additional studies will need to explore its long-term effects on the kidney in MetS. The anesthetic regimen used in this study might have also masked a rise in blood pressure, as previous studies have shown that a combination of ketamine and xylazine decreases blood

pressure by approximately 30% (48). These drugs may have also induced acute hyperglycemia (49) or modulated plasma insulin levels, which could impact our assessment of insulin resistance (50). Future studies are needed to test whether MetS-induced renal changes and the renoprotective properties of ELAM are observed in conscious pigs. Despite increased RBF and GFR in MetS pigs, PRA levels remain unchanged in MetS, consistent with our previous findings in swine obesity (51) and renovascular disease (52). ELAM did not affect PRA levels, arguing against regulation of the systemic renin/angiotensin system, consistent with our previous finding that ELAM does not directly regulate vascular tone (13). Further studies will be needed to explore an effect on blood pressure.

In summary, our study shows that MetS induces mitochondrial injury and early structural and functional changes in the kidney, characterized by glomerular hyperfiltration and medullary hypoxia, possibly related to increased oxidative stress, and mild tubular injury. Interestingly, the majority of the early changes were observed in the medulla, suggesting regional differences in renal susceptibility to MetS. Possibly, cellular apoptosis might have served to protect cortical tubular cells. Notably, chronic mitoprotection preserved mitochondrial bioenergetics and mTAL matrix density, and improved renal structure. Although ELAM is rapidly removed from the plasma compartment, its action in the inner mitochondrial membrane is sustained for a longer period of time, prolonging its protective effects (53). Therefore, our observations support a role for renal mitochondrial injury and dysfunction in experimental MetS. Further exploration of the role of mitochondria in MetS-induced renal disease could contribute to the development of adequate therapies to protect the kidney in these patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

MetS	metabolic syndrome
GFR	glomerular filtration rate
ROS	reactive oxygen species
Ox-LDL	oxidized low-density lipoprotein
ELAM	elamipretide
mPTP	mitochondrial permeability transition pore
PBS	phosphate-buffered saline
PRA	plasma renin activity

HOMA-IR	homeostasis model assessment of insulin resistance
MDCT	multi-detector computed tomography
BOLD-MRI	blood oxygen level dependent magnetic resonance imaging
RVR	renal vascular resistance
CRLS	cardiolipin synthase
mTAL	medullary thick ascending limb
PT	proximal tubular
H₂O₂	hydrogen peroxide
Taz	taffazin
COX	cytochrome oxidase
SOD	superoxide dismutase
TRX	thioredoxin
PPAR	peroxisome proliferator-activated receptor
OPA	optic atrophy
DRP	dynamamin-related protein
LDH	lactate dehydrogenase
TUNEL	terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling
Bax	B-cell lymphoma (Bcl)-xl and Bcl2-associated X protein
DHE	dihydroethidium
PAS	Periodic acid-Schiff

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Brief Commentary

Our study demonstrates that metabolic syndrome induces early structural and functional changes in the kidney, associated with renal mitochondrial abnormalities and dysfunction, particularly in the medullary thick ascending limb. Notably, chronic mitoprotection with the mitochondria targeted peptide elamipretide restores mitochondrial structure and function, improves medullary oxygenation, and decreases renal injury. Therefore, our observations implicate mitochondrial damage in renal injury and position mitochondria as a therapeutic target in experimental early metabolic syndrome.

Figure 1.

A: Schematic of the experimental protocol. B: Renal cardiolipin content decreased in MetS, but normalized in MetS+ELAM (n=7/group). C: Lyso-cardiolipin content, increased in MetS compared to lean, but decreased in ELAM-treated pigs (n=7/group). D: Renal expression of cardiolipin synthase (CRLS)-1 did not differ among the groups. E: Renal expression of taffazin (Taz)-1 decreased in MetS, but normalized in MetS+ELAM. *p 0.05 vs. Lean, †p 0.05 vs. MetS+ELAM.

Figure 2.

A: Transmission electron microscopy and quantification of mitochondrial density, mitochondrial area, and matrix density (5 mitochondria/cell) in proximal tubular (PT) and medullary thick ascending limb (mTAL) cells in study groups (n=7/group). B: The ratio of ATP/ADP content in isolated renal cortical and medullary mitochondria decreased in MetS compared to Lean, but normalized in ELAM-treated pigs (n=7/group). C: ELAM decreased medullary mitochondrial hydrogen peroxide (H₂O₂) production (n=7/group). *p 0.05 vs. Lean, †p 0.05 vs. MetS+ELAM. Scale bar = 200nm.

Figure 3.

A: ELAM decreased the number of terminal deoxynucleotidyl transferase-mediated dUTP nick end endlabeling (TUNEL)+ and caspase-3+ cells/field in the cortex and medulla (n=7/group). B: Renal protein expression and ratio of B-cell lymphoma (Bcl)-xl and Bcl2-associated X protein (Bax) and Bcl-xl (n=7/group). C: The ratio of mitochondrial/whole tissue cytochrome-c expression decreased in MetS compared to Lean, but normalized in ELAM-treated pigs (n=7/group). D: Lactate dehydrogenase (LDH) levels were elevated in the MetS medulla compared to Lean, but normalized in MetS+ELAM (n=7/group). *p < 0.05 vs. Lean, †p < 0.05 vs. MetS+ELAM.

Figure 4.

A: Representative kidney dihydroethidium (DHE) staining (40X) and its quantification, showing increased renal oxidative stress that ELAM ameliorated (n=7/group). B: Kidney oxidized low-density lipoprotein (ox-LDL) increased in MetS, but normalized in ELAM-treated pigs (n=7/group). C: Representative kidney Periodic acid-Schiff (PAS) staining (40X) and its quantification, showing increased renal tubular injury that ELAM ameliorated (n=7/group). D: Tubulointerstitial fibrosis (trichrome staining) did not differ among the groups (n=7/group). *p < 0.05 vs. Lean, †p < 0.05 vs. MetS+ELAM. Scale bar = 50µm.

Table 1

Systemic characteristics and single-kidney function (mean±standard deviation) in anesthetized pigs (n=7 per group) at 16 weeks.

Parameter	Lean	MetS	MetS+ELAM
Body Weight (Kg)	70.0±4.5	93.2±1.0 *	92.5±3.3 *
Mean blood pressure (mmHg)	101.3±3.9	124.2±4.0 *	114.3±4.8 *
Fasting glucose (mg/dl)	115.6±31.2	109.2±26.6	108.2±28.0
Fasting insulin (μU/ml)	0.4±0.1	0.8±0.2 *	0.7±0.1 *
HOMA-IR score	0.6±0.09	1.7±0.3 *	1.6±0.8 *
Total cholesterol (mg/dl)	73.2±2.8	569.0±71.9 *	609.0±62.3 *
HDL cholesterol (mg/dl)	43.1±1.5	134.2±10.4 *	132.2±10.3 *
LDL cholesterol (mg/dl)	28.7±2.8	391.7±49.0 *	390.8±48.9 *
Triglycerides (mg/dl)	7.1±0.7	13.8±2.3 *	14.8±1.6 *
Isoprostane (pg/ml)	84.1±10.6	163.8±33.5 * [‡]	130.5±180.8 *
Serum Cr (mg/dl)	1.67±0.21	1.55±0.20	1.66±0.37
Plasma renin activity (ng/ml/h)	0.16±0.11	0.15±0.08	0.12±0.08
Total Renal Volume (cc)	135.8±9.0	220.9±10.3 *	228.5±14.1 *
Cortical Volume (cc)	105.9±8.5	180.3±11.4 *	205.9±14.2 *
Medullary Volume (cc)	29.8±1.2	40.6±2.1 * [‡]	22.6±1.6 *
Cortical Perfusion (ml/min/cc)	4.0±0.4	4.1±0.2	4.1±0.5
Medullary Perfusion (ml/min/cc)	3.0±0.5	2.8±0.7	3.0±1.0
RBF (ml/min)	505.3±31.5	861.0±106.1 *	892.3±72.5 *
RVR (mmHg/ml/min)	0.20±0.01	0.15±0.02 *	0.13±0.01 *
GFR (ml/min)	74.2±5.2	145.1±8.5 *	139.2±3.9 *
Cortical hypoxia (R2 [*])	14.7±1.3	15.5±1.5	15.2±0.8
Medullary hypoxia (R2 [*])	23.7±1.1	27.8±0.1 * [‡]	25.9±1.1 *

* p 0.05 vs. Lean,

[‡] p 0.05 vs. MetS+ELAM.

ELAM: Elamipretide, HOMA-IR: Homeostasis model assessment of insulin resistance, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, Cr: Creatinine, RBF: renal blood flow, RVR: renal vascular resistance, GFR: glomerular filtration rate.