Mitochondrial Dysfunction in Kidney Cortex and Medulla of Subtotally Nephrectomized Rats

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Summary

Five-sixths nephrectomy is a widely used experimental model of chronic kidney disease (CKD) that is associated with severe mitochondrial dysfunction of the remnant tissue. In this study, we assessed the effect of CKD on mitochondrial respiration separately in the rat kidney cortex and medulla 10 weeks after induction of CKD by subtotal 5/6 nephrectomy (SNX). Mitochondrial oxygen consumption was evaluated on mechanically permeabilized samples of kidney cortex and medulla using high-resolution respirometry and expressed per mg of tissue wet weight or IU citrate synthase (CS) activity. Mitochondrial respiration in the renal cortex of SNX rats was significantly reduced in all measured respiratory states if expressed per unit wet weight and remained lower if recalculated per IU citrate synthase activity, i.e. per mitochondrial mass. In contrast, the profound decrease in the activity of CS in SNX medulla resulted in significantly elevated respiratory states expressing the OXPHOS capacity when Complexes I and II or II only are provided with electrons, LEAK respiration after oligomycin injection, and Complex IV-linked oxygen consumption per unit CS activity suggesting compensatory hypermetabolic state in remaining functional mitochondria that is not sufficient to fully compensate for respiratory deficit expressed per tissue mass. The results document that CKD induced by 5/6 nephrectomy in the rat is likely to cause not only mitochondrial respiratory dysfunction (in the kidney cortex), but also adaptive changes in the medulla that tend to at least partially compensate for mitochondria loss.

Key words

Chronic kidney disease • Subtotal nephrectomy • Mitochondrial respiration • Cortex • Medulla

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Introduction

Chronic kidney disease (CKD) represents a serious health issue being associated with poor prognosis and enormous socioeconomic burden [1,2]. Among the key factors contributing to the onset and progression of the disease, mitochondrial dysfunction is frequently mentioned since the kidney is an organ with high-energy demand and rich in mitochondria [3]. Mitochondria are dynamic organelles that play a pivotal role not only in the conversion of energy stored in nutrients into high-energy phosphates and reactive oxygen species (ROS) generation, but also in the cellular calcium homeostasis, intermediary metabolism, thermogenesis, inflammation, differentiation, or apoptosis [4]. In addition, the number of functional mitochondria in the cell is regulated by sophisticated processes working in concert as fission and fusion (mitochondrial dynamics), mitochondrial biogenesis and mitophagy [5]. On the one hand, all these functions can be affected by other pathological processes underlying CKD; on the other

hand, they themselves are suspected to contribute to the onset and progression of the disease resulting in deterioration of basic renal functions, i.e. glomerular filtration and tubular reabsorption/ secretion [6].

Studies on the impact of CKD on renal mitochondrial oxygen consumption performed on mitochondria isolated from the whole organ or renal cortex are relatively numerous [7,8]. Surprisingly, only a small number of original research articles report parameters of mitochondrial respiration in the kidney medulla in general [9] and those related to renal pathophysiology in particular [10].

Thus, the aim of our study was to assess the effect of CKD on mitochondrial oxygen consumption separately in the rat kidney cortex and medulla 10 weeks after induction of CKD by subtotal nephrectomy (SNX).

Materials and Methods

Animals and experimental protocol

Adult Wistar male rats at the age of 4 months (Velaz, Prague, Czech Republic) underwent 5/6 nephrectomy performed in two steps (SNX; n=15) or sham-operation (SHAM; n=15) as described previously [11,12]. In the course of the following 10 weeks, serum concentrations of creatinine, urea, and potassium were monitored weekly in the blood samples obtained from the tail vein. At the end of the experiment, the animals were placed in metabolic cages and urine was collected for 24 h. Creatinine, urea and potassium concentrations in serum and urine were measured using a routine autoanalyzer system (Olympus AU 640, Mishima, Japan). Glomerular filtration rate (GFR) was estimated by determination of creatinine clearance; clearance of urea and fractional excretion of potassium were also calculated. Systolic and diastolic blood pressures were measured on days 10, 30, 50, and 70 after completed surgery using the tail-cuff method (RTBP apparatus; Kent Scientific Co., USA). After general anesthesia (urethane; 1 g/kg body weight), the rats were sacrificed by cervical dislocation and the kidneys were dissected and weighed. All experimental procedures were conducted in accordance with the relevant Guidelines of the Czech Ministry of Agriculture for scientific experimentation on animals, European legislation (European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes; 86/609/EU) and were approved by the Animal Welfare Advisory Committee at the Faculty of Medicine in Pilsen, Charles University.

High-resolution respirometry

Mitochondrial oxygen consumption was measured separately in the renal cortex and medulla in O2k oxygraphs by high-resolution respirometry (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria). The kidneys were placed in the preservation solution BIOPS of the following composition (in mmol/l): CaK_2EGTA 2.77, K_2EGTA 7.23, imidazole 20, taurine 20, MES hydrate 50, dithiothreitol 0.5, $MgCl_2 \cdot 6H_2O$ 6.56, Na_2ATP 5.77, Na_2phosphocreatine 15, pH 7.1. Samples of the kidney cortex and outer layer of the medulla (approx. 10 mg) were dissected under the magnifying glass and gently mechanically permeabilized using two pairs of sharp forceps in the respiration medium MiR05 composed of 0.5 mmol/l EGTA, 3 mmol/l $MgCl_2 \cdot 6H_2O$, 60 mmol/l potassium lactobionate, 20 mmol/l taurine, 10 mmol/l KH_2PO_4, 20 mmol/l HEPES, 110 mmol/l sucrose, and 1 g/l fatty acid free bovine serum albumin, pH 7.0 [13]. Samples were weighed (approx. 2 mg of the kidney cortex and 4 mg of the medulla) and placed into the pre-calibrated oxygraph chambers containing MiR05 solution with oxygen content equilibrated with air at 37 °C.

After closing the oxygraph chambers, substrates and inhibitors of individual complexes of the respiratory system were sequentially injected into the oxygraphs to determine the state LEAK (L; oxygen consumption needed to maintain mitochondrial membrane potential), OXPHOS (P; coupled oxygen consumption during oxidative phosphorylation when Complexes I, I+II, II are activated), ETSC (E; uncoupled respiration estimating the electron transport system capacity when oxidation is not coupled to phosphorylation), ROX (residual oxygen consumption) and CIV (Complex IV activity after stimulation with an artificial substrate). Oxygen consumption in individual respiratory states was determined online as a negative time derivative of oxygen concentration in the chamber. The following substrate-uncoupler-inhibitor-titration protocol (SUIT) [13] was used for both cortex and medulla: malate (M; 2 mmol/l) – glutamate (G; 10 mmol/l) – pyruvate (P; 5 mmol/l) [L_I] – adenosine diphosphate (D; 5 mmol/l) – cytochrome c (c; 10 µmol/l) [P_I] – succinate (S; 10 mmol/l) [P_{I+II}] – rotenone (Rot; 0.5 µmol/l) [P_{II}] – oligomycin (Omy; 2 µg/ml) [L_{OMY}], carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP; 0.5 µmol/l titrations) [E_{II}] – antimycin A (Ama; 2.5 µg/ml) [ROX] – N,N,N′,N′-te-

tramethyl-p-phenylenediamine (TMPD; 0.5 mmol/l) in the presence of ascorbate (Asc; 2 mmol/l) – sodium azide (Azd; 100 mmol/l) [C_{IV} – calculated by subtraction of Azd oxygen consumption from TMPD/Asc respiration].

A representative graph of a respirometric experiment in permeabilized kidney medulla and a scheme of the SUIT protocol are depicted in Figure 1.

The following control ratios were calculated: coupling control ratio (L_{OMY}/E_{II}) to reveal potential dyscoupling of SNX mitochondria, N/NS pathway control ratio (P_I/P_{I+II}) to determine relative contribution of Complex I-related phosphorylating respiration to that including Complexes I and II, and P_{II}/E_{II} control ratio expressing the limitation of OXPHOS capacity by the phosphorylation system.

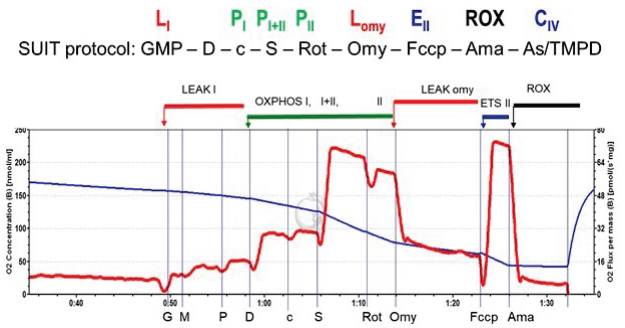

Fig. 1. A representative graph of a respirometric experiment in mechanically permeabilized kidney medulla. Red line – oxygen consumption rate, blue line – oxygen concentration in the O2k chamber. L_I (LEAK I), leak respiration in the presence of substrates providing electrons to Complex I (glutamate [G], malate [M], pyruvate, [P]), but absence of ADP (D); P_I (OXPHOS I), active phosphorylating respiration related to Complex I; P_{I+II} (OXPHOS I+II), active phosphorylating oxygen consumption when a substrate of the Complex II succinate (S) is added; P_{II} (OXPHOS II), phosphorylating state after the Complex I inhibitor rotenone (Rot); L_{OMY} (LEAK omy), leak state after ATP synthase inhibitor oligomycin; E_{II} (ETS II), electron-transporting system capacity after uncoupler (FCCP) injection; ROX, residual oxygen consumption after Antimycin A (Ama) addition. For the concentrations of substrates, inhibitors and uncoupler utilized and precise definition of respiratory states, see Methods.

Citrate synthase (CS) activity

CS activity is used to estimate mitochondrial mass in tissues. In the present study, it was measured in the homogenized content of each oxygraph chamber using the protocol described previously [14,15]. Briefly, 950 µl of the assay medium consisting of 0.1 mmol/l 5,5-dithio-bis-(2-nitrobenzoic) acid, 0.25 % Triton-X, 0.5 mmol/l oxaloacetate, 0.31 mmol/l acetyl coenzyme A, 5 µmol/l EDTA, 5 mmol/l triethanolamine hydrochloride, and 0.1 mol/l Tris-HCl, pH 8.1, was mixed with 50 µl of the sample and the rate of absorbance change was monitored over 200 s at 412 nm and 30 °C using the Life Science UV/Vis spectrophotometer DU®730 (Brea, CA, United States).

Data presentation and statistics

Blood pressure values are given in mm Hg, CS activity in IU/g tissue wet weight. Oxygen consumptions in individual respiratory states were corrected for ROX and expressed in pmol.s^{-1}.mg^{-1} or pmol.s^{-1}.IU^{-1} CS activity. In the graphs, data are presented as means ± SD. After testing for normality of distribution (Smirnov-Kolmogorov test) and, if needed, logarithmic transformation, the mean differences were analyzed by unpaired two-tailed Student's *t*-test (respirometric data, serum concentrations, creatinine clearance etc.), by the two-way ANOVA (CS activity; factors tissue type and intervention) or by the two-way mixed design ANOVA (one repeated-measures factor for analysis of the progression of parameter in time and one between-groups factor for SHAM and SNX rats) followed with *post hoc* Tukey test (blood pressure). The analyses were performed using the software Origin 2019 (OriginLab, Corp., Northampton, MA, United States). Values of $p<0.05$ were considered significant.

Results

Effects of 5/6 nephrectomy

Ten weeks after 5/6 nephrectomy, SNX rats had significantly lower body weight (395±12 g) than the SHAM animals (446±15 g). Table 1 summarizes data on some renal glomerular and tubular functions of the SHAM and SNX rats documenting severe decline in urea and creatinine clearance and an increase in potassium serum levels associated with an increase in fractional excretion of potassium in SNX rats 10 week after 5/6 nephrectomy. Remarkable hypertrophy of the remnant kidney was always present in surviving SNX animals. In 5/6 nephrectomized rats, both systolic and diastolic pressures rose significantly from day 10 after surgery. The diastolic pressure remained relatively stable from the day 30 onwards; systolic pressure values displayed a continuously increasing trend (Fig. 2).

High-Resolution Respirometry

As shown in Figure 3, mechanically permeabilized cortex and medulla displayed significantly different patterns of mitochondrial respiration. With the exception of phosphorylating Complex I activity (state P_I), all other respiratory states were significantly lower in

the medulla compared to the cortex of the control (SHAM) rats (Fig. 3A). This difference was not related to potentially different density of mitochondria, since the same characteristics was obtained when O_2 fluxes were recalculated per IU of CS activity (Fig. 3B).

Table 1. Impact of 5/6 nephrectomy on renal parameters.

	SHAM	n	SNX	n
$C_{creatinine}$ *(ml/min)*	2.24 ± 0.45	10	0.86 ± 0.38*	10
C_{urea} *(ml/min)*	1.36 ± 0.38	10	0.45 ± 0.24*	10
S_{K+} *(mmol/l)*	5.2 ± 1.1	10	6.6 ± 1.7*	10
FE_{K+} *(%)*	15.61 ± 2.36	10	38.72 ± 6.83*	10
Kidney weight (g)	1.36 ± 0.17	15	2.23 ± 0.46*	10

Creatinine and urea clearance (C), concentration of potassium in serum (S_{K+}), fractional excretion of potassium (FE_{K+}) calculated as a per cent ratio of potassium to creatinine clearance, and kidney weight of a single organ in the SHAM rats and the stump of the remnant kidney in 5/6 nephrectomized (SNX) rats. * $p<0.05$, compared to SHAM rats.

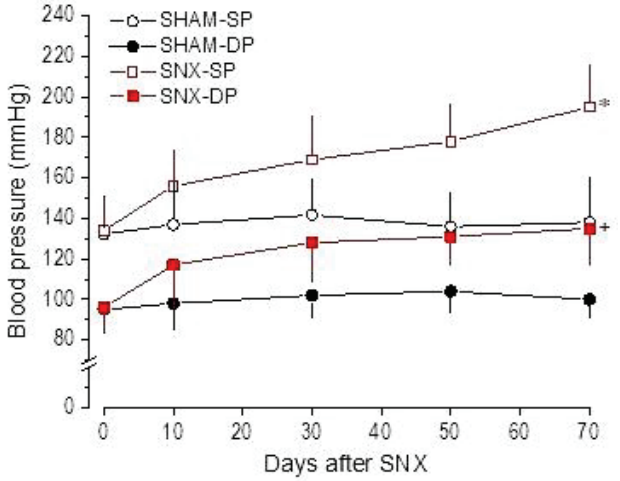

Fig. 2. Systolic (open symbols) and diastolic (closed symbols) blood pressures (SP, DP) in the control (SHAM; black lines) and 5/6 nephrectomized rats (SNX; red lines). * $p<0.05$, SP significantly higher compared to SHAM rats; + $p<0.05$, DP significantly higher compared to SHAM rats.

Renal mass reduction had an interesting impact on mitochondrial respiration of both kidney cortex and medulla. When expressed per mg of the tissue wet weight, all respiratory states were significantly lower in SNX samples compared to the SHAM ones, with the exception of the LEAK state and C_{IV} activity in the kidney medulla (Fig. 4A, C). However, normalization of oxygen fluxes to CS activity revealed that in the cortex, only states P_{I+II}, P_{II}, and E_{II} remained significantly lower in SNX than in the SHAM rats. Moreover, kidney medulla displayed significantly higher values of P_{I+II}, P_{II} and L_{OMY} in SNX compared to SHAM samples (Fig. 4B, D). Kidney medulla of SNX rats also displayed changes in the respiratory control ratios: coupling control ratio estimated as L_{OMY}/E_{II} significantly increased after 5/6 nephrectomy as did the P_{II}/E_{II} control ratio. Conversely, CI control ratio determined as P_I/P_{I+II} was decreased in the medulla of SNX rats (Fig. 4E).

Citrate synthase activity

The CS activity was slightly, but significantly higher in the kidney medulla than cortex dissected from the SHAM rats. Renal mass reduction resulted in a significant decrease in CS activity in both tissues; however, the diminution was more expressed in the medulla than in the cortex (Fig. 4F).

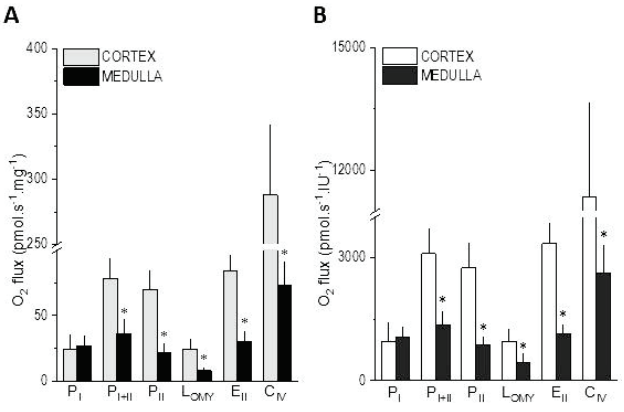

Fig. 3. Mitochondrial oxygen consumption in mechanically permeabilized samples of kidney cortex and medulla expressed per mg tissue wet weight (**A**) or IU citrate synthase activity (**B**). P_I, active phosphorylating respiration in the presence of substrates providing electrons to Complex I (glutamate, malate, pyruvate), ADP and cytochrome c; P_{I+II}, active phosphorylating oxygen consumption when a substrate of the Complex II succinate is added; P_{II}, phosphorylating state after the Complex I inhibitor rotenone; L_{OMY}, leak state after ATP synthase inhibitor oligomycin; E_{II}, electron-transporting system capacity after uncoupler injection; C_{IV}, respiration related to the Complex IV activity. * $p<0.05$, compared to the same respiratory state in the cortex.

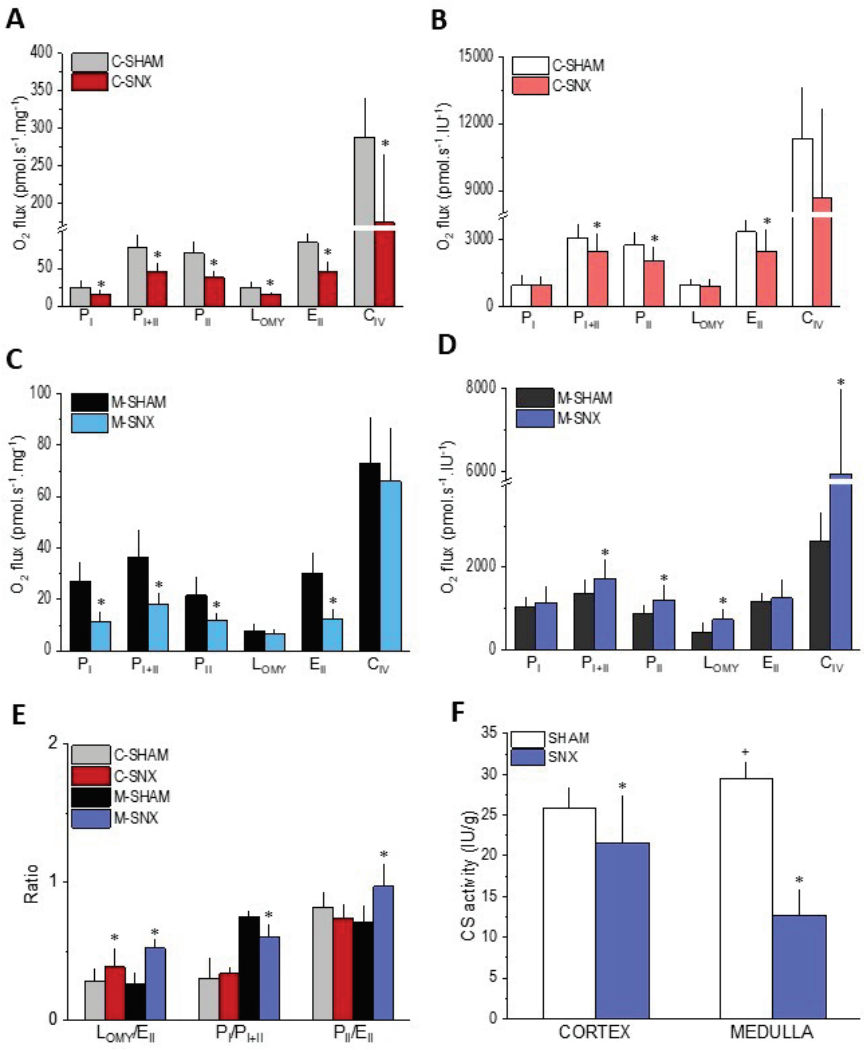

Fig. 4. Mitochondrial oxygen consumption in mechanically permeabilized samples of kidney cortex (**A, B**) and medulla (**C, D**) expressed per mg tissue wet weight (A, C) or IU citrate synthase activity (B, D); coupling control ratio (L_{OMY}/E_{II}), N/NS pathway control ratio (P_I/P_{I+II}), P_{II}/E_{II} control ratio expressing the limitation of OXPHOS capacity by the phosphorylation system (**E**), citrate synthase (CS) activity in the cortex and medulla of sham- operated (SHAM) and subtotally nephrectomized (SNX) rats (**F**). C, cortex; M, medulla; P_I, active phosphorylating respiration in the presence of substrates providing electrons to Complex I, ADP and cytochrome c; P_{I+II}, active phosphorylating oxygen consumption when a substrate of the Complex II succinate is added; P_{II}, phosphorylating state after the Complex I inhibitor rotenone; L_{OMY}, leak state after ATP synthase inhibitor oligomycin; E_{II}, electron-transporting system capacity after uncoupler injection; C_{IV}, respiration related to the Complex IV activity. * $p<0.05$, compared to the same respiratory state in the respective SHAM samples, $^{+}$ $p<0.05$, compared to CS activity in the SHAM cortex.

Discussion

CKD is characterized by progressive nephron loss associated with reduced glomerular filtration rate and increase in markers of renal damage, like albuminuria or blood creatinine levels [2]. This condition usually develops after repeated acute insults or gradually over many months or even years. In the experimental practice, renal mass reduction procedures on rats are widely acknowledged as a model suitable to study the pathophysiology of CKD, although the onset of the kidney injury is in fact sharp followed by mixed compensatory processes and progression of injury caused by compromised renal function and oxidative stress [8]. Among them, the model of 5/6 nephrectomy performed in two stages is the most frequently used one [16]. Typical symptoms, as nephron hypertrophy, reduced glomerular filtration rate, deterioration of tubular functions, and severe arterial hypertension usually develop within one week after renal mass reduction [11]. Also the present study confirmed decreased clearance of creatinine and urea, altered excretion of potassium resulting in elevated serum potassium levels and marked hypertrophy of the stump of the remnant kidney in SNX rats at week 10 after surgery.

The results of our study indicate that subtotal nephrectomy and consequent CKD are associated with different impact on mitochondrial oxygen consumption in the kidney cortex and outer medulla 10 weeks after completed 5/6 renal mass reduction. Mitochondrial respiration in the renal cortex was significantly reduced in all measured respiratory states if expressed per unit wet weight and remained lower in states P_{I+II}, P_{II} and E_{II} if expressed per IU citrate synthase activity, i.e. per mitochondrial mass. In contrast, the profound decrease in the activity of CS in SNX medulla resulted in significantly elevated values of mitochondrial respiratory states P_{I+II}, P_{II}, L_{OMY}, and C_{IV} per unit of CS activity suggesting compensatory hypermetabolic state in remaining functional mitochondria that is not sufficient

anyway to fully compensate for respiratory deficit expressed per tissue mass.

It should be noted that majority of studies dealing with mitochondrial oxygen consumption, activity of individual respiratory complexes or ATP production in the rat kidneys at various time points after 5/6 nephrectomy were performed on mitochondria isolated either from the whole organ or renal cortex disregarding the potential impact of CKD on mitochondrial respiration in the kidney medulla [17-19]. It has been clearly shown that individual nephron segments display remarkable differences not only in their transport processes, related membrane proteins and intracellular enzymes equipment, but also in morphology and functional properties of mitochondria [20-23]. A study using high-resolution respirometry to measure oxygen consumption in the permeabilized homogenates of the kidney cortex and medulla reported exactly the same pattern of mitochondrial respiration that we observed in our experiments: higher oxygen fluxes in the cortex with the exception of Complex I-linked phosphorylating oxygen consumption (P_I in our study) that tended to be higher in the medulla [10]. In addition, several studies suggested higher susceptibility of cortical compared to outer medullary mitochondria to ischemic or toxic challenges [24,25]. The reason is unclear, one theory states that medullary mitochondria might employ a strategy to sustain ATP production in a suboptimal environment [9].

So far reported data on renal mitochondrial dysfunction associated with CKD are not fully consistent, although majority of them agree on compromised respiratory complexes activity, reduced mitochondrial membrane potential, and decreased ATP content [8]. However, the extent of mitochondrial damage seems to be dependent on time. Early after the renal mass reduction (up to 10 days) in rats, compensatory response of remaining kidney tissue results in hypertrophy of proximal tubular cells, increased activity of membrane transporters including Na-K-ATPase and hypermetabolic state associated with increased mitochondrial respiration if succinate is used as the electron donor [26]. This initial compensatory reaction is accompanied with elevated production of reactive oxygen species that make the remnant kidney more susceptible to potential damage caused by various mitochondrial toxicants [26]. At the later stages (i.e. from week 4 after 5/6 nephrectomy onwards), studies consistently report compromised OXPHOS capacity, elevated cytochrome c release, decreased mitochondrial membrane potential, diminished expression of relevant proteins, mitochondria swelling and fragmentation [17,18,27]. Our research confirmed some of these findings in the permeabilized cortex (decreased OXPHOS and ETS capacities, increased decoupling reflected in L/E ratio), but not in the renal medulla where remaining mitochondrial mass reflected in profound decrease in CS activity seemed to maximally compensate for threatening bioenergetics failure.

Conclusions

The results of our study document that CKD induced by 5/6 nephrectomy in the rat is likely to cause not only mitochondrial respiratory dysfunction (in the kidney cortex), but also adaptive changes in the medulla that tend to at least partially compensate for mitochondrial loss. The increase in the P_{II}/E_{II} ratio in the SNX medulla suggests that the control of electron transport by the phosphorylation system could be less tight to allow the maximal use of electrons for ATP generation. In addition, Complex II stimulation might help to compensate for increased LEAK respiration and might be implicated in the metabolic adaptation to challenging conditions [28].

Study limitations

Metabolic switch from oxidative to glycolytic metabolism leading to compromised mitochondrial respiration cannot be excluded in the kidney cortex challenged by CKD especially with regard to enormous hypertrophy of the remnant tissue that can lead to limited oxygen diffusion [29]. Further studies dealing with the rate of ATP and lactate production are needed to judge on real consequences of CKD on the whole organ bioenergetics

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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References

1. Coresh J, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers P, Van LF, Levey AS: Prevalence of chronic kidney disease in the United States. JAMA 2007;298: 2038-2047. https://doi.org/10.1001/jama.298.17.2038
2. Shafi T, Coresh J. Chronic kidney disease: Definition, epidemiology, cost, and outcomes. In: *Chronic Kidney Disease, Dialysis, and Transplantation*. HIMMELFARB J, IKIZLER TA (eds), Elsevier, Amsterdam, 2010, pp. 3-21. https://doi.org/10.1016/B978-1-4377-0987-2.00001-7
3. Galvan DL, Green NH, Danesh FR. The hallmarks of mitochondrial dysfunction in chronic kidney disease. Kidney Int 2017;92:1051-1057. https://doi.org/10.1016/j.kint.2017.05.034
4. Chiong M, Cartes-Saavedra B, Norambuena-Soto I, Mondaca-Ruff D, Morales PE, García-Miguel M, Mellado R. Mitochondrial metabolism and the control of vascular smooth muscle cell proliferation. Front Cell Dev Biol 2014;2:72. https://doi.org/10.3389/fcell.2014.00072
5. Tang C, Cai J, Yin XM, Weinberg JM, Venkatachalam MA, Dong Z. Mitochondrial quality control in kidney injury and repair. Nat Rev Nephrol 2021;17:299-318. https://doi.org/10.1038/s41581-020-00369-0
6. Che R, Yuan Y, Huang S, Zhang A. Mitochondrial dysfunction in the pathophysiology of renal diseases. Am J Physiol Renal Physiol 2014;306:F367-F378. https://doi.org/10.1152/ajprenal.00571.2013
7. Aparicio-Trejo OE, Rojas-Morales P, Avila-Rojas SH, León-Contreras JC, Hernández-Pando R, Jiménez-Uribe AP, Prieto-Carrasco R, Sánchez-Lozada LG, Pedraza-Chaverri J, Tapia E. Temporal alterations in mitochondrial β-oxidation and oxidative stress aggravate chronic kidney disease development in 5/6 nephrectomy induced renal damage. Int J Mol Sci 2020;21:6512. https://doi.org/10.3390/ijms21186512
8. Aparicio-Trejo OE, Tapia E, Sánchez-Lozada LG, Pedraza-Chaverri J. Mitochondrial bioenergetics, redox state, dynamics and turnover alterations in renal mass reduction models of chronic kidney diseases and their possible implications in the progression of this illness. Pharmacol Res 2018;135:1-11. https://doi.org/10.1016/j.phrs.2018.07.015
9. Schiffer TA, Gustafsson H, Palm F. Kidney outer medulla mitochondria are more efficient compared with cortex mitochondria as a strategy to sustain ATP production in a suboptimal environment. Am J Physiol Renal Physiol 2018;315:F677-F681. https://doi.org/10.1152/ajprenal.00207.2018
10. Woodman AG, Mah R, Keddie DL, Noble RMN, Holody CD, Panahi S, Gragasin FS, Lemieux H, Bourque SL. Perinatal iron deficiency and a high salt diet cause long-term kidney mitochondrial dysfunction and oxidative stress. Cardiovasc Res 2020;116:183-192. https://doi.org/10.1093/cvr/cvz029
11. Svíglerová J, Kuncová J, Nalos L, Holas J, Tonar Z, Rajdl D, Stengl M. Cardiac remodeling in rats with renal failure shows interventricular differences. Exp Biol Med (Maywood) 2012;237:1056-1067. https://doi.org/10.1258/ebm.2012.012045
12. Nalos L, Švíglerová J, Rajdl D, Jedlička J, Dejmek J, Štengl M, Kuncová J. Norepinephrine turnover in the left ventricle of subtotally nephrectomized rats. Physiol Res 2019;68(Suppl 3):S233-S242. https://doi.org/10.33549/physiolres.934354
13. Pesta D, Gnaiger E. High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. Methods Mol Biol 2012;810:25-58. https://doi.org/10.1007/978-1-61779-382-0_3
14. Kuznetsov AV, Strobl D, Ruttmann E, Königsrainer A, Margreiter R, Gnaiger E. Evaluation of mitochondrial respiratory function in small biopsies of liver. Anal Biochem 2002;305:186-194. https://doi.org/10.1006/abio.2002.5658
15. Grundmanová M, Jarkovská D, Süß A, Tůma Z, Marková M, Grundman Z, El-Kadi A, Čedíková M, Štengl M, Kuncová J. Propofol-induced mitochondrial and contractile dysfunction of the rat ventricular myocardium. Physiol Res 2016;65(Suppl 5):S601-S609. https://doi.org/10.33549/physiolres.933537
16. Bao YW, Yuan Y, Chen JH, Lin WQ. Kidney disease models: tools to identify mechanisms and potential therapeutic targets. Zool Res 2018;39:72-86. https://doi.org/10.24272/j.issn.2095-8137.2017.055

17. Aparicio-Trejo OE, Tapia E, Molina-Jijón E, Medina-Campos ON, Macías-Ruvalcaba NA, León-Contreras JC, Hernández-Pando R, García-Arroyo FE, Cristóbal M, Sánchez-Lozada LG, Pedraza-Chaverri J. Curcumin prevents mitochondrial dynamics disturbances in early 5/6 nephrectomy: Relation to oxidative stress and mitochondrial bioenergetics. Biofactors 2017;43:293-310. https://doi.org/10.1002/biof.1338
18. Correa F, Buelna-Chontal M, Hernández-Reséndiz S, García-Niño WR, Roldán FJ, Soto V, Silva-Palacios A, Amador A, Pedraza-Chaverrí J, Tapia E, Zazueta C. Curcumin maintains cardiac and mitochondrial function in chronic kidney disease. Free Radic Biol Med 2013;61:119-129. https://doi.org/10.1016/j.freeradbiomed.2013.03.017
19. Hui Y, Lu M, Han Y, Zhou H, Liu W, Li L, Jin R. Resveratrol improves mitochondrial function in the remnant kidney from 5/6 nephrectomized rats, Acta Histochem 2017;119:392-399. https://doi.org/10.1016/j.acthis.2017.04.002
20. Hall AM, Unwin RJ, Parker N, Duchen MR. Multiphoton imaging reveals differences in mitochondrial function between nephron segments. J Am Soc Nephrol 2009;20:1293-1302. https://doi.org/10.1681/ASN.2008070759
21. Bagnasco S, Good D, Balaban R, Burg M. Lactate production in isolated segments of the rat nephron. Am J Physiol 1985;248:F522-F526. https://doi.org/10.1152/ajprenal.1985.248.4.F522
22. Tomar N, Zhang X, Kandel SM, Sadri S, Yang C, Liang M, Audi SH, Cowley AW, Jr., Dash RK. Substrate-dependent differential regulation of mitochondrial bioenergetics in the heart and kidney cortex and outer medulla. Biochim Biophys Acta Bioenerg 2021;1863:148518. https://doi.org/10.1016/j.bbabio.2021.148518
23. Tuma Z, Kuncova J, Mares J, Matejovic M. Mitochondrial proteomes of porcine kidney cortex and medulla: foundation for translational proteomics. Clin Exp Nephrol 2016;20:39-49. https://doi.org/10.1007/s10157-015-1135-x
24. Brezis M, Shanley P, Silva P, Spokes K, Lear S, Epstein FH, Rosen S. Disparate mechanisms for hypoxic cell injury in different nephron segments. Studies in the isolated perfused rat kidney. J Clin Invest 1985;76: 1796-1806. https://doi.org/10.1172/JCI112171
25. Jung K, Pergande M. Different susceptibility of cortical and medullary rat kidney mitochondria to ischemic injury. Biomed Biochim Acta 1988;47:455-460.
26. Lash LH, Putt DA, Horky SJ, Zalups RK. Functional and toxicological characteristics of isolated renal mitochondria: impact of compensatory renal growth. Biochem Pharmacol 62;2001:383-395. https://doi.org/10.1016/S0006-2952(01)00673-6
27. Prieto-Carrasco R, García-Arroyo FE, Aparicio-Trejo OE, Rojas-Morales P, León-Contreras JC, Hernández-Pando R, Sánchez-Lozada LG, Tapia E, Pedraza-Chaverri J. Progressive reduction in mitochondrial mass is triggered by alterations in mitochondrial biogenesis and dynamics in chronic kidney disease induced by 5/6 nephrectomy. Biology (Basel) 2021;10:349. https://doi.org/10.3390/biology10050349
28. Acín-Pérez R, Carrascoso I, Baixauli F, Roche-Molina M, Latorre-Pellicer A, Fernández-Silva P, Mittelbrunn M, Sanchez-Madrid F, Pérez-Martos A, Lowell CA, Manfredi G, Enríquez JA. ROS-triggered phosphorylation of complex II by Fgr kinase regulates cellular adaptation to fuel use. Cell Metab 2014;19:1020-1033. https://doi.org/10.1016/j.cmet.2014.04.015
29. Zhu Z, Hu J, Chen Z, Feng J, Yang X, Liang W, Ding G. Transition of acute kidney injury to chronic kidney disease: role of metabolic reprogramming. Metabolism 2022;131:155194. https://doi.org/10.1016/j.metabol.2022.155194