

Cannabidiol attenuates kidney injury and mitochondrial calcium dysregulation in experimental cardiorenal syndrome.

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Article

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Abstract

Cardiorenal syndrome (CRS) is characterized by bidirectional heart–kidney dysfunction in which oxidative stress, inflammation, and mitochondrial dysfunction drive progressive kidney injury, but the role of mitochondrial calcium (Ca^{2+}) handling through the mitochondrial calcium uniporter (MCU) complex remains unclear. Here we show, in a mouse model of CRS induced by angiotensin II and L-NAME in male mice (C57BL/6), that renal mitochondria exhibit increased MCU expression, reduced Ca^{2+} retention capacity, and greater susceptibility to Ca^{2+} -induced permeability transition, alongside impaired respiration, reduced PGC-1 α and TFAM expression, oxidative stress, and inflammatory activation. Cannabidiol (CBD) attenuates renal dysfunction and structural injury, restores mitochondrial respiratory capacity and complex I activity, normalizes MCU expression, and partially improves mitochondrial Ca^{2+} handling, independently of blood pressure reduction. These findings identify MCU-dependent mitochondrial Ca^{2+} dysregulation as a candidate mechanism linking mitochondrial dysfunction to renal injury in CRS, and a potential target for CBD's nephroprotective effects.

1. Introduction

Heart failure (HF) remains one of the leading causes of morbidity and mortality worldwide despite substantial advances in pharmacological and device-based therapies^{1,2}. Renal dysfunction develops in nearly half of patients with HF and is associated with increased hospitalization, progression to chronic kidney disease, and mortality, making it one of the strongest predictors of adverse clinical outcomes^{3–6}. This bidirectional interaction between the failing heart and the kidney, clinically recognized as cardiorenal syndrome (CRS), results from a complex interplay of hemodynamic alterations, persistent neurohormonal activation, oxidative stress, inflammation, endothelial dysfunction, and metabolic remodeling rather than from reduced renal perfusion alone^{5–8}. Despite significant advances in HF therapy, the molecular mechanisms responsible for progressive renal injury during CRS remain incompletely understood, limiting the development of targeted therapies.

Mitochondrial dysfunction has emerged as a central mechanism contributing to organ injury during CRS. Beyond their role in ATP production, mitochondria regulate Ca^{2+} homeostasis, reactive oxygen species (ROS) generation, redox signaling, and inflammatory responses, thereby maintaining cellular energetic and metabolic homeostasis^{9–12}. Disruption of these processes promotes energetic failure, oxidative stress, and tissue remodeling, thereby contributing to progressive cardiac and renal dysfunction^{9,12}. Among the molecular pathways regulating mitochondrial function, mitochondrial Ca^{2+} homeostasis is essential for coupling oxidative metabolism to cellular energy demand^{13,14}. Ca^{2+} entry into the mitochondrial matrix is mediated by the mitochondrial Ca^{2+} uniporter (MCU) complex, whose activity is tightly regulated by the regulatory proteins MICU1 and MICU2^{15,16}. While physiological mitochondrial Ca^{2+} uptake stimulates oxidative phosphorylation, excessive calcium accumulation induces ROS production, opening of the mitochondrial permeability transition pore (mPTP), mitochondrial swelling, bioenergetic collapse, and cell death^{15–17}. Although dysregulation of the MCU complex has been

implicated in several cardiovascular and renal disorders, its contribution to renal mitochondrial dysfunction during CRS remains poorly understood^{17,18}.

Cannabidiol (CBD), a non-psychoactive phytocannabinoid, exhibits anti-inflammatory, antioxidant, and mitochondria-protective properties in several experimental models of cardiovascular and renal disease^{19–21}. In addition, our group recently demonstrated that CBD improves cardiac function while preserving mitochondrial Ca^{2+} homeostasis and mitochondrial function in an experimental model of HF¹⁸. However, whether similar mechanisms underlie the renoprotective effects of CBD during CRS remains unknown.

Therefore, we investigated whether alterations in renal mitochondrial Ca^{2+} homeostasis are associated with kidney injury in an experimental model of CRS and whether CBD preserves renal mitochondrial function by restoring mitochondrial Ca^{2+} homeostasis. We hypothesized that preservation of mitochondrial Ca^{2+} homeostasis contributes to the nephroprotective effects of CBD in CRS. Here we show that experimental CRS increases renal MCU expression, impairs mitochondrial Ca^{2+} retention and respiratory capacity, and reduces PGC-1 α and TFAM expression, and that CBD attenuates renal injury and mitochondrial dysfunction while normalizing MCU expression and partially restoring mitochondrial Ca^{2+} handling, independently of blood pressure reduction.

2. Methods

- 2.1 Experimental Cardiorenal Syndrome (CRS) mouse model.** All animal experiments were approved by the Institutional Animal Care and Use Committee of Tecnológico de Monterrey (Protocol No. 2023-007) and performed following NOM-062-ZOO-1999 and ARRIVE guidelines. Male C57BL/6J mice (11 weeks old; total n = 50; CoreLab–Preclinical Research, Tecnológico de Monterrey) were housed under standard conditions (12-hour light cycle 6:00–18:00) with ad libitum access to food and water. Animals were wild-type, and had not undergone any previous procedures prior to enrollment in this study. Mice were group-housed (4 animals/cage) in individually ventilated cages with cardboard tunnels provided as environmental enrichment. Animals were subjected to one-week acclimatization period before experimental procedures. Experimental CRS was induced by 1% NaCl and 0.1% L-NAME in drinking water for 7 days, followed by continuous angiotensin II infusion (0.7 mg/kg/day) via subcutaneous micro-osmotic pumps (Alzet, model 1004), obtained the same mortality rate as reported²², as post-surgery care was administered one dose of meloxicam (2 mg/kg, s.c.). Also, animals were monitored twice daily, human endpoints include a 20% loss in initial body weight, severe lethargy, or signs of pain and respiratory distress. Sham animals underwent identical surgical handling without pump implantation. Another group of CRS mice started cannabidiol treatment on the day pump implantation (CBD; 1 or 10 mg/kg in PEG-400) subcutaneously every 3 days for 28 days; controls received vehicle. An additional cohort of healthy mice received CBD (1 mg/kg) to assess potential renal CBD side effects. CBD was provided by Cardiol Therapeutics. Obtaining five groups: 1. Control (n = 11), 2. Control + 1 mg/kg- CBD (n = 7), 3.

CRS (n = 13), 4. CRS + 1 mg/kg-CBD (n = 11), 5. CRS 10 mg/kg-CBD (n = 8). No formal a priori sample-size/power calculation was performed for the present study; group sizes were determined empirically based on the effect sizes for mitochondrial Ca^{2+} handling and function parameters observed in our previous study in an experimental heart failure model¹⁸, and are consistent with group sizes typically used in comparable rodent cardiorenal syndrome models. Animals were assigned to experimental groups using simple randomization generated with a random number table, and investigators performing biochemical, molecular, and functional analyses were blinded to group assignment. Cage position within the housing rack and the order in which animals underwent surgical procedures, dosing and outcome measurements were alternated across experimental groups to minimize potential confounding related to cage location or procedural order. Group allocation and drug/vehicle administration were performed by veterinary staff, who was not blinded due to the practical need to prepare treatment solutions. Investigators performing biochemical, molecular, histological and functional analyses, as well as image and data analysis, were blinded to group identity; group codes were revealed only after all measurements and statistical analyses were completed. The experimental unit was an individual mouse; each mouse contributed one independent biological replicate.

2. **2.2 Echocardiographic and Renal Doppler Assessment.** Transthoracic echocardiography was performed using a Prospect T1 high-frequency ultrasound imaging system. Post-CBD treatment mice were anesthetized with inhaled sevoflurane delivered at 3.5% and positioned supine on a temperature-controlled imaging platform. Two-dimensional B-mode imaging was used to obtain standard parasternal long- and short-axis views and to guide Doppler and M-mode acquisitions. Pulmonary artery flow was assessed in the parasternal long-axis view using pulsed-wave Doppler, and transmitral inflow was evaluated with the sample volume positioned at the mitral valve leaflet tips to measure E and A waves and derived diastolic parameters, including E/A ratio, deceleration time, isovolumic intervals and ejection time. Left ventricular systolic function was assessed by M-mode imaging in the parasternal short-axis view at the papillary muscle level.

Renal Doppler ultrasound was performed using abdominal mode. The right kidney was identified in short-axis orientation by B-mode imaging, and renal blood flow was localized using Doppler guidance. Pulsed-wave Doppler recordings were obtained from the right renal vascular signal with the Doppler angle aligned as parallel as possible to blood flow. Peak systolic velocity and end-diastolic velocity were measured from stable consecutive waveforms, and the renal resistive index was calculated as $\text{RI} = (\text{PSV} - \text{EDV}) / \text{PSV}^{23}$.

1. **2.3 Noninvasive Blood Pressure Assessment.** Arterial blood pressure was measured noninvasively using the CODA tail-cuff system (Kent Scientific) as previously described. During the last week of CBD-treatment, mice were placed on a warming platform with tail temperature maintained at 32°C. After discarding the first five cycles, 15 measurement cycles were recorded, and at least three accepted readings per animal were used for analysis²⁴.
2. **2.4 Euthanasia and tissue collection.** At 28 days after pump implantation, mice were anesthetized with sevoflurane (4.5%); urine and blood (inferior vena cava) were collected, and humanely euthanasia was confirmed by cardiectomy. Kidneys were removed, weighed, and processed for mitochondrial assays and histology; remaining tissue was stored at – 80°C.
3. **2.5 Histological analysis.** Kidney tissue was fixed, paraffin-embedded, and stained with Masson's trichrome (fibrosis) or H&E (mesangial expansion and glomerular hypertrophy), as previously described^{25–27}. Glomerular diameter was measured in ≥ 130 glomeruli/group (NIS Elements) across five subcortical fields per kidney and organized by rank, since glomeruli can be sectioned at variable poles. Evaluations were performed blinded to group.
4. **2.6 Plasma and urinary creatinine concentrations.** Plasma and urinary creatinine were measured using the LabAssay™ Creatinine Kit (FujiFilm) per manufacturer's instructions (Jaffé reaction)²⁸, with absorbance read on a Synergy HT plate reader (BioTek). These parameters, in conjunction with histological analysis were used as primary outcome to determine if CBD protects renal function in an experimental CRS.
5. **2.7 Mitochondrial isolation.** Renal tissue from each group was rapidly excised and placed in ice-cold isolation buffer containing (in mmol/L): 250 sucrose, 10 Tris-base and 1 EGTA (pH 7.3). The tissue was minced and homogenized using a 2 mL tissue grinder tube. Homogenates were centrifuged at 2500 rpm for 10 minutes (min) at 4°C. The mitochondrial pellet was washed with isolation buffer supplemented with 0.2% fatty acid-free BSA, followed by centrifugation at 9000 rpm for 10 min at 4°C. Finally, the mitochondrial pellet was resuspended in isolation buffer, and protein concentration was determined using the Lowry assay.
6. **2.8 Mitochondrial respiration, membrane potential, and oxidative stress assessment.** Mitochondrial respiratory function was evaluated in isolated kidney mitochondria (0.1 mg/mL) using high-resolution respirometry (Oroboros Instruments), with succinate/rotenone as substrate and sequential addition of ADP (state 3) and FCCP (maximal uncoupled respiration), as previously described¹⁸. Mitochondrial membrane potential ($\Delta\Psi_m$) was determined by safranin O fluorescence and calculated using the Nernst equation, as previously described^{29,30}. Complex I activity was measured by NADH-dependent ferricyanide reduction³¹. Mitochondrial H₂O₂ production was assessed using the Amplex Red/horseradish peroxidase assay³², with simultaneous measurement of oxygen consumption. Protein carbonylation and the GSH/GSSG ratio were quantified using commercial kits (Abcam), and lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS)³³, all per manufacturer's/published protocols.
7. **2.9 Mitochondrial Ca²⁺ handling and swelling assays.** Mitochondrial Ca²⁺ retention capacity (CRC) and swelling were assessed in isolated mitochondria energized with succinate/rotenone, using Ca²⁺

Green-5N fluorescence (sequential 15 $\mu\text{mol/L}$ Ca^{2+} pulses) and absorbance at 540 nm (150 $\mu\text{mol/L}$ Ca^{2+} challenge), respectively, as previously described^{34,35}.

8. **2.10 Immunoblotting.** Urinary SerpinA3K, a marker of renal injury^{36,37}, and renal PGC-1 α , TFAM, MCU, and OXPHOS complexes were evaluated by standard SDS-PAGE/Western blotting with primary antibodies detailed in Supplementary Table 1. Band densitometry (ImageJ) was normalized to urinary creatinine (SerpinA3K), GAPDH (PGC-1 α , TFAM, MCU), or Coomassie blue staining (OXPHOS complexes).
9. **2.11 RNA isolation and quantitative PCR (qPCR).** Total RNA was extracted (TRIzol) and reverse-transcribed, and quantitative PCR was performed using SYBR Green chemistry (QuantStudio; primers in Supplementary Table 2) to measure *Nfkb1*, *Il6*, *Ccl2*, *Tnfa*, *Edn1*, *Agtr1a*, and *Agtr2a* mRNA, as previously described^{18,38}. Relative expression was calculated by the $2^{-\Delta\Delta\text{Ct}}$ method using β -actin or 18S rRNA as internal control.
10. **2.12 Statistics and Reproducibility.** Data are expressed as mean $\pm$ SEM. There was not a predefined inclusion/exclusion criterion. Shapiro wilk test was used as normality test. Comparisons among ≥ 3 groups used one-way ANOVA with Tukey's post hoc test (or Kruskal–Wallis/Dunn's for non-normal data); Holm-Sidak procedure was used to control the family-wise error rate (FWER) for multiple comparisons of renal Doppler parameters (Fig. 2) as indicated in each figure legend. Glomerular diameter distribution was analyzed by contingency analysis on $> = 130$ glomeruli per group from five subcortical fields per kidney. Histological evaluation was performed blinded to group assignment. $P < 0.05$ was considered statistically significant. All analyses were performed using GraphPad Prism (v10). No animals or data points were excluded from the analyses. Variation in group sizes across assays reflects the allocation of a limited amount of tissue/sample to multiple downstream analyses (biochemical, molecular, histology and functional), rather than attrition or exclusion.

3. Results

3.1 Cannabidiol attenuates renal injury and preserves glomerular architecture in experimental CRS.

Animals exposed to Ang II plus L-NAME developed cardiac dysfunction as previously reported¹⁸, concomitant with severe renal dysfunction, as evidenced by a 1.6-fold increase in plasma creatinine, elevated urinary protein excretion, and increased urinary SerpinA3K excretion, a biomarker of renal injury, compared with control animals (Fig. 1A–C). Administration of cannabidiol (CBD) at both 1 and 10 mg/kg significantly attenuated these alterations, improving renal function and reducing renal injury markers toward control values. Both doses produced comparable improvements in the renal parameters evaluated. Structurally, CRS was associated with glomerular hypertrophy and mesangial expansion, consistent with renal remodeling (Fig. 1D–E). Morphometric analysis revealed a shift toward larger glomerular diameters in CRS animals compared with controls; however, the difference in the 101–125 μm range did not reach statistical significance. In contrast, CBD treatment preserved glomerular morphology and maintained glomerular diameter comparable to that of control animals (yellow squares in Fig. 1D). Mesangial expansion was markedly increased in CRS animals and was significantly attenuated by CBD treatment, further supporting its nephroprotective effects.

Although CBD did not significantly reduce systemic blood pressure, it substantially improved renal function and attenuated renal injury, indicating that mechanisms beyond blood pressure reduction contribute to its renoprotective effects. Hemodynamic analyses were performed exclusively in the 1 mg/kg CBD group (Fig. 1F–H and Supplementary Fig. 1). CRS animals developed a pronounced hypertensive phenotype, characterized by increased systolic, diastolic, and mean arterial pressures compared with control animals (Fig. 1F–H). Importantly, CRS animals also exhibited impaired cardiac function, as evidenced by reduced ventricular ejection fraction, fractional shortening, and increased cardiac *Nppb* expression, consistent with systolic dysfunction (Supplementary Fig. 1). In addition, altered pulmonary flow parameters were observed, supporting the presence of cardiopulmonary dysfunction (Supplementary Table 3). CBD improved these cardiovascular parameters, consistent with our previous findings¹⁸. Together, the renal and cardiovascular alterations confirmed the successful establishment of the experimental CRS model. Because both CBD doses produced comparable improvements in renal function and histopathological injury (Fig. 1A–E), whereas comprehensive hemodynamic analyses were available only for the 1 mg/kg group, this dose was selected for all subsequent studies.

3.2. Renal Doppler assessment demonstrates preserved intrarenal hemodynamics despite reduced peak systolic velocity in experimental cardiorenal syndrome. To determine whether the renoprotective effects of CBD were associated with changes in intrarenal hemodynamics, renal blood flow was evaluated by Doppler ultrasound. Representative Doppler waveforms are shown in Fig. 2A. Quantitative analysis revealed a significant reduction in peak systolic velocity (PSV) in both the CRS and CRS + CBD groups compared with controls, whereas end-diastolic velocity (EDV) remained unchanged across experimental groups (Fig. 2B–C). Despite the reduction in PSV, the renal resistive index (RI) did not differ significantly among groups (Fig. 2D), indicating preserved intrarenal vascular resistance. The isolated decrease in PSV, in the absence of changes in EDV or RI, likely reflects alterations in arterial compliance or systemic hemodynamic adaptations rather than increased intrarenal vascular resistance. These findings indicate that the renoprotective effects of CBD are not associated with major changes in intrarenal hemodynamics and are likely mediated through mechanisms independent of systemic blood pressure or renal vascular resistance.

3.3 Cannabidiol reduces oxidative stress and suppresses inflammatory and vasoactive signaling in experimental cardiorenal syndrome. Since CBD improved renal function without significantly altering intrarenal hemodynamics, the potential contribution of oxidative stress and inflammatory signaling to its renoprotective effects was investigated. CRS animals exhibited a pronounced pro-oxidant state, as evidenced by increased lipid peroxidation and protein carbonylation, together with a significant reduction in the GSH/GSSG ratio, indicating a shift toward an oxidized intracellular environment (Fig. 3A–C). Mitochondrial H₂O₂ production was also significantly increased in CRS animals (Fig. 3D). CBD treatment effectively reversed these alterations, restoring redox balance to levels comparable to controls while having no effect under basal conditions. Given the well-established interplay between oxidative stress and inflammation, we next assessed the expression of key inflammatory mediators. CRS induced a robust upregulation of *Nfkb1* mRNA, accompanied by increased expression of the pro-inflammatory

cytokines *Il6*, *Ccl2* (MCP-1), and *Tnfa* (TNF- α) (Fig. 3E–H). CBD markedly suppressed the expression of these inflammatory markers, consistent with attenuation of the inflammatory response.

In addition to oxidative stress and inflammatory activation, CRS animals exhibited increased renal *Edn1* (endothelin-1, ET-1) mRNA expression, consistent with enhanced intrarenal vasoconstrictive signaling (Fig. 3I). CBD normalized *Edn1* expression to levels comparable to those of control animals, further supporting its renoprotective effects. Whereas *Agtr1a* expression remained unchanged among experimental groups, *Agtr2a* expression was significantly increased in CRS animals compared with controls (Fig. 3J–K). However, CBD treatment did not significantly modify angiotensin receptor expression, suggesting that its protective effects are unlikely to be mediated through major changes in renal angiotensin receptor abundance. The interplay between oxidative stress and endothelin signaling suggests that increased ET-1 expression may contribute to the mitochondrial and cellular dysfunction associated with experimental CRS. Collectively, these findings demonstrate that CBD attenuates oxidative stress, inflammatory activation, and endothelin signaling, thereby limiting key mechanisms that contribute to renal mitochondrial dysfunction in experimental CRS.

3.4 Cannabidiol improves mitochondrial respiratory function and restores mitochondrial biogenesis-associated signaling. To determine whether the improvements in oxidative stress and inflammatory signaling were associated with enhanced mitochondrial function, mitochondrial respiration was evaluated in isolated renal mitochondria. Experimental CRS significantly reduced the respiratory control ratio (RCR), indicating impaired oxidative phosphorylation efficiency (Fig. 4A). CBD treatment restored RCR to control levels, indicating recovery of mitochondrial bioenergetic function. This impairment was characterized by reduced ADP-stimulated (state 3) respiration and increased uncoupled (state 3u) respiration in mitochondria isolated from CRS animals; both parameters were normalized by CBD treatment (Supplementary Fig. 2). Consistent with these findings, CRS was associated with reduced expression and activity of electron transport chain complex I, demonstrating selective impairment of mitochondrial respiration (Fig. 4B–D). CBD treatment reversed these alterations, restoring both complex I protein abundance and enzymatic activity. In contrast, the expression of electron transport chain complexes II, III, IV, and V remained unchanged across experimental groups (Supplementary Fig. 2), indicating that complex I was selectively affected in experimental CRS.

Mitochondrial biogenesis was further assessed by measuring the expression of PGC-1 α , a transcriptional coactivator that regulates the expression of nuclear-encoded mitochondrial proteins, and TFAM, a key regulator of mitochondrial DNA transcription and replication. CRS animals exhibited marked downregulation of both PGC-1 α and TFAM, consistent with impaired mitochondrial biogenesis and maintenance (Fig. 4E–F). Restoration of these regulators by CBD may have contributed to improved mitochondrial resilience against oxidative and Ca²⁺-mediated stress, thereby promoting recovery of mitochondrial homeostasis. Collectively, these findings demonstrate that CBD restores mitochondrial bioenergetic efficiency and biogenic capacity, supporting recovery of mitochondrial function in experimental CRS.

3.5 Cannabidiol attenuates mitochondrial Ca^{2+} dysregulation in experimental cardiorenal syndrome. To investigate the mechanisms underlying mitochondrial dysfunction in experimental CRS, we evaluated mitochondrial Ca^{2+} homeostasis, a critical regulator of oxidative phosphorylation, bioenergetics, and mitochondrial integrity. Mitochondria isolated from CRS animals exhibited greater susceptibility to Ca^{2+} -induced mitochondrial permeability transition pore (mPTP) opening, as evidenced by increased mitochondrial swelling following Ca^{2+} challenge (Fig. 5A). Consistent with this finding, mitochondria from CRS animals displayed reduced Ca^{2+} retention capacity (CRC), indicating impaired mitochondrial Ca^{2+} buffering and decreased tolerance to repeated Ca^{2+} pulses (Fig. 5B). Although CBD treatment did not fully normalize these parameters, it consistently shifted both mitochondrial swelling ($p = 0.073$) and CRC ($p = 0.052$) toward the control phenotype, suggesting partial recovery of mitochondrial resistance to Ca^{2+} -induced stress.

At the molecular level, CRS induced a significant increase in the expression of the MCU, whereas CBD restored MCU expression to levels comparable to those of control animals (Fig. 5C). These findings suggest that enhanced MCU expression contributes to mitochondrial Ca^{2+} dysregulation during experimental CRS and that modulation of MCU-dependent Ca^{2+} handling represents a key mechanism underlying the mitochondrial protective effects of CBD.

To determine whether the effects of CBD were specific to the pathological setting of experimental CRS, rather than intrinsic to healthy kidneys, CBD was administered to normotensive control mice. CBD alone did not affect plasma creatinine, urinary protein excretion, lipid peroxidation, protein carbonylation, renal inflammatory gene expression (*Nfkb1*, *Il6*, *Ccl2*, and *Tnfa*), or mitochondrial respiratory function compared with untreated controls (Supplementary Figs. 3 and 4). These findings indicate that the renoprotective and mitochondrial effects of CBD are specific to experimental CRS and do not reflect nonspecific actions in healthy animals.

4. Discussion

Experimental CRS caused broad renal mitochondrial dysfunction, characterized by impaired bioenergetics, oxidative-inflammatory activation, and dysregulated mitochondrial Ca^{2+} homeostasis with increased MCU expression. CBD markedly attenuated these abnormalities and improved renal function, mitochondrial respiration, and biogenic signaling, extending prior evidence implicating MCU in the regulation of bioenergetics under cellular stress^{13–16,18} (Fig. 6).

Renal injury in CRS reflects convergent neurohormonal activation, oxidative stress, inflammation, and metabolic disturbance rather than hemodynamics alone^{5–7}; our Ang II + L-NAME model reproduced these features. Doppler revealed reduced peak systolic velocity that was not normalized by CBD, whereas the resistive index remained unchanged. Because renal Doppler indices reflect vascular resistance, compliance, and systemic hemodynamics^{39,40}, these findings suggest that CBD-mediated renoprotection is not explained by restoration of renal vascular hemodynamics or blood pressure.

In our study, CRS showed coordinated oxidative-inflammatory activation, characterized by increased lipid peroxidation and H₂O₂, reduced GSH/GSSG, and upregulated *Nfkb1*, *Il6*, *Ccl2*, and *Tnfa*, consistent with an Ang II–driven ROS-NF-κB inflammatory cycle^{41,42}. CBD attenuated these abnormalities, consistent with its established antioxidant and anti-inflammatory effects^{20,21}.

CRS also impaired oxidative phosphorylation, as reflected by reduced respiratory control ratio and complex I activity, and downregulated PGC-1α/TFAM; CBD attenuated these abnormalities. Oxidative stress can suppress PPAR-γ/PGC-1α signaling in renal tubular cells^{43,44}, whereas Ang II/ROS can inhibit PPARα, partly through NLRP3-dependent mechanisms, contributing to metabolic reprogramming in cardiorenal disease^{45,46}. Thus, restoration of PGC-1α/TFAM and mitochondrial function with CBD may partly reflect normalization of PPAR-dependent signaling. Because PPAR signaling was not directly manipulated in the present study, however, this remains a plausible upstream pathway rather than an established mediator. This interpretation is supported by evidence that CBD-mediated mitochondrial protection in experimental HF is associated with PPARγ activation¹⁸, and by other work showing that CBD ameliorates mitochondrial dysfunction through PPARγ activation⁴⁷.

Mitochondrial Ca²⁺ normally couples energy demand to ATP production^{13,14,16}, whereas sustained Ca²⁺ accumulation promotes ROS generation, mPTP opening, and injury^{15,17,48}. CRS mitochondria exhibited increased Ca²⁺-induced permeability transition and reduced Ca²⁺ retention capacity, both partially improved by CBD. These findings were accompanied by increased MCU expression in CRS, consistent with involvement of the mitochondrial Ca²⁺ uptake pathway, whose excessive activity can promote oxidative stress and mPTP opening⁴⁹. CBD also normalized MCU expression, consistent with a potential role for altered mitochondrial Ca²⁺ handling in renal injury and with previous studies showing that MCU inhibition protects against renal ischemia-reperfusion injury^{34,49,50}. However, direct mitochondrial Ca²⁺ flux was not measured, and the contribution of other MCU-complex regulators, including MICU1/2, EMRE, and MCUB, remains to be determined.

Beyond mitochondrial Ca²⁺ handling, CBD normalized renal *Edn1* expression, consistent with the role of endothelin-1 in oxidative, inflammatory, and mitochondrial injury^{51,52}. In contrast, *Agtr1a* and *Agtr2a* expression was unchanged, suggesting that CBD-mediated renoprotection is not associated with changes in angiotensin receptor expression.

These findings build upon existing evidence that cannabinoid signaling can influence renal injury. For example, CB1 blockade protects renal mitochondria in hypoxic injury⁵³, CB1 inhibition limits renal fibrosis⁵⁴, and cannabinoid effects differ among compounds, as CBGA but not CBD prevented cisplatin nephrotoxicity in one model⁵⁵. Unlike most previous cannabinoid–kidney studies, the present work offers greater mechanistic depth by directly examining mitochondrial Ca²⁺ handling and MCU expression as a defined molecular node. This approach provides a more specific mechanistic basis for the renoprotective effects of CBD in experimental CRS.

These findings should be interpreted in the context of established cardiorenal therapies that modulate mitochondrial function. Angiotensin receptor blocker treatment has been shown to improve cardiac mitochondrial structure without fully restoring mitochondrial biogenesis or respiration⁵⁶. In contrast, SGLT2 inhibitors reduce oxidative stress and may promote mitochondrial biogenic programs through metabolic and nutrient-sensing pathways, including AMPK/SIRT1 signaling^{57–59}. Evidence that mitochondrial dysfunction is a convergent feature across HF and related cardiorenal conditions⁶⁰ suggests that mitochondrial preservation may represent a common cellular component of cardiorenal protection, rather than a mechanism unique to a specific therapy. Within this framework, the present findings implicate mitochondrial Ca^{2+} handling and MCU expression as potential contributors to CBD-mediated protection. However, the specific mitochondrial vulnerabilities and upstream pathways may remain organ-dependent.

From a translational perspective, these findings support further investigation of purified CBD as a potential modulator of mitochondrial dysfunction in CRS; however, they do not establish its clinical efficacy or safety. Clinical evidence in chronic kidney disease (CKD) remains limited, and recent pharmacokinetic data indicate that advanced CKD can alter exposure to CBD, THC, and their metabolites, highlighting the need for dedicated studies to determine appropriate dosing and safety⁶¹. It is important to distinguish purified CBD from THC-containing cannabis preparations, as THC presents additional concerns in CKD, including psychoactive effects, potential drug interactions, and reported associations between synthetic cannabinoids and acute kidney injury⁶². Therefore, purified CBD warrants evaluation in cardiorenal disease through studies that specifically address efficacy, pharmacokinetics, drug interactions, safety, and renal-adjusted dosing.

Several limitations should be considered. Although CBD normalized MCU expression, this finding does not establish that MCU mediates its renoprotective effects; genetic or pharmacological manipulation of MCU will be required to establish causality^{35,50}. Direct mitochondrial Ca^{2+} flux was not measured, and other components of the MCU complex were not evaluated. Finally, only male mice were studied, limiting assessment of potential sex-dependent effects^{63,64}.

5. Conclusions

CBD attenuated renal injury in experimental CRS beyond blood pressure reduction and improved mitochondrial bioenergetics, redox-inflammatory balance, and Ca^{2+} handling. These findings implicate mitochondrial Ca^{2+} dysregulation and MCU as potential components of renal mitochondrial dysfunction in CRS and support the mitochondrial Ca^{2+} regulatory axis as a candidate therapeutic target for further investigation.

Protocol registration

The study protocol, including the research question, key design features and analysis plan, was not formally pre-registered prior to data collection.

Abbreviations

CBD

Cannabidiol

CKD

Chronic kidney disease

CRS

Cardiorenal syndrome

MCU

Mitochondrial Ca^{2+} Uniporter

PGC

1 α -Peroxisome proliferator-activated receptor gamma coactivator 1- α

TFAM

Mitochondrial transcription factor A

PSV

Peak systolic velocity

ROS

Reactive oxygen species

HF

Heart failure

RI

Renal resistive index

EDV

End-diastolic velocity

Declarations

Competing interests:

Synthetic cannabidiol used in this study was provided by Cardiol Therapeutics. Cardiol Therapeutics had no role in the study design, data collection, analysis, interpretation, or the decision to submit this manuscript for publication. All other authors declare no competing interests.

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Author Contributions:

HCHV: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing – original draft; Writing – review & editing.

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Data availability:

All data generated or analyzed during this study are included in the published article and its Supplementary Information. Additional data are available from the corresponding author upon reasonable request. This study did not generate custom code or algorithms. All statistical analyses were performed using GraphPad Prism (v10), a commercially available software package.

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Figures

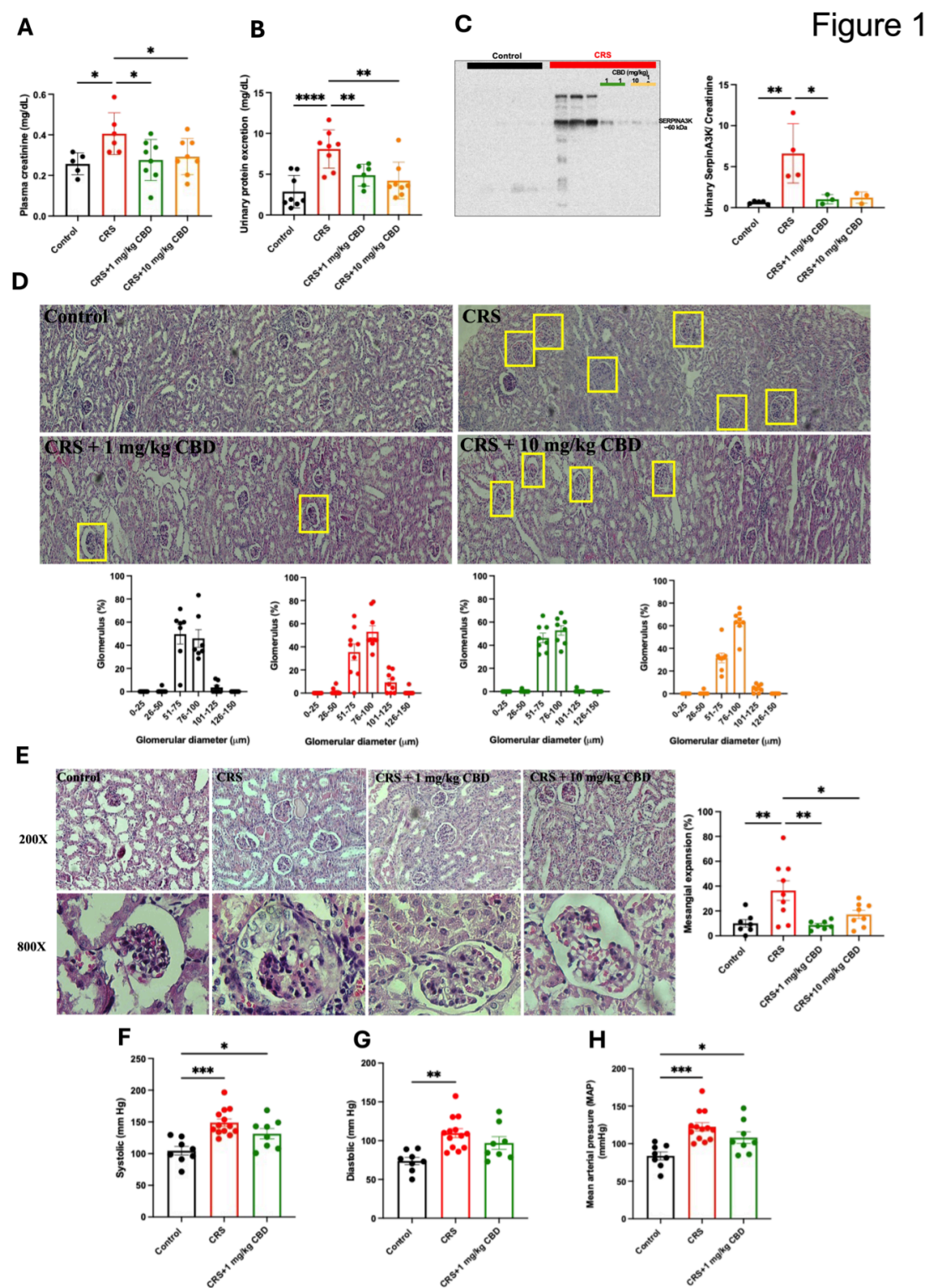


Figure 1

Cannabidiol attenuates renal dysfunction and injury in cardiorenal syndrome. Plasma creatinine (A; n = 5 Control, 6 CRS, 8 CRS+CBD 1 mg/kg, 8 CRS+CBD 10 mg/kg), urinary protein excretion (B; n = 8, 8, 6, 8), and urinary SerpinA3K normalized to creatinine, with representative blot (C; n = 5,4,3,3). Glomerular hypertrophy (D; n = 7,9,8,8) and mesangial expansion (E; n = 7,9,8,8), with representative histology. Systolic (F; n = 8 Control, 13 CRS, 8 CRS+CBD 1 mg/kg), diastolic (G; n = 8,13,8) and mean arterial pressure (H; n = 8,13,8). CRS + CBD received 1 or 10 mg/kg CBD for renal analyses; hemodynamics were assessed only in the 1 mg/kg group. Mean $\pm$ SEM; one-way ANOVA, Tukey's test. * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001.

Figure 2

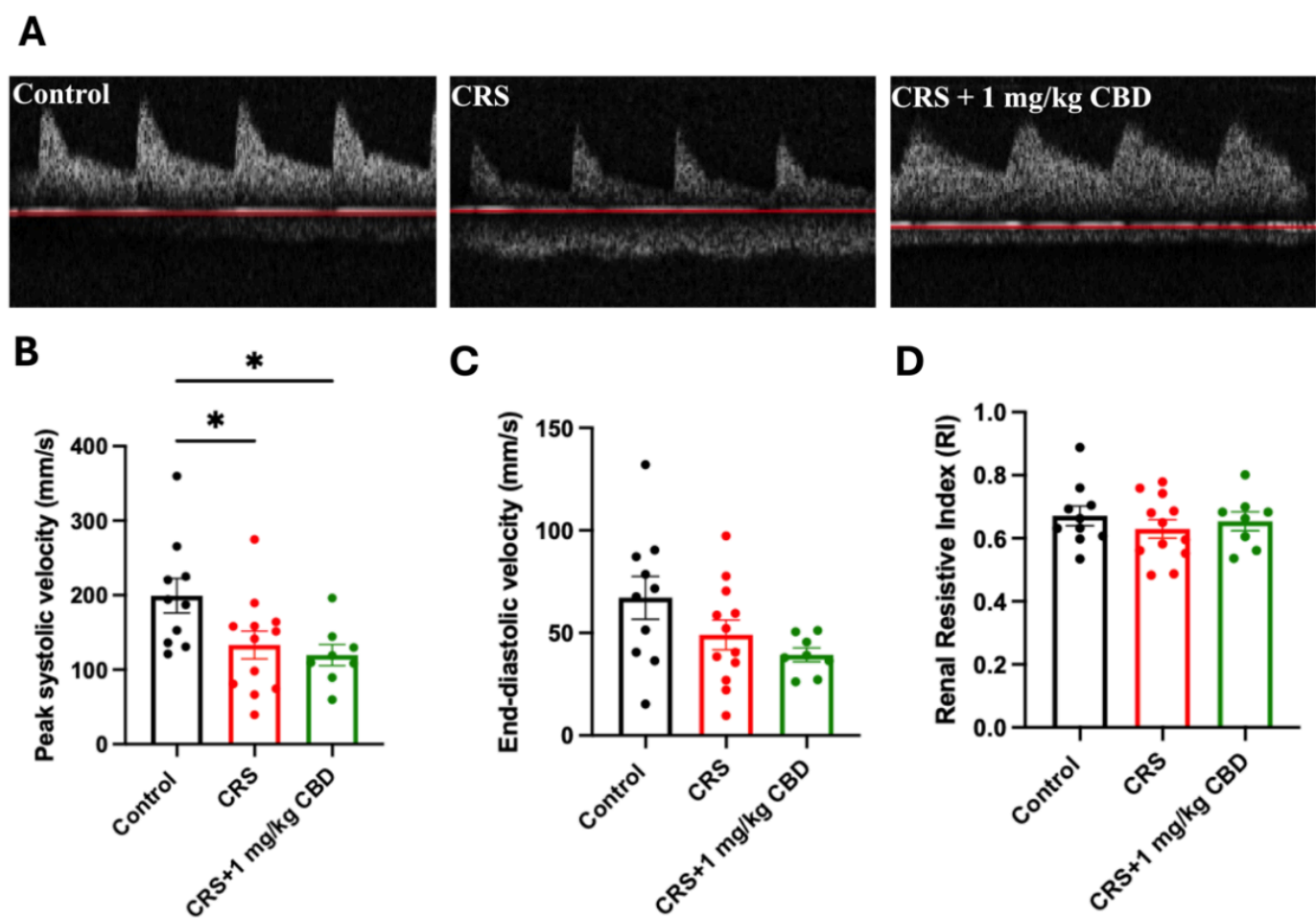


Figure 2

Cannabidiol does not alter intrarenal vascular resistance in experimental cardiorenal syndrome.

Representative renal Doppler images (A). Renal artery peak systolic velocity (PSV, B; n = 10 Control, 12 CRS, 8 CRS+CBD) and end-diastolic velocity (EDV, C; n = 10,12,8). Renal resistive index (RI = [PSV-EDV]/PSV, D; n = 10,12,8). CRS + CBD received 1 mg/kg CBD. Mean $\pm$ SEM; one-way ANOVA, Holm-Sidak test. * p <0.05 vs Control.

Figure 3

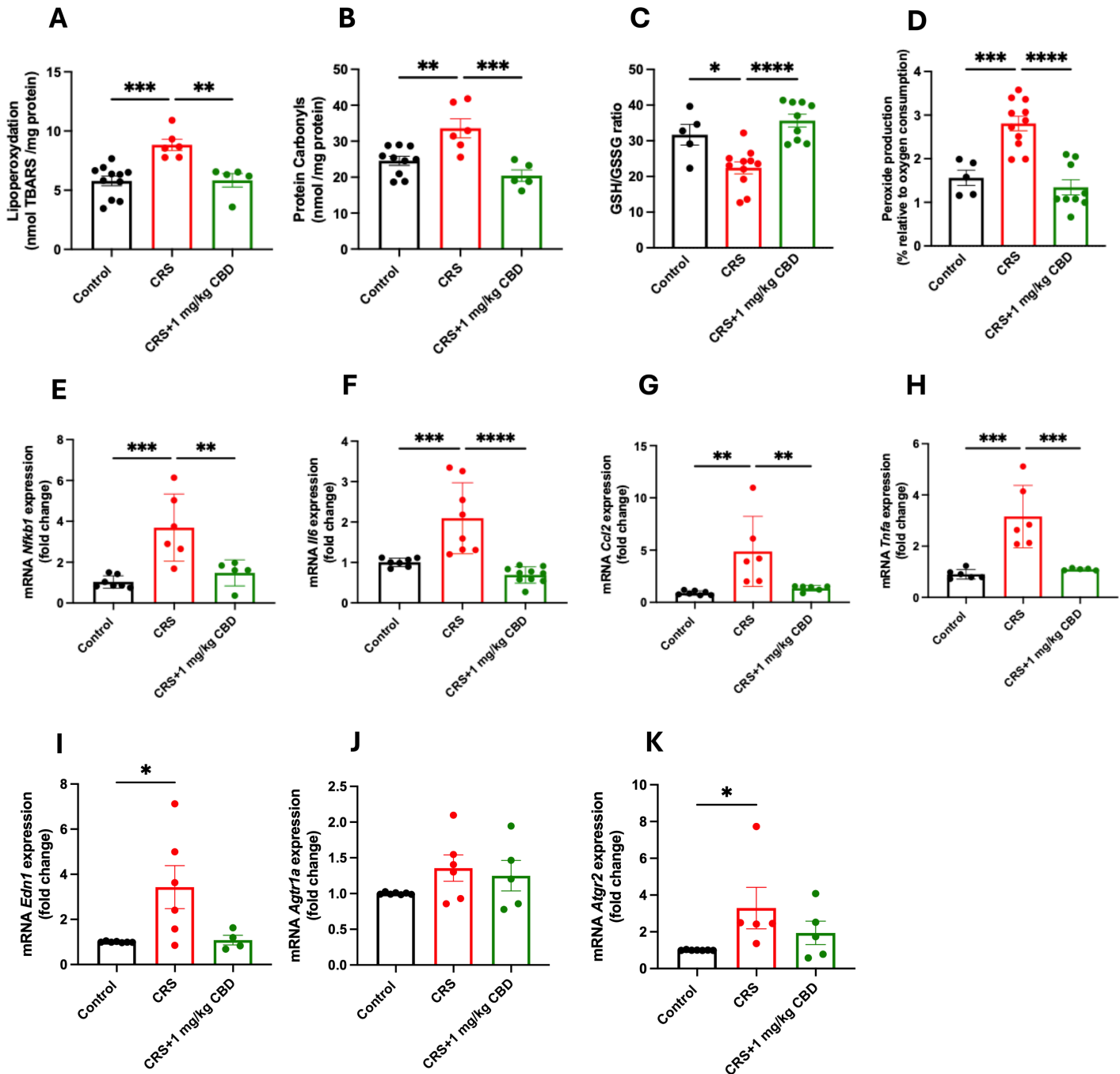


Figure 3

Cannabidiol reduces oxidative stress, inflammatory and vasoactive signaling in experimental cardiorenal syndrome. Lipid peroxidation (TBARS, A; n = 11 Control, 6 CRS, 5 CRS+CBD), protein carbonyls (B; n = 10,6,5), GSH/GSSG ratio (C; n = 5,11,9), and mitochondrial H₂O₂ (Amplex Red, D; n = 5,11,9). Renal mRNA expression of *Nfkb1* (E; n = 7,6,5), *Il6* (F; n = 7,8,10), *Ccl2* (G; n = 7,6,6), *Tnfa* (H; n = 6,6,5), *Edn1* (I; n = 7,6,4), *Agtr1a* (J; n = 7,6,5), and *Agtr2a* (K; n = 7,5,5), normalized to β -actin. CRS + CBD received 1 mg/kg CBD. Mean $\pm$ SEM; one-way ANOVA, Tukey's test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, *****p<0.00001.

****p<0.0001. TBARS: thiobarbituric acid reactive substances; GSH/GSSG: reduced/oxidized glutathione ratio.

Figure 4

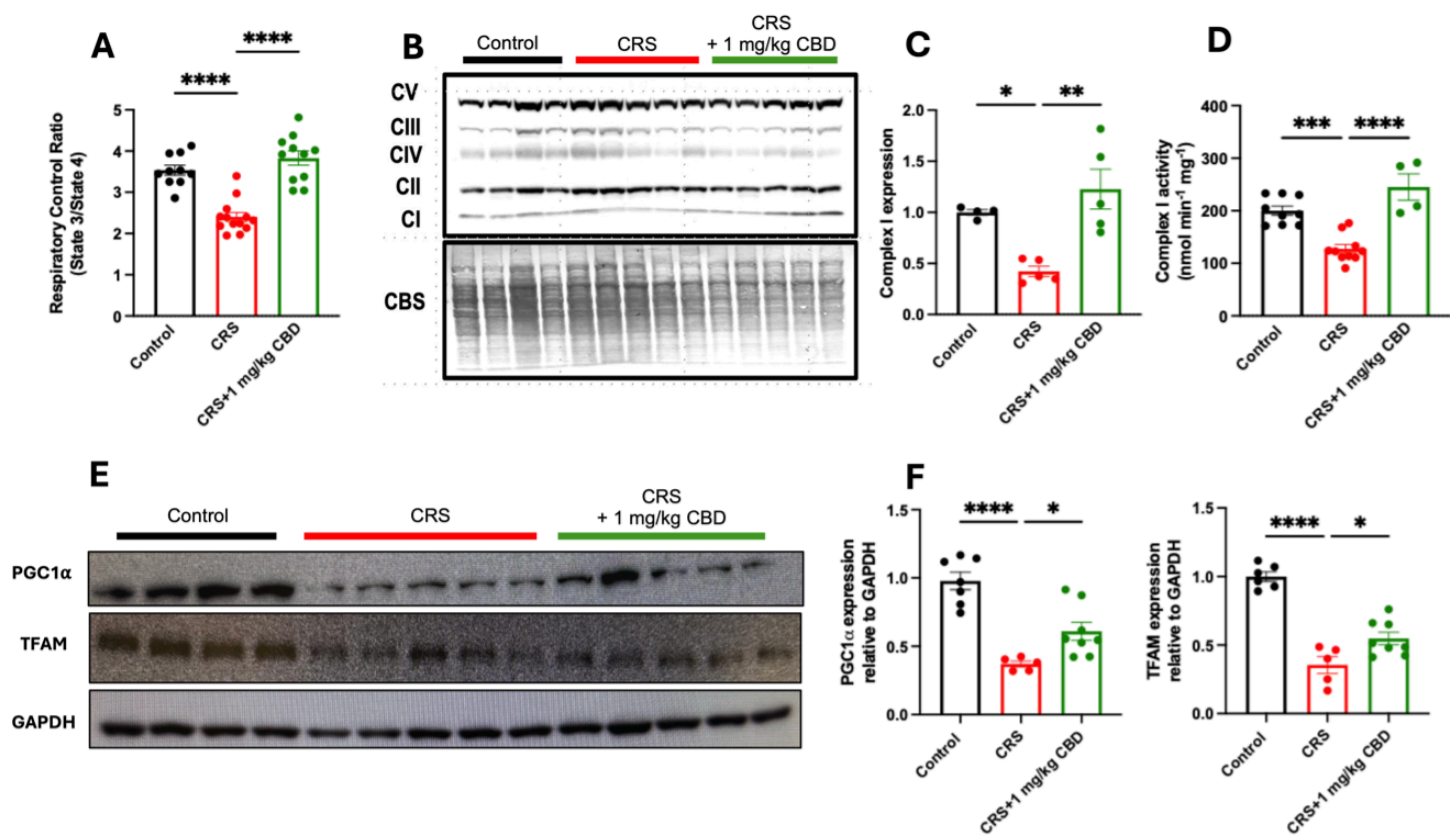


Figure 4

Cannabidiol improves mitochondrial respiratory function and biogenesis in renal tissue. Respiratory control ratio (state 3/state 4, A; n = 10 Control, 13 CRS, 11 CRS+CBD); representative blot of complexes CI–CV, with Coomassie Blue Staining (CBS) as loading control (B); complex I protein expression (C; n = 4,5,5) and activity (D; n = 9,10,4); representative blot of PGC-1α and TFAM (E; n = 4,5,5); PGC-1α protein expression (F; n = 7,5,8) and TFAM (F; n = 6,5,8) normalized to GAPDH. CRS + CBD received 1 mg/kg CBD. Mean ± SEM; one-way ANOVA, Tukey's test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Figure 5

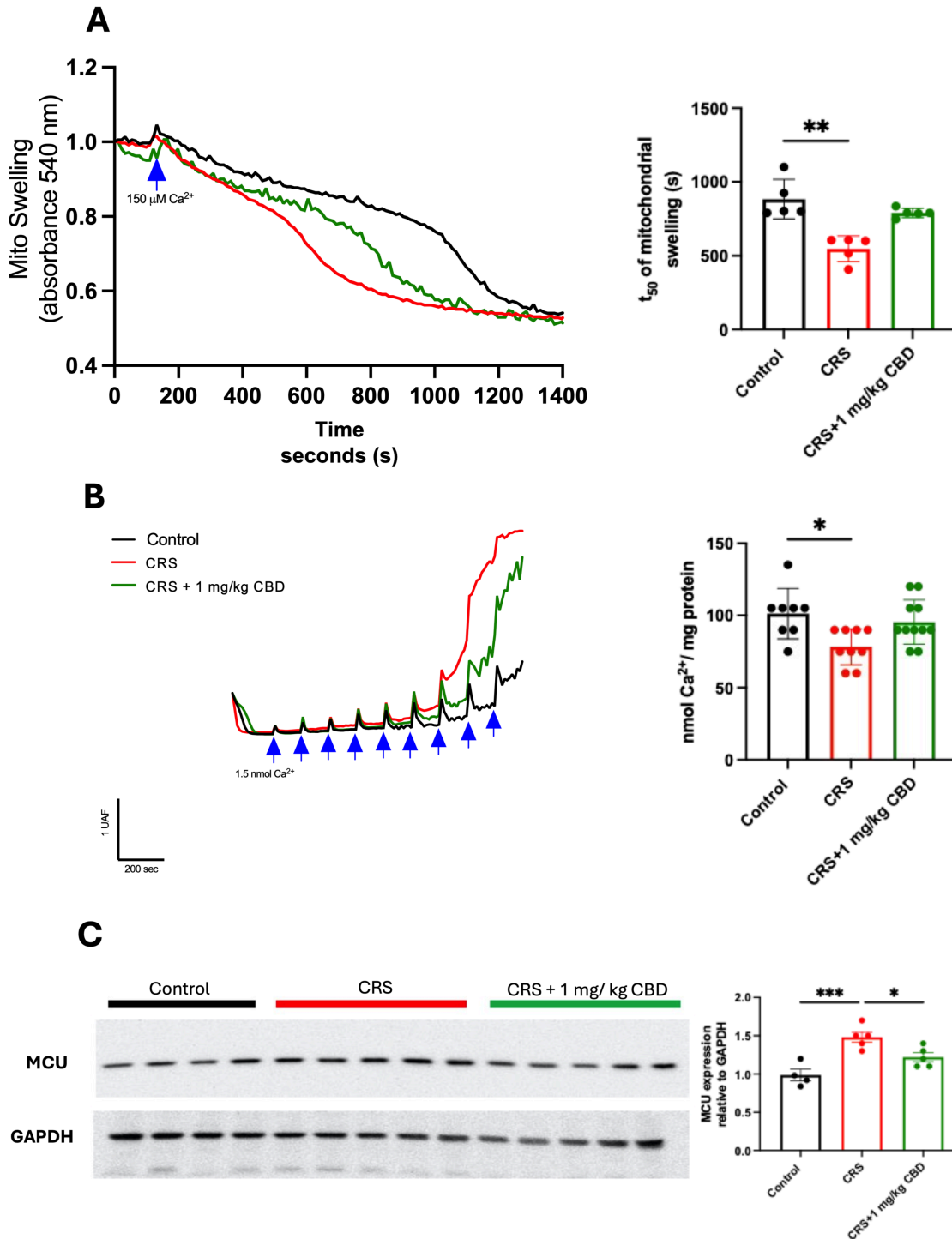


Figure 5

Cannabidiol restores mitochondrial calcium homeostasis in renal tissue. (A) Mitochondrial swelling (absorbance) after Ca^{2+} challenge ($n = 5$ Control, 5 CRS, 5 CRS+CBD); CRS+CBD showed a trend toward increased t_{50} relative to CRS ($p=0.073$, not significant). (B) Calcium retention capacity (CRC), representative traces and quantification ($n = 8,9,11$); arrows indicate 15 μ mol/L Ca^{2+} additions; CRS+CBD showed a trend toward increased CRC relative to CRS ($p=0.052$, not significant). (C) MCU expression

normalized to GAPDH, with representative blot (n = 4,5,5). CRS + CBD received 1 mg/kg CBD. Mean ± SEM; one-way ANOVA, Tukey's test (n = 4–11). *p<0.05, **p<0.01, ***p<0.001. MCU: mitochondrial calcium uniporter; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Figure 6

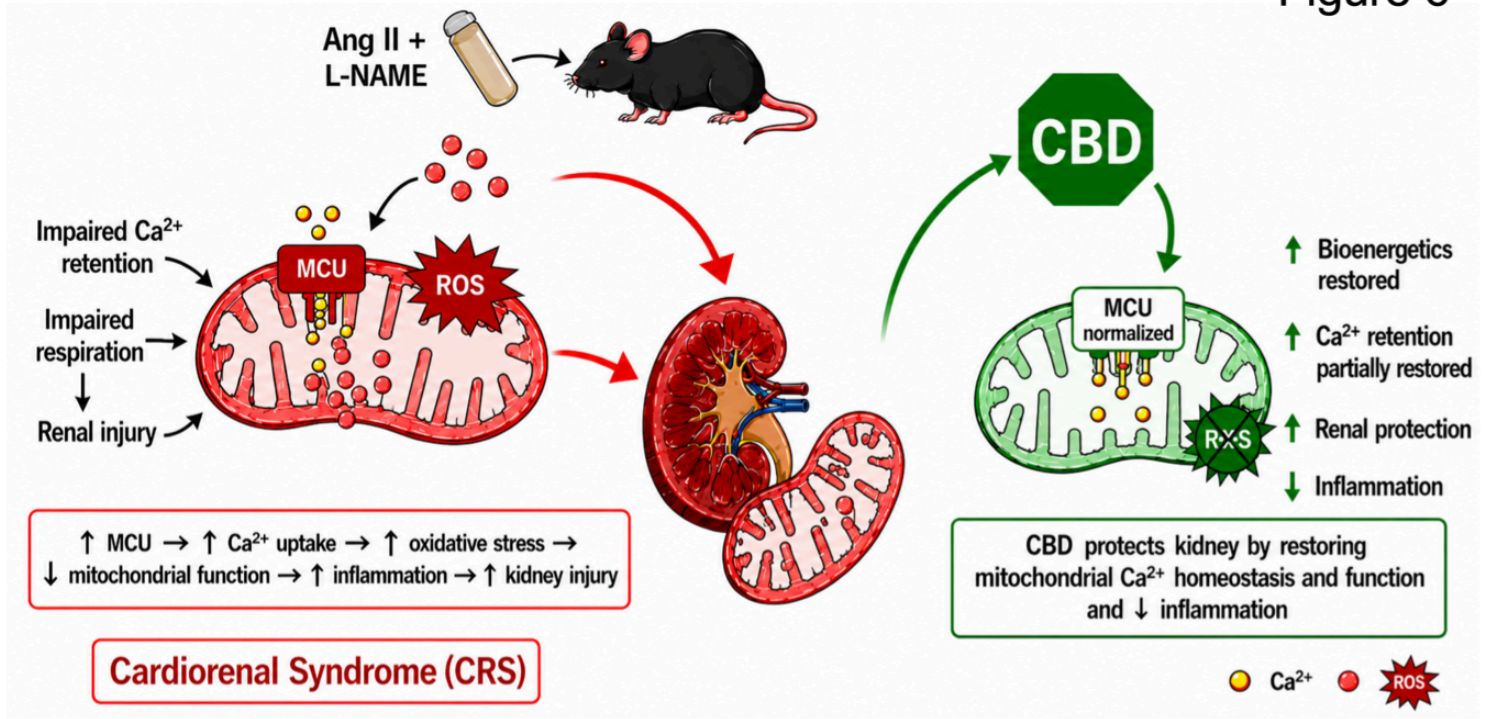


Figure 6

Cannabidiol protects the kidney by restoring mitochondrial calcium homeostasis in experimental cardiorenal syndrome. Schematic representation summarizing the main findings of the study. In the experimental cardiorenal syndrome (CRS) model, induced by angiotensin II and L-NAME, mitochondrial overexpression of the calcium uniporter (MCU) increases Ca²⁺ uptake, thereby reducing mitochondrial function, increasing reactive oxygen species (ROS), and promoting renal dysfunction. Cannabidiol (CBD) normalizes MCU expression, partially restores Ca²⁺ retention and mitochondrial bioenergetics, suppresses ROS generation, and reduces renal inflammation. Together, these effects demonstrate that CBD protects the kidney by restoring mitochondrial Ca²⁺ homeostasis and function in experimental CRS.

Supplementary Files

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