PORTLAND
PRESS

Commentary

The CAT-1 is out of the bag: endothelial Cationic Amino Acid Transporter-1 is a critical player in cardiorenal syndrome type 2

Michael P. Hutchens[1] and Carmen De Miguel[2]

[1]Department of Anesthesiology and Perioperative Medicine, Oregon Health and Science University, Portland, OR, U.S.A.; [2]Section of Cardio-Renal Physiology and Medicine, Division of Nephrology, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, U.S.A.

Correspondence: Carmen De Miguel (mdemiguelgarijo@uabmc.edu)

Although the numbers of patients affected by cardiorenal syndrome keeps increasing, we lack a complete understanding of the molecular pathways involved in its development and progression. Nitric oxide synthase (NOS) may play a role in cardiorenal syndrome, particularly cardiorenal syndrome type 2 (CRS2). However, complexities and paradoxical clinical findings have limited translation. In the current *Clinical Science*, Giam et al. (*Clinical Science* (2020) **134**, 2755–2769) highlight the role of a key NOS substrate transporter, the cationic amino acid transporter-1, in preserving renal function in CRS2. In this commentary, we introduce the cardiorenal syndrome and the putative role that nitric oxide (NO) may play in the development of this disease and discuss the exciting findings of Giam et al. (*Clinical Science* (2020) **134**, 2755–2769) and their tantalizing translational implications.

Chronic heart failure accounts for a considerable portion of the excess mortality attributed to cardiovascular disease, and precisely because of its chronicity, disproportionally contributes to suffering. Patients with chronic heart failure experience increasing limitations and symptoms, and with advancing disease, progressive organ failure. Cardiorenal syndrome type 2 (CRS2) occurs as renal function is progressively impaired (perhaps in part by episodes of CRS1) in chronic heart failure, increasing risk of dialysis and death. This syndrome has long been recognized by physicians treating heart failure and kidney disease, but challenges of investigating across disciplines hampered mechanistic understanding until recently. In particular, when viewed separately, the cardiorenal syndromes (summarized in Table 1) have sometimes been reductively conceived either as kidney disease induced by lack of blood flow, or as heart disease induced by lack of solute clearance. However, in the past 15 years a series of influential consensus definitions and organizing reviews have identified that heart–kidney interactions and signals themselves represent an intervenable target which may be shared across the syndromes. With this recognition came a challenge: mechanistic understanding in cardiorenal syndromes would require investigators with expertise in both organ systems to discern the signaling pathways between them. Nitric oxide (NO) signaling is an informative example of this challenge.

Table 1 Cardiorenal syndrome classification

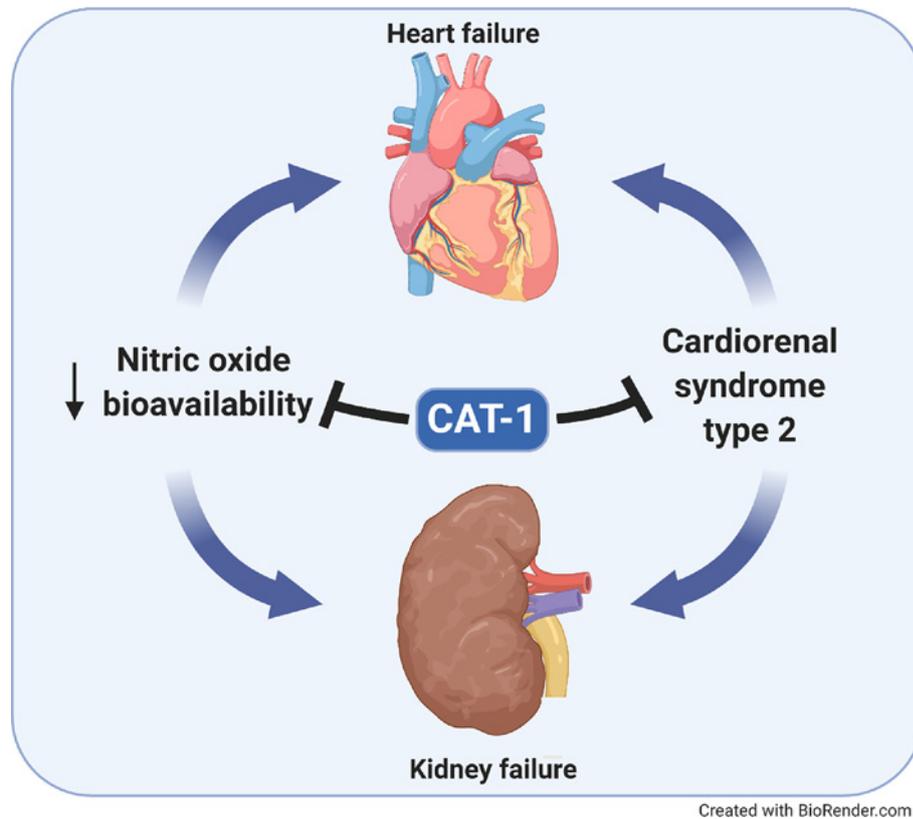| Syndrome	Acuity of inciting disease	Inciting organ	Affected organ
CRS1	Acute	Heart	Kidney
CRS2	Chronic	Heart	Kidney
CRS3	Acute	Kidney	Heart
CRS4	Chronic	Kidney	Heart
CRS5	Acute	Systemic	Both

Figure 1. Endothelial CAT-1 at the center of complex heart–kidney interactions in CRS2
Heart failure causes chronic kidney disease, while chronic kidney disease influences and can worsen heart failure. NO is a key signal molecule in the cardiovascular system, and is less bioavailable in heart failure, kidney injury and CRS2. CAT-1 transports L-arginine and makes this critical substrate available to NOS for NO biosynthesis. CAT-1 is, therefore, an attractive therapeutic target which could interrupt or reverse CRS2.

Since its Nobel Prize winning recognition as an endothelial-derived relaxing factor, NO is increasingly recognized as a foundation of cardiovascular disease and health. NO has recognized roles in heart failure, regulating myocardial contractility, the response to adrenergic stimulation, and vascular tone; NO dysfunction is a crucial contributor to heart failure [1]. Highlighting its role at the nexus of heart–kidney interactions, NO is also central to kidney disease. All isoforms of NO synthase (NOS) use L-arginine as substrate for the production of NO [2]. Numerous studies have demonstrated reduced renal and systemic NO and L-arginine in chronic kidney disease [3], and extensive evidence shows that deficient transport of L-arginine results in reduced NO bioavailability in animal models of kidney disease [4–8]. Uremic disease may further impair intrarenal NO synthesis by inhibition of NOS; asymmetric dimethyl-larginine, an accumulated uremic toxin is a competitive inhibitor of renal NOS [9]. Meanwhile, systemic levels of L-arginine, the critical substrate for NO production, are reduced in heart failure [10]. Adding fuel to the fire, systemic and myocardial L-arginine transport are deficient in heart failure patients and associated with a deficit in the cationic anion transporter-1 (CAT-1) [11]. However, supplementation with L-arginine and other related interventions for heart failure have produced mixed results. Taken together, these data suggest a co-regulatory environment, in which abnormalities of NO metabolism in the kidney and those mediated by heart failure conspire to worsen CRS2, potentially further worsening heart failure (Figure 1).

In the present issue of *Clinical Science*, Giam et al. [12] step into this loop, methodically evaluating the concept that reduced CAT-1 expression in heart failure leads to reduced NO production and results in decreased kidney function. Two mouse models of CRS2 were used in these studies. The DCM mouse is an accepted heart failure model which overexpresses the mammalian sterile 20-like kinase 1 (Mst1) in cardiomyocytes. On the other hand, the novel HFCAT-1 mouse overexpresses Mst1 in cardiomyocytes and the CAT-1 specifically in endothelial cells. Using these animal models, the authors elegantly demonstrate that endothelial overexpression of CAT-1 in a mouse model of heart failure restores plasma NO levels and leads to improvement of the renal function, fibrosis and inflammation.

Despite the persistent cardiac dysfunction, the HFCAT-1 mice presented with a significant attenuation of the urinary albumin/creatinine ratio, decreased kidney T-cell infiltration and diminished collagen deposition in the kidney and heart relative to their DCM controls. T-cell infiltration and renal dysfunction were reduced by similar quantities, while anti-inflammatory renal interleukin-10 was increased threefold, suggesting concurrent mediation of inflammation and renal function through the CAT-1 axis. Importantly, overexpression of CAT-1 did not reduce systemic blood pressure, a potential challenge for translational studies, and a limitation of therapeutic trials using L-arginine. CAT-1 overexpression, however, was not a panacea, as cardiac function was not improved in HFCAT-1 mice. Nevertheless, this observation reinforces the complex nature of heart–kidney interactions: renal function was improved by CAT-1 overexpression without alterations in heart function.

One of the limitations of the current study is that the protective renal effects of overexpressing CAT-1 in the endothelium were studied using solely male mice. In general, premenopausal females show lower prevalence of cardiovascular disease than males of the same age, including heart failure and renal disease. However, this apparent protection disappears once they reach menopausal age and the incidence becomes comparable or even greater than in the male population [13]. This evidence highlights the fact that different molecular mechanisms may be involved in males and females, and investigation of these molecular pathways in both sexes is desired to improve health outcomes in both men and women in our society. Similarly, it is important to investigate the long-term effects of endothelial CAT-1 overexpression/activity. Beyond being a substrate for NOS, L-arginine can also be metabolized by other enzymes, for instance arginase or glycine amidinotransferase [14]. Arginase 2 in particular, is highly expressed in the kidney and endothelial cells [15]. It was recently reported that selective inhibition of endothelial arginase 2 protects against renal fibrosis in a mouse model of chronic kidney disease [16]. Moreover, up-regulation of arginase was described in heart failure patients [17]. Thus, increasing the transport of L-arginine by overexpressing CAT-1 in the endothelium might result in increased arginase 2 activity, and possibly worsen kidney damage and heart failure in the long run. Development of therapies targeting CAT-1 expression or activity will need to test effects in these other enzymes that also metabolize L-arginine.

The findings by Giam et al. [12] raise an interesting question: could pharmacological targeting of L-arginine transport in the endothelium reduce the renal consequences of heart failure and improve CRS2? As mentioned in the manuscript, there are currently no available drugs which increase CAT-1 activity. Certainly, new therapeutic strategies to reach that goal would be welcomed by the medical community treating CRS and merit further investigation. In the absence of such novel therapeutics, however, manipulation of CAT-1 regulation may offer options. For example, post-translational regulation of this system was long suspected and microRNA-145 was recently found to down-regulate CAT-1 [18], as does miR-122 [19]. Interference with miR-145 or miR-122 could be expected to liberate CAT-1 from this repression, potentially increasing CAT-1 function and improving CRS2. Regardless, the exciting finding of Giam et al. [12] opens new and hopeful possibilities in investigation of CRS2.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Funding

Abbreviations
CAT-1, cationic anion transporter-1; CRS2, cardiorenal syndrome type 2; Mst1, mammalian sterile 20-like kinase 1; NO, nitric oxide; NOS, NO synthase.

References
1 Farah, C., Michel, L.Y.M. and Balligand, J.-L. (2018) Nitric oxide signalling in cardiovascular health and disease. *Nat. Rev. Cardiol.* **15**, 292–316,
https://doi.org/10.1038/nrcardio.2017.224

2 Förstermann, U. and Sessa, W.C. (2012) Nitric oxide synthases: regulation and function. *Eur. Heart J.* **33**, 829–837,
https://doi.org/10.1093/eurheartj/ehr304

3 Baylis, C. (2006) Arginine, arginine analogs and nitric oxide production in chronic kidney disease. *Nat. Clin. Pract. Nephrol.* **2**, 209–220,
https://doi.org/10.1038/ncpneph0143

4 Rajapakse, N.W., Nanayakkara, S. and Kaye, D.M. (2015) Pathogenesis and treatment of the cardiorenal syndrome: Implications of L-arginine-nitric oxide pathway impairment. *Pharmacol. Ther.* **154**, 1–12, https://doi.org/10.1016/j.pharmthera.2015.05.011

5   Kakoki, M., Kim, H.S., Arendshorst, W.J. and Mattson, D.L. (2004) L-Arginine uptake affects nitric oxide production and blood flow in the renal medulla. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287**, R1478–R1485, https://doi.org/10.1152/ajpregu.00386.2004

6   Kakoki, M., Wang, W. and Mattson, D.L. (2002) Cationic amino acid transport in the renal medulla and blood pressure regulation. *Hypertension* **39**, 287–292, https://doi.org/10.1161/hy0202.102700

7   Rajapakse, N.W., Karim, F., Straznicky, N.E., Fernandez, S., Evans, R.G., Head, G.A. et al. (2014) Augmented endothelial-specific L-arginine transport prevents obesity-induced hypertension. *Acta Physiol. (Oxf.)* **212**, 39–48, https://doi.org/10.1111/apha.12344

8   Rajapakse, N.W. and Mattson, D.L. (2013) Role of cellular L-arginine uptake and nitric oxide production on renal blood flow and arterial pressure regulation. *Curr. Opin. Nephrol. Hypertens.* **22**, 45–50, https://doi.org/10.1097/MNH.0b013e32835a6ff7

9   Kielstein, J.T., Fliser, D. and Veldink, H. (2009) Asymmetric dimethylarginine and symmetric dimethylarginine: axis of evil or useful alliance? *Semin. Dial.* **22**, 346–350, https://doi.org/10.1111/j.1525-139X.2009.00578.x

10   Hanssen, H., Brunini, T.M.C., Conway, M., Banning, A.P., Roberts, N.B., Mann, G.E. et al. (1998) Increased l-arginine transport in human erythrocytes in chronic heart failure. *Clin. Sci.* **94**, 43–48, https://doi.org/10.1042/cs0940043

11   Kaye, D.M., Parnell, M.M. and Ahlers, B.A. (2002) Reduced myocardial and systemic L-arginine uptake in heart failure. *Circ. Res.* **91**, 1198–1203, https://doi.org/10.1161/01.RES.0000047506.52381.90

12   Giam, B., Nomura, H., Kuruppu, S., Chu, P.-Y., Essid, S., Kiriazis, H. et al. (2020) Endothelial specific overexpression of cationic amino acid transporter-1 prevents loss of renal function in cardiorenal syndrome type 2. *Clin. Sci.* **134**, 2755–2769, https://doi.org/10.1042/CS20200087

13   Reckelhoff, J.F. and Samson, W.K. (2015) Sex and gender differences in cardiovascular, renal and metabolic diseases. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **309**, R1057–9, https://doi.org/10.1152/ajpregu.00417.2015

14   Wu, G. and Morris, Jr, S.M. (1998) Arginine metabolism: nitric oxide and beyond. *Biochem. J.* **336**, 1–17, https://doi.org/10.1042/bj3360001

15   Morris, Jr, S.M. (2009) Recent advances in arginine metabolism: roles and regulation of the arginases. *Br. J. Pharmacol.* **157**, 922–930, https://doi.org/10.1111/j.1476-5381.2009.00278.x

16   Wetzel, M.D., Stanley, K., Wang, W.W., Maity, S., Madesh, M., Reeves, W.B. et al. (2020) Selective inhibition of arginase-2 in endothelial cells but not proximal tubules reduces renal fibrosis. *JCI Insight* **5**, https://doi.org/10.1172/jci.insight.142187

17   Quitter, F., Figulla, H.R., Ferrari, M., Pernow, J. and Jung, C. (2013) Increased arginase levels in heart failure represent a therapeutic target to rescue microvascular perfusion. *Clin. Hemorheol. Microcirc.* **54**, 75–85, https://doi.org/10.3233/CH-2012-1617

18   Wang, Y. and Jin, L. (2018) miRNA-145 is associated with spontaneous hypertension by targeting SLC7A1. *Exp. Ther. Med.* **15**, 548–552

19   Bhattacharyya, S.N., Habermacher, R., Martine, U., Closs, E.I. and Filipowicz, W. (2006) Relief of microRNA-mediated translational repression in human cells subjected to stress. *Cell* **125**, 1111–1124, https://doi.org/10.1016/j.cell.2006.04.031