



24 Renal transforming growth factor-beta (TGF- $\beta$ ) has been implicated in the pathogenesis of  
25 hypertension. Renal TGF- $\beta$ 1 protein expression increased along with blood pressure in male  
26 Dahl salt-sensitive (DS) rats in response to a high sodium diet (6) and in male stroke-prone  
27 spontaneously hypertensive rats (SHR) when the drinking water contained 1% sodium (8).  
28 Moreover, a neutralizing antibody to TGF- $\beta$  prevented the sodium-induced increase in mean  
29 arterial pressure (MAP) in the male DS rats. Less is known regarding the role renal TGF- $\beta$  plays  
30 in hypertension in females since TGF- $\beta$  protein expression did not increase with MAP in the DS  
31 female rat in response to a high sodium diet (6) and no females were included in the above  
32 stroke-prone SHR study (8).

33 Rigor and reproducibility (R&R) are the foundation of science; however, there is a  
34 growing realization in the scientific community that practices that optimize R&R have not been  
35 adequately employed which has led to irreproducible findings and wasted resources (2). The  
36 National Institutes of Health (NIH) now expects research grants to address R&R in their  
37 proposals and comprehensive educational programs in R&R must be included in all individual  
38 and institutional training and career development grant applications. As of January 2016, NIH  
39 requires all grant applicants to consider the biological variable of sex as an important aspect of  
40 R&R since one's sex impacts the incidence, age of onset, manifestation, severity and rate of  
41 progression as well as response to treatment in diverse pathologies including cardiovascular,  
42 renal and metabolic diseases (1, 7).

43 Not reporting findings or interpreting data by sex has contributed to the lack of  
44 reproducibility. Mirabito et al. (5) found the angiotensin type 2 receptor plays a protective role in  
45 the pressure-natriuresis relationship in female but not in male rats. Ji et al. (3) showed that T  
46 cells from males but not from females increase the magnitude of angiotensin II-dependent  
47 hypertension in mice. These two examples illustrate how negative findings in one sex could

48 mask positive effects in the other if the results were not separated by sex and how extrapolating  
49 positive results discovered in one sex could lead to erroneous conclusions in the other.

50 In this issue, Tipton et al. (10) shows that maturation from ~1 to 4 months of age doubled  
51 renal TGF- $\beta$ 1 protein expression in the female but not male SHR. Thus, this study demonstrates  
52 a sex-specific role for renal TGF- $\beta$ 1 in the maturing female SHR kidney and supports a previous  
53 report showing that renal TGF- $\beta$  protein expression was nearly 3-fold higher in the 12 vs 4  
54 month old female DS rat maintained on a low sodium diet (4). The Sullivan laboratory previously  
55 found that the frequency of renal Foxp3<sup>+</sup>-CD4<sup>+</sup> T regulatory cells correlated with systolic blood  
56 pressure in female but not in male SHR (9). While hydrochlorothiazide-reserpine (HCTZ-Res)  
57 reduced systolic blood pressure in both male and female SHR, the frequency of T regulatory  
58 cells was reduced only in the female rat.

59 In this current study (10), HCTZ-Res reduced both MAP and renal TGF- $\beta$ 1 protein  
60 expression in the female SHR and HCTZ-Res treatment prevented the age-associated increase  
61 in MAP and renal TGF- $\beta$ 1 protein. The authors also reported that treating female SHR with a  
62 monoclonal antibody to TGF- $\beta$  did not result in lowering MAP. These findings suggest that the  
63 rise in renal TGF- $\beta$ 1 is a consequence of the increased MAP rather than the cause. Further  
64 studies are needed, however, to confirm that the antibody treatment fully neutralized TGF- $\beta$ .  
65 Research is also needed to compare the role of renal TGF- $\beta$  in various male models of  
66 hypertension since renal TGF- $\beta$ 1 protein expression correlated with MAP in DS (6) and stroke-  
67 prone SHR (8) under high sodium ingestion but not in the current study of male SHR on a  
68 normal sodium diet.

69 This article is important not only because it provides mechanistic insight into the role of  
70 renal TGF- $\beta$ 1 in hypertension but also because the study improves our fundamental  
71 understanding of female renal pathophysiology. Few investigators study renal mechanisms in  
72 the female even though kidney disease afflicts both men and women. A review of all papers

73 published this year between January and July in the *American Journal of Physiology - Renal*  
74 *Physiology*, which publishes "information on kidney and urinary tract physiology, epithelial cell  
75 biology, and control of body fluid volume and composition" (<http://ajprenal.physiology.org>),  
76 showed that less than 15% of the animal studies included both sexes and of these, half did not  
77 specify the n value by sex. Furthermore, there were 5 studies conducted solely in male animals  
78 to every study performed in females (Fig. 1A). This male:female (M:F) ratio has *not* improved  
79 over the past ten years since a review of all animal studies published in this journal in January  
80 2007 revealed a similar 4.5:1 M:F ratio. Two other prominent journals including *Kidney*  
81 *International* (11:1 M:F; Fig. 1B) and the *Journal of the American Society of Nephrology* (16:1  
82 M:F; Fig. 1C) which also publish key findings in renal physiology and pathophysiology, exhibited  
83 even greater male bias.

84 The dearth of research in female animal models has contributed to the lack of  
85 reproducibility since the biological variable of sex is often not considered in the interpretation of  
86 results. The National Institute of Diabetes and Digestive and Kidney Diseases held a workshop  
87 this past July on *Sex and the Kidneys: Sex Differences in Renal Disease* to raise awareness of  
88 this problem. These journal statistics serve as one metric for assessing the efficacy of efforts led  
89 by NIH and others in the scientific community to improve consideration of biological sex in  
90 mechanisms of renal physiology and pathology. Hopefully, the intriguing findings arising from  
91 the Sullivan lab will inspire more investigators to study disease mechanisms in the female  
92 kidney.

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#### 98 **AUTHOR CONTRIBUTIONS**

99 K.S., A.V.P. and T.M. drafted, edited and approved the manuscript; T.M. conducted the research,  
100 analyzed the data and prepared the figures.

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### 136 **Figure Legends**

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138 Fig. 1 The number of articles published monthly between January-July 2017 in A) the *American*  
139 *Journal of Physiology-Renal Physiology (Am J Physiol Renal Physiol)* B) *Kidney International*  
140 (*Kidney Int*), and C) the *Journal of the American Society of Nephrology (J Am Soc Nephrol)* as a  
141 function of the sex of the animals studied  $\pm$  SEM. In addition to reviewing the methods, figure  
142 and table legends for each article, the entire article was electronically searched for the terms:  
143 male, man, men, female, woman, women, sex, and gender.

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