Impact of casein- versus grain-based diets on rat renal sodium transporters’ abundance and regulation

Alicia A. McDonough,* Luciana C. Veiras,** Brandon McFarlin,* Donna L. Ralph*

*Department of Physiology and Neuroscience, Keck School of Medicine of USC, Los Angeles, CA;
†current address: Department of Biomedical Sciences, Cedar Sinai Medical Center, Los Angeles, CA 90048

Correspondence:

Alicia A. McDonough, Ph.D.
Department of Physiology and Neuroscience
1333 San Pablo St, MMR 508, Los Angeles, CA 90033
phone: (323) 442-1238, fax: (323) 442-2411; email: mcdonoug@usc.edu
KEY POINTS:

- "Control" diets that are casein- versus grain- based differentially impact baseline abundance of sodium transporters all along the nephron
- Renal sodium transporters' responses to angiotensin II treatment are differentially impacted by casein- versus grain- based diets
- Investigators must pair control and treated groups to the same diet so that effects can be ascribed to the treatment (not the diet)

Hypertension - related mortality is on the rise and evidence ranks hypertension as the top global disease burden, thus, an important public health challenge.¹ A component of the hypertension trend can be assigned to lifestyle trends including consumption of higher sodium/lower potassium diets suggesting that reducing these trends could reduce the incidence of hypertension.¹-⁴ For these reasons, definition of molecular mechanisms connecting dietary electrolyte consumption to blood pressure are essential. Pre-clinical studies varying electrolyte intake in rodents utilize synthetic casein-based chows in which the composition (e.g. sodium, potassium, chloride, bicarbonate) can be well defined. However, in studies not focused on diet, rodents are usually bred, maintained and studied on grain-based chow. A few studies have noted an impact of chow composition on renal function. For example, maintaining and breeding Dahl salt sensitive rats on grain-chow blunts the offspring's propensity to develop hypertension and renal injury when fed high salt casein-chow compared to offspring of Dahl rats bred and maintained on casein-chow;⁵ and doubling dietary protein composition of high salt casein-chow exacerbates hypertension, renal damage and immune infiltration.⁶

Recent experiments in our group revealed a significant impact of casein-chow versus grain-chow on the abundance of rat renal sodium transporters (transporters, channels, and claudins) along the
nephron both at baseline and in response to angiotensin II (AngII) infusion model of hypertension (AngII-HTN). We previously reported that distal Na-Cl cotransporter (NCC) was stimulated during AngII-HTN in male Sprague Dawley rats (SDR) fed grain-chow. In another study, we reported that NCC stimulation by AngII was blunted when males were fed K+ supplemented chow, necessarily casein based. Since female SDR exhibit higher NCC and lower baseline plasma [K+], we proceeded to examine their response to AngII-infusion ± K+ supplementation in casein based diets. The pattern of sodium transporter regulation by AngII evident in females fed control K+ casein chow was quite distinct from what we previously reported in males fed control K+ grain-based chow. To clarify whether this was a sexual dimorphism vs. a diet effect, we assessed the impact of AngII-HTN in female SDR fed control K+ grain-chow and discovered a pattern of transporter regulation very similar to that we previously reported for AngII infused control K+ grain-fed male SDR. Our study of the impact of AngII-HTN in female SDR fed grain-based chow was recently published. The aim of this brief communication is to present, the effects of casein-based versus grain-based diets on sodium transporters' abundance and their regulation as a "cautionary tale" to investigators who, like us, utilize both grain- and casein-based chows to study renal transporter regulation.

All studies were approved by the Institutional Animal Care and Use Committee of the Keck School of Medicine of the University of Southern California and adhered to the NIH Guide for the Care and Use of Laboratory Animals. Female SDR were all obtained from Envigo. The grain-based chow (LabDiet 5001, www.labdiet.com) and casein-based chow (Envigo Teklad Diet TD.88239 supplemented to 1% potassium, www.envigo.com) list similar levels of constituents (listed as % weight in grain, casein, respectively): sodium (0.4, 0.3%) potassium (1% in both), protein (24, 18%), carbohydrates (58, 63%) and fat (5.2, 5.3%). Minor differences in these and other constituents may impact kidney sodium transporter expression directly or secondarily. Methods, previously described in detail and abstracted in legends, were applied uniformly over a period of several months by the same personnel.
to the casein-chow and grain-chow fed rat series. Rats in both series were infused with 400 ng/kg/min AngII via osmotic mini-pumps for 14 days (AngII-HTN) or sham treated (control).

Differences in sodium transporters’ abundance at baseline were evident in cortical homogenates from sham treated casein-chow versus grain-chow fed female rats (Fig. 1): proximal tubule (PT) Na⁺/H⁺ exchanger isoform 3 (NHE3) and its phosphorylated form NHE3pS552 were 27% and 57% more abundant, respectively, while phosphorylated forms of thick ascending limb (TAL) apical Na⁺-K⁺-2Cl⁻ co-transporter isoform 2 (NKCC2p) and distal convoluted tubule (DCT) Na⁺-Cl⁻-2Cl⁻ co-transporter (NCCp) were both 30% less abundant in casein- versus grain-chow fed SDR. NCC, NKCC2 and epithelial Na⁺ channel gamma subunit abundance (γ-ENaC) were not significantly different between diets. Taken together, the differences predict higher fractional reabsorption of sodium in proximal versus distal nephron of casein-chow fed rats at baseline (or lower fractional reabsorption in grain- versus casein-chow fed rats).

Equivalent efficacy of AngII infusion on series of rats fed the two different diets is supported by 3 measurements: 1) Similar differences in systolic blood pressure between control and AngII infused groups (measured by tail cuff in mmHg): casein-chow fed (110 ± 3 [control] and 193 ± 8 [AngII]) and grain-chow fed (114 ± 2 [control] and 191 ± 1 [AngII]); 2) Similar 20-fold rise in aldosterone in both casein-chow fed (550 ± 61 to 12,949 ± 3,143 pg/ml plasma) and as reported recently for grain-chow fed rats, 3) Pressure-diuresis increased 2-fold in casein-chow fed (not shown) and 4-fold in grain-chow fed rats. Chow-dependent responses to AngII-HTN were evident all along the nephron, summarized in Fig. 2. Data represent fold-changes in transporters’ abundance with AngII-HTN groups normalized to abundance in their control sham- treated rats, defined as 1 (bold dotted line at 1.0) for both casein-chow and grain-chow fed groups. Overall, the responses to AngII-HTN were more robust (*P < 0.01) in the grain-fed versus casein-fed SDRs including: DCT NCC, NCCp and claudin-7, as well as cortical collecting duct α and γ ENaC subunits. NHE3 and medullary TAL
NKCC2 pool sizes, which contribute to pressure diuresis, were smaller in grain- versus casein-chow fed females. Abundance of claudin-2, cortical NKCC2 and beta-ENaC increased similarly during AngII-HTN, that is, independent of diet. We cannot conclude that the same differences would be evident in males.

In conclusion, the results of our analyses comparing sodium transporter profiles in rats subjected to the same protocols and AngII treatment, but fed two different “control” diets, illustrate heretofore unexplored effects of diet on sodium transporters’ (transporters, claudins and channels) abundance along the nephron. Multiple mechanisms may contribute to the transporter-specific responses arising over the 14 day feeding including: differences in chow composition that may influence signaling and metabolism such as sources of proteins, carbohydrates and lipids or differences in gut-microbiome on casein vs grain based diets. In any case, these findings demonstrate that investigators should pair control and experimental diets to the same base, and apply caution in interpreting findings in studies from rats fed different commonly employed “control” diets even if they have similar percentages of electrolytes, protein, fat and carbohydrate.
DISCLOSURES: A. McDonough reports Honoraria: ASN - small stipend for writing a NephSAP review, Merck stipend for consulting on scientific question (not ongoing), Swiss NCCR stipend for evaluating a scientific program; Scientific Advisor or Membership: American Journal of Physiology, Journal of the American Society of Nephrology, Kidney360; Other Interests/Relationships: American Heart Association KCVD committee volunteer. All remaining authors have nothing to disclose.

FUNDING INFORMATION: NIH: Alicia McDonough, Luciana Veiras, Brandon McFarlin, Donna Ralph, NIDDK 2R01DK083785; NIH: Brandon McFarlin, F31 DK1264571

AUTHOR CONTRIBUTIONS: A McDonough: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Validation; Writing - original draft; Writing - review and editing

L Veiras: Conceptualization; Data curation; Investigation; Methodology; Writing - original draft; Writing - review and editing

B McFarlin: Data curation; Formal analysis; Investigation; Writing - review and editing

D Ralph: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Visualization; Writing - review and editing
REFERENCES


### Table 1. Antibody and immunoblot protocol details

<table>
<thead>
<tr>
<th>Antibody Target</th>
<th>~kDa</th>
<th>Protein/lane cortex (µg)</th>
<th>Protein/lane medulla (µg)</th>
<th>Primary antibody supplier</th>
<th>Dilution</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin-2</td>
<td>23</td>
<td>15, 7.5 NA</td>
<td></td>
<td>ThermoFisher (#32-5600)</td>
<td>1:2000</td>
<td>11</td>
</tr>
<tr>
<td>Claudin-7</td>
<td>23</td>
<td>15, 7.5 NA</td>
<td></td>
<td>ThermoFisher (#34-9100)</td>
<td>1:1000</td>
<td>11</td>
</tr>
<tr>
<td>ENaC-α</td>
<td>~100</td>
<td>80, 40 20, 10</td>
<td></td>
<td>Loffing (Zurich)</td>
<td>1:5000</td>
<td>12, 13</td>
</tr>
<tr>
<td></td>
<td>~30</td>
<td>20, 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENaC-β</td>
<td>~100</td>
<td>60, 30 20, 10</td>
<td></td>
<td>Loffing (Zurich)</td>
<td>1:15000</td>
<td>12, 13</td>
</tr>
<tr>
<td>ENaC-γ</td>
<td>~80</td>
<td>60, 30 20, 10</td>
<td></td>
<td>Palmer (Cornell)</td>
<td>1:1000</td>
<td>12, 13</td>
</tr>
<tr>
<td></td>
<td>~60</td>
<td>20, 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCC</td>
<td>150</td>
<td>60, 30 NA</td>
<td></td>
<td>McDonough</td>
<td>1:5000</td>
<td>7</td>
</tr>
<tr>
<td>NCCpS71</td>
<td>150</td>
<td>15, 7.5 NA</td>
<td></td>
<td>Loffing (Zurich)</td>
<td>1:5000</td>
<td>14</td>
</tr>
<tr>
<td>NHE3</td>
<td>~83</td>
<td>15, 7.5 8, 4</td>
<td></td>
<td>McDonough</td>
<td>1:2000</td>
<td>15</td>
</tr>
<tr>
<td>NHE3pS552</td>
<td>~83</td>
<td>5, 2.5 8, 4</td>
<td></td>
<td>Santa Cruz (sc-53962)</td>
<td>1:1000</td>
<td>16</td>
</tr>
<tr>
<td>NKCC2</td>
<td>160</td>
<td>15, 7.5 8, 4</td>
<td></td>
<td>DSHB (Iowa)</td>
<td>1:6000</td>
<td>17</td>
</tr>
<tr>
<td>NKCC2-pT96T101</td>
<td>160</td>
<td>15, 7.5 8, 4</td>
<td></td>
<td>Forbush (Yale)</td>
<td>1:2000</td>
<td>18</td>
</tr>
<tr>
<td>Na,K-ATPase α1</td>
<td>~100</td>
<td>1, 0.5 1, 0.5</td>
<td></td>
<td>Kashgarian (Yale)</td>
<td>1:2000</td>
<td>19</td>
</tr>
</tbody>
</table>

See legends to Figure 1,2 for abbreviations.
FIGURE LEGENDS

FIGURE 1 Effect of casein-based chow versus grain-based chow on select sodium transporters in female Sprague Dawley rats. Rats were acclimated to each diet for 14 days; n = 5-6/group (all samples shown). Abundance of renal transporters was determined by semi-quantitative immunoblot in homogenates from renal cortex as described. For each transporter, samples from casein-based chow fed rats and grain-based chow fed rats were prepared with the same protocol, processed and quantified on the same blot. Both 1 and ½ amounts were assessed to verify linearity of the detection system and loading was verified by quantifying a parallel Coomassie stained gel; Table 1 provides protein loading and protocols. Data were collected and analyzed as arbitrary density units using LI-COR Odyssey Infrared Imaging System. Region of interest is indicated by blues boxes on the last sample on the right; broad bands reflect posttranslational processing of glycoproteins; data were normalized to the mean density of the grain-fed group defined as 1. Box-and-whiskers graphs (error bars indicate minimum and maximum values, boxes indicate quartiles, line indicates median, + indicates mean) plot the relative abundance of each transporter for rats fed casein- vs grain-based chow. *P < 0.01 by unpaired Student’s t-test. “mw” indicates molecular weight (kD) markers.

Abbreviations: ENaC: epithelial Na⁺ channel (fl=full length, cl=cleaved) γ subunit, NCC: Na⁺-Cl⁻ co-transporter, NCCp: phosphorylated at S71 associated with more activity, NHE3: Na⁺/H⁺ exchanger isoform 3, NHE3p: NHE3pS552 associated with less activity, NKCC2: apical Na⁺-K⁺-2Cl⁻ co-transporter isoform 2, NKCC2p: phosphorylated at Thr 96, Thr 101, associated with more activity.

FIGURE 2 Effect of Angiotensin II hypertension on sodium transporter profiles in female Sprague Dawley rats fed casein-based chow versus grain-based chow. Rats were infused with 400 ng/kg/min AngII for 14 days (AngII-HTN) or sham treated (control) while on casein or grain based diets; n = 5-6/group (all samples shown). Abundance of renal transporters was determined by semi-quantitative immunoblot in homogenates prepared from renal cortex and medulla (“m” prefix); areas

...
dissected superimposed on photo of bisected female kidney. Samples from casein- chow fed rats and
grain- chow fed rats were analyzed separately. As in Fig 1, both 1 and \( \frac{1}{2} \) amounts were assessed on
the same immunoblot to verify linearity of the detection system; only one amount shown. Data were
collected and analyzed as detailed in Fig 1 legend. Table 1 provides protein loading and protocols.
AngII treated samples were normalized to the control densities of each transporter within each diet
defined as =1 (bold dotted line).\(^8,10\) The box-and-whiskers graphs, defined in Fig 1, plot the fold
change in each transporter in AngII infused rats fed casein- or grain-based chow. \(*P < 0.01\) by
unpaired Student’s t-test with Benjamini, Krieger, & Yekutieli procedure for controlling false discovery
rate using Graph Pad Prism. Apparent molecular weights (mw KD) indicated on the right of blots.
Abbreviations as in Fig 1 and: AngII: angiotensin II, Cldn: claudin family members -2 and -7, \( \alpha \)NKA:
\( \alpha \)1 sodium pump catalytic subunit.