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Impact of Casein- versus Grain-Based Diets on Rat Renal Sodium Transporters' Abundance and Regulation

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Key Points

- "Control" diets that are casein- versus grain-based differentially affect baseline abundance of sodium transporters all along the nephron.
- Renal sodium transporters' responses to angiotensin II treatment are differentially affected 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 and 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 BP is essential. Preclinical studies varying electrolyte intake in rodents utilize synthetic casein-based chows in which the composition (*e.g.*, sodium, potassium, chloride, and 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 effect 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 with 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 effect 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 the angiotensin II 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 (SDRs) fed grain chow (7). In another study, we reported that NCC stimulation by angiotensin II (AngII) was blunted when males were fed K^+-supplemented chow, necessarily casein based (8). Because female SDRs exhibit higher NCC and lower baseline plasma [K^+] (9), 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 versus a diet effect, we assessed the effect of AngII-HTN in female SDRs fed control K^+ grain chow and discovered a pattern of transporter regulation very similar to that we had previously reported for AngII-infused control K^+ grain-fed male SDRs. Our study of the effect of AngII-HTN in female SDRs fed grain-based chow was recently published (10). 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 National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (11). Female SDRs were all obtained from Envigo. The grain-based chow (LabDiet 5001; www.labdiet.com) and the casein-based chow (Envigo Teklad Diet TD.88239 supplemented to 1% potassium; www.envigo.com) list similar levels of constituents (listed as percentage weight in grain or casein, respectively): sodium (0.4% and 0.3%, respectively) potassium (1% in both), protein (24% and 18%, respectively), carbohydrates (58% and 63%, respectively), and fat (5.2% and 5.3%, respectively). Minor differences in these and other constituents may affect kidney sodium transporter expression directly or secondarily. Methods, previously described in detail (10) 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 per minute AngII *via* osmotic minipumps for 14 days (AngII-HTN) or sham treated (control).

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Differences in sodium transporters' abundance at baseline were evident in cortical homogenates from sham-treated casein chow– versus grain chow–fed female rats (Figure 1): proximal tubule Na^+/H^+ exchanger isoform 3 (NHE3) and its phosphorylated form NHE3pS552 were 27% and 57% more abundant, respectively, whereas phosphorylated forms of thick ascending limb apical Na^+-K^+-$2Cl^-$ cotransporter isoform 2 (NKCC2) and distal convoluted tubule Na^+-Cl^- cotransporter (NCC) were both 30% less abundant in casein chow– versus grain chow–fed SDRs. NCC, NKCC2, and epithelial Na^+ channel γ-subunit (ENaC-γ) abundances were not significantly different between diets. Taken together, the differences predict higher fractional reabsorption of sodium in proximal versus distal

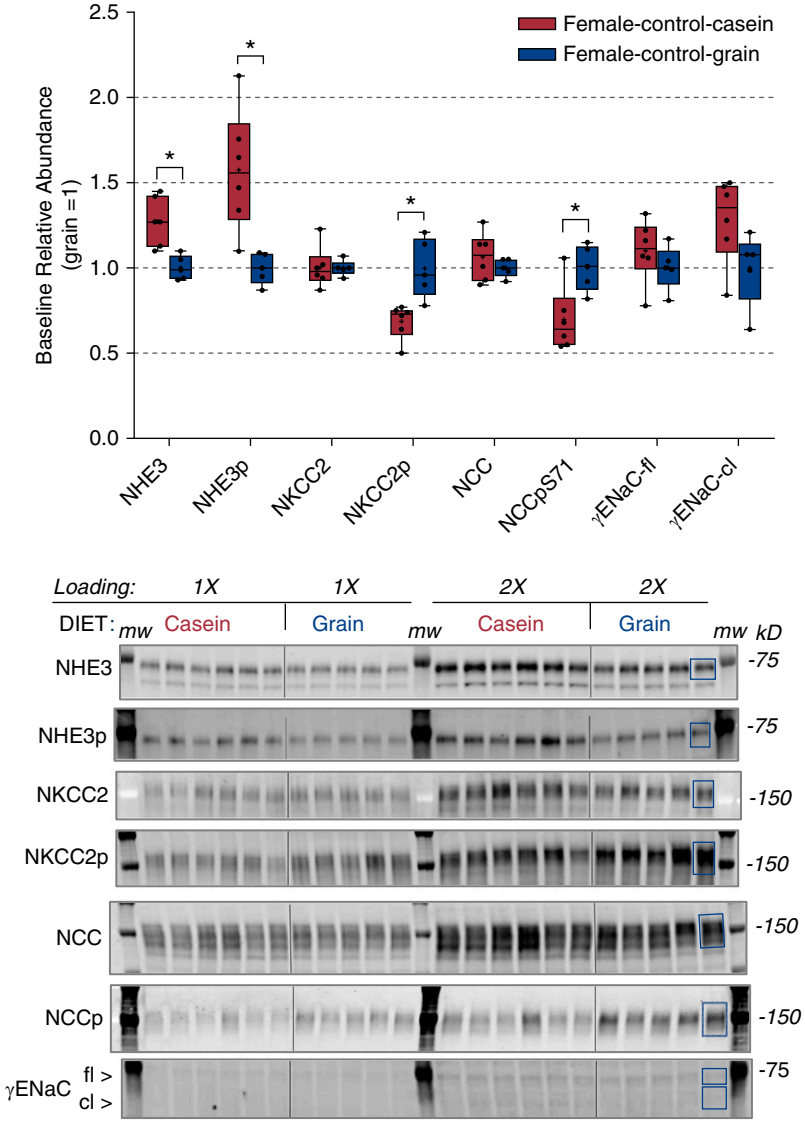

Figure 1. | **Casein-based chow versus grain-based chow differentially impact select sodium transporters in female Sprague Dawley rats**. Rats were acclimated to each diet for 14 days (n=5–6 per group; all samples shown). Abundance of renal transporters was determined by semiquantitative immunoblot in homogenates from renal cortex as described (10). For each transporter, samples from casein chow–fed rats and grain chow–fed rats were prepared with the same protocol, processed, and quantified on the same blot. Both one and 1/2 amounts were assessed to verify linearity of the detection system, and loading was verified by quantifying a parallel Coomassie-stained gel (10); Table 1 provides protein loading and protocols. Data were collected and analyzed as arbitrary density units using the LI-COR Odyssey Infrared Imaging System. Region of interest is indicated by blue boxes in the last sample on the right; broad bands reflect post-translational processing of glycoproteins. Data were normalized to the mean density of the grain-fed group defined as equal to one. Box-and-whiskers graphs (error bars indicate minimum and maximum values, boxes indicate quartiles, lines indicate medians, and + indicates mean) plot the relative abundance of each transporter for rats fed casein- versus grain-based chow. cl, cleaved; γENaC, epithelial Na^+ channel γ-subunit; fl, full length; mw, molecular mass (kilodaltons) markers; NCC, Na^+-Cl^- cotransporter; NCCp, phosphorylated form of Na^+-Cl^- cotransporter (phosphorylated at S71 and associated with more activity); NHE3, Na^+/H^+ exchanger isoform 3; NHE3p, phosphorylated form of Na^+/H^+ exchanger isoform 3 (NHE3pS552 associated with less activity); NKCC2, Na^+-K^+-$2Cl^-$ cotransporter isoform 2; NKCC2p, phosphorylated form of Na^+-K^+-$2Cl^-$ cotransporter isoform 2 (phosphorylated at Thr 96 and Thr 101 and associated with more activity). *P=0.01 by unpaired t test.

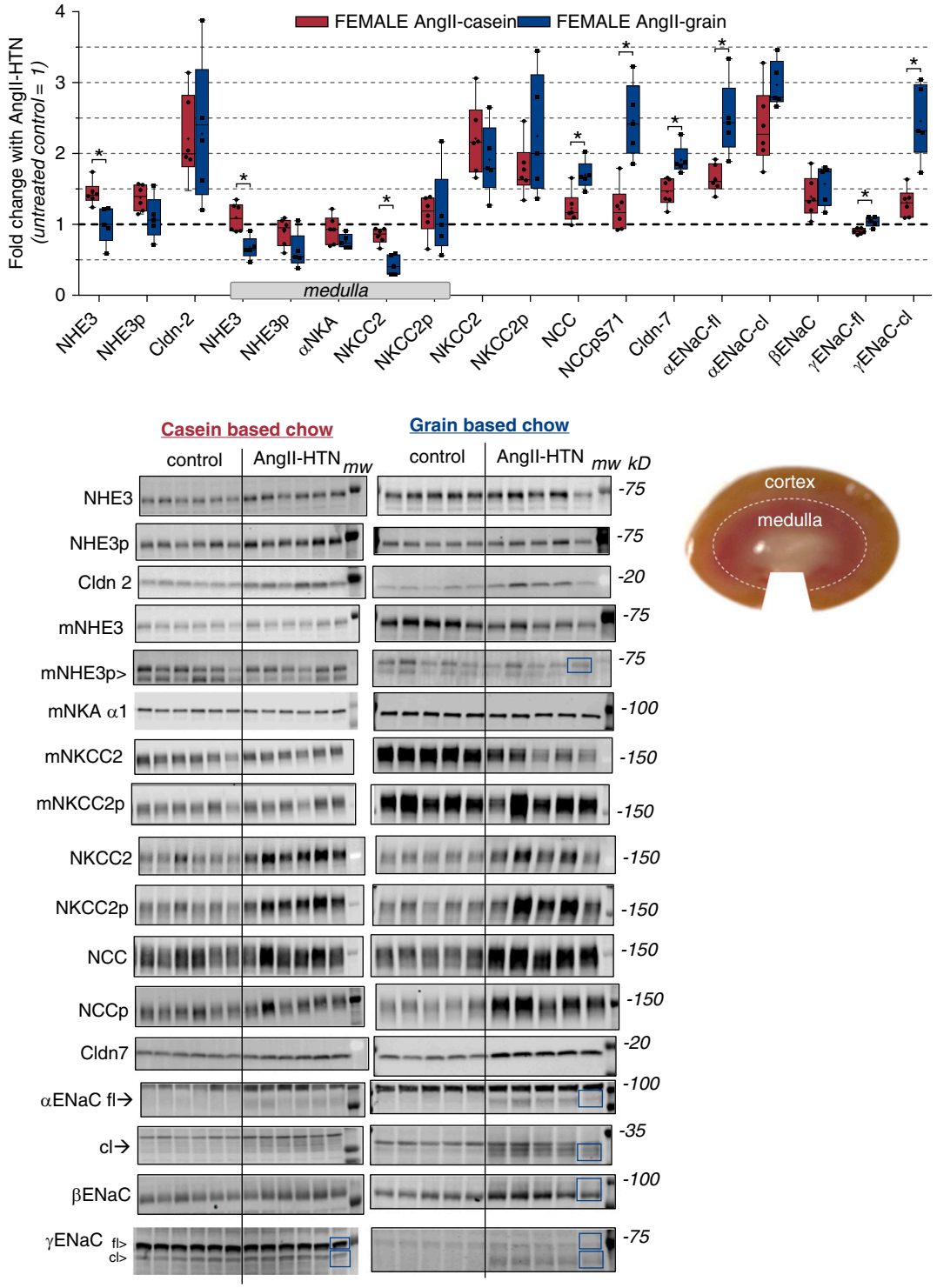

Figure 2. | **Angiotensin II (AngII) hypertension impact on sodium transporter profiles depends on whether female Sprague Dawley rats are fed casein-based chow versus grain-based chow.** Rats were infused with 400 ng/kg per minute AngII for 14 days (angiotensin II infusion model of hypertension [AngII-HTN]) or sham treated (control) while on casein- or grain-based diets (n=5–6 per group; all samples shown). Abundance of renal transporters was determined by semiquantitative immunoblot in homogenates prepared from renal cortex and medulla ("m" prefix); areas dissected are superimposed on the photo of the bisected female kidney. Samples from casein chow–fed rats and grain chow–fed rats were analyzed separately. As in Figure 1, both one and 1/2 amounts were assessed on the same immunoblot to verify linearity of the detection system; only one amount is shown. Data were collected and analyzed as detailed in Figure 1. Table 1 provides protein loading and protocols. AngII-treated samples were normalized to the control densities of each transporter within each diet defined as equal to one (bold dotted line) (8,10). The box-and-whiskers graphs, defined in Figure 1, plot the fold change in each transporter in AngII-infused rats fed casein- or grain-based chow. Apparent molecular masses (mw; in kilodaltons) are indicated on the right of blots. Cldn-2, claudin family member-2; Cldn-7, claudin family member-7; αNKA, α1-sodium pump catalytic subunit. *P=0.01 by unpaired t test with Benjamini, Krieger, and Yekutieli procedure for controlling false discovery rate using Graph Pad Prism.

Table 1. Antibody and immunoblot protocol details

Antibody Target	Approximately, kDa	Protein/Lane Cortex, μg	Protein/Lane Medulla, μg	Primary Antibody Supplier	Dilution	Reference
Claudin-2	23	15, 7.5	NA	ThermoFisher (#32–5600)	1:2000	12
Claudin-7	23	15, 7.5	NA	ThermoFisher (#34–9100)	1:1000	12
ENaC-α						
Full length >	100	80, 40	20, 10	Loffing (Zurich)	1:5000	13,14
Cleaved >	30					
ENaC-β	100	60, 30	20, 10	Loffing (Zurich)	1:15,000	13,14
ENaC-γ						
Full length >	80	60, 30	20, 10	Palmer (Cornell)	1:1000	13,14
Cleaved >	60					
NCC	150	60, 30	NA	McDonough	1:5000	7
NCCpS71	150	15, 7.5	NA	Loffing (Zurich)	1:5000	15
NHE3	83	15, 7.5	8, 4	McDonough	1:2000	16
NHE3pS552	83	5, 2.5	8, 4	Santa Cruz (sc-53962)	1:1000	17
NKCC2	160	15, 7.5	8, 4	DSHB (Iowa)	1:6000	18
NKCC2-pT96T101	160	15, 7.5	8, 4	Forbush (Yale)	1:2000	19
Na,K-ATPase–α1	100	1, 0.5	1, 0.5	Kashgarian (Yale)	1:200	20

ENaC-α, epithelial Na^+ channel α-subunit; ENaC-β, epithelial Na^+ channel β-subunit; ENaC-γ, epithelial Na^+ channel γ-subunit; NCC, Na^+-Cl^- cotransporter; NCCpS71, phosphorylated form of Na^+-Cl^- cotransporter (phosphorylated at S71); NHE3, Na^+/H^+ exchanger isoform 3; NHE3pS552, phosphorylated form of Na^+/H^+ exchanger isoform 3 (phosphorylated at S552); NKCC2, Na^+-K^+-$2Cl^-$ cotransporter isoform 2; NKCC2-pT96T101, phosphorylated form of Na^+-K^+-$2Cl^-$ cotransporter isoform 2 (phosphorylated at Thr 96 and Thr 101).

nephron of casein chow–fed rats at baseline (or lower fractional reabsorption in grain chow– versus casein chow–fed rats).

Equivalent efficacy of AngII infusion on series of rats fed the two different diets is supported by three measurements: (*1*) similar differences in systolic BP between control and AngII-infused groups (measured by tail cuff in millimeters of mercury): casein chow fed (110±3 [control] and 193±8 [AngII]) and grain chow fed (114±2 [control] and 191±1 [AngII]) (10); (*2*) similar 20-fold rise in aldosterone in both casein chow–fed rats (550±61–12,949±3143 pg/ml plasma) and as reported recently for grain chow–fed rats (10); and (*3*) pressure diuresis increased two-fold in casein chow–fed rats (not shown) and four-fold in grain chow–fed rats (10). Chow-dependent responses to AngII-HTN were evident all along the nephron and are summarized in Figure 2. Data represent fold changes in transporters' abundance with AngII-HTN groups normalized to abundance in their control sham-treated rats, defined as one (bold dotted line at 1.0 in Figure 2) 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 distal convoluted tubule NCC, phosphorylated form of NCC, and claudin-7, as well as cortical collecting duct ENaC-α and ENaC-γ. NHE3 and medullary thick ascending limb NKCC2 pool sizes, which contribute to pressure diuresis, were smaller in grain chow– versus casein chow–fed females. Abundance of claudin-2, cortical NKCC2, and ENaC-β 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 days of 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- versus 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 used "control" diets, even if they have similar percentages of electrolytes, protein, fat, and carbohydrate.

Disclosures

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Author Contributions

A.A. McDonough, D.L. Ralph, and L.C. Veiras conceptualized the study; A.A. McDonough, B.E. McFarlin, and D.L. Ralph were responsible for formal analysis; A.A. McDonough was responsible for funding acquisition; A.A. McDonough, B.E. McFarlin, D.L. Ralph, and L.C. Veiras were responsible for investigation; A.A. McDonough, D.L. Ralph, and L.C. Veiras were responsible for methodology; A.A. McDonough and D.L. Ralph were responsible for project administration; A.A. McDonough and D.L. Ralph were responsible for validation; B.E. McFarlin, D.L. Ralph, and L.C. Veiras were responsible for data curation; A.A. McDonough and D.L. Ralph provided supervision; D.L. Ralph was responsible for visualization; A.A. McDonough and L.C. Veiras wrote the original draft; and A.A. McDonough, B.E. McFarlin, D.L. Ralph, and L.C. Veiras reviewed and edited the manuscript.

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