Title page

The solute carrier SLC16A12 is critical for creatine and guanidinoacetate handling in the kidney

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ABSTRACT

A heterozygous mutation (c.643C.A; p.Q215X) in the creatine transporter *SLC16A12* was proposed to cause a syndrome with juvenile cataracts, microcornea and glucosuria in humans. To further explore the role of SLC16A12 in renal physiology and decipher the mechanism underlying the phenotype of humans with the SLC16A12 mutation, we studied *Slc16a12* knock-out (KO) rats. *Slc16a12* KO rats had lower plasma levels and increased absolute and fractional urinary excretion of creatine and its precursor guanidinoacetate (GAA). *Slc16a12* KO rats displayed lower plasma and urinary creatinine levels, but GFR was normal. The phenotype of heterozygous rats was indistinguishable from wild-type (WT) rats. Renal artery to vein (RAV) concentration differences in WT rats were negative for GAA and positive for creatinine. However, RAV differences for GAA were similar in *Slc16a12* KO rats, indicating incomplete compensation of urinary GAA losses by renal GAA synthesis. Together, our results reveal that *Slc16a12* in the basolateral membrane of the proximal tubule is critical for reabsorption of creatine and GAA. Our data suggest a dominant-negative mechanism underlying the phenotype of humans affected by the heterozygous *SLC16A12* mutation. Furthermore, in the absence of *Slc16a12*, urinary losses of GAA are not adequately compensated by increased tubular synthesis, likely caused by feedback inhibition of the rate limiting enzyme L-arginine:glycine amidinotransferase by creatine in proximal tubular cells.

Keywords: SLC16A12, GAA, creatine, GATM, GAMT

Number of words: 2800 words

NEW AND NOTEWORTHY

SLC16A12 is a recently identified creatine transporter of unknown physiological function. A heterozygous mutation in the human *SLC16A12* gene causes juvenile cataracts and reduced plasma guanidinoacetate (GAA) levels with an increased fractional urinary excretion of GAA. Our study with transgenic SLC16A12 deficient rats reveals that SLC16A12 is critical for tubular reabsorption creatine and GAA in the kidney. Our data furthermore indicate a dominant-negative mechanism underlying the phenotype of humans affected by the heterozygous *SLC16A12* mutation.

INTRODUCTION

Creatine and phosphocreatine provide a spatial and temporal energy buffering system that is essential for the maintenance of ATP supply in tissues with high energy demands. Due to the spontaneous and irreversible conversion of creatine to creatinine, there is a continued need for creatine replacement in the body (8, 12, 25). In humans, creatine is replaced by both dietary intake and biosynthesis. *De novo* creatine synthesis involves two enzymes, L-arginine:glycine amidinotransferase (GATM) and guanidinoacetate methyltransferase (GAMT). GATM catalyzes the transfer of a guanidine group from arginine to glycine to form ornithine and guanidinoacetate (GAA), and GAMT catalyzes the transfer of a methyl group from S-adenosylmethionine to GAA to form creatine and S-adenosylhomocysteine. Regulation of creatine biosynthesis occurs at the level of GATM, high creatine levels lower GATM enzyme activity (18). The bulk of GAA synthesis occurs in the proximal tubule of the kidney, but GATM activity is also found at other sites, including pancreas, brain, spleen and testis tubules (19, 23). GAA produced by the kidney (and other sites) is released into the circulation and methylated to creatine by the liver. Creatine enters cells devoid of creatine biosynthesis through the Na^+- and Cl^--dependent creatine transporter SLC6A8 (also known as CRT1). In humans, mutation in *SLC6A8* leads to an X-linked cellular creatine-deficiency syndrome with mental retardation, increased plasma and urinary creatine levels, and resistance to creatine supplementation(14, 21). Blood and urine levels of the creatine precursor GAA are normal in patients with *SLC6A8* mutations.

SLC16A12 (also known as MCT12) is a recently identified Na^+- and Cl^-- independent creatine transporter of the monocarboxylate transporter (*SLC16*) gene family. Whereas SLC6A8 also transports the creatine precursor GAA, we and others found that SLC16A12 is selective for creatine and does not accept GAA as a substrate (2, 6, 17). We previously reported a unique Swiss family with heterozygous nonsense mutation (c.643C>A; p.Q215X) in exon 6 of the *SLC16A12* gene (6). Affected patients exhibited juvenile cataracts,

91 microcornea and glucosuria. The latter, however, was assumed to be due to a digenic
92 syndrome in the index family with a concomitant heterozygous *SLC5A2* (also known as
93 *SGLT2*) missense mutation (c.265G>A; p.A89T) (6).
94 Creatine and GAA are eliminated by glomerular filtration and reclaimed by SLC6A8 residing
95 in the apical membrane of proximal tubular cells(2),(10). We previously demonstrated that
96 SLC16A12 localizes to the basolateral membrane in proximal tubular cells and speculated
97 that SLC16A12 mediates basolateral exit of creatine (5). The basolateral exit pathway of
98 GAA, which is both reclaimed and synthesized by proximal tubular cells, remains currently
99 unknown.
100 Previous i*n vitro* studies revealed retention of the mutant SLC16A12 protein in the
101 endoplasmatic reticulum while full-length SLC16A12 trafficked to the plasma membrane,
102 suggesting a dominant-negative mechanism in the lens due to protein misfolding (3).
103 Unexpectedly, however, while plasma and urine creatine concentrations were normal in
104 affected patients, they had a reduced plasma GAA concentration with an increased fractional
105 urinary excretion of GAA (6). Hence, the role of SLC16A12 in renal tubular function,
106 systemic creatine and GAA homeostasis and the mechanisms responsible for the renal
107 phenotype observed in affected members of the index family with the *SLC16A12* mutation
108 remain unknown. To address these questions, we studied creatine metabolism and renal
109 tubular function in transgenic *Slc16a12* rats.
110
111 **RESULTS**
112 **GAA, creatine and creatinine levels in blood and urine in *Slc16a12* KO rats.**
113 Transgenic *Slc16a12* rats were generated by transposon insertional mutagenesis. The mutation
114 carried by this strain consists of an insertion of a Sleeping Beauty transposable element gene
115 trap construct into the first intron of the *Slc16a12* gene (11). Heterozygotes rats (HET) were
116 crossed to obtain WT, HET and *Slc16a12* KO rats. Rats were born at expected mendelian

ratios. Complete or partial deletion of *Slc16a12* was confirmed by quantitative PCR in *Slc16a12* KO or HET rats, respectively (Fig.1A). At 9 weeks of age, S*lc16a12* KO rats displayed a small but significant reduction in body weight compared to WT littermates (Fig.1B), similar as observed previously in *Gatm* and *Gamt* KO mice(4, 24), but food and water intake as well as weights of liver, kidney and heart were similar between rats with different genotypes (Fig.1C-G).

In a first step, we subjected rats to metabolic cage studies. As depicted in Table 1, plasma and 24-hour urine analyses did not reveal differences between WT and *Slc16a12* KO rats.

We then quantified creatine, creatinine and GAA in plasma and 24-hour urines of WT, HET and *Slc16a12* KO rats (Fig.2). We observed significantly lower plasma levels of GAA, creatine and creatinine with an increased urinary excretion of GAA and creatine in *Slc16a12* KO compared to WT rats. HET rats did not display differences compared to WT rats.

To estimate the clearance of creatine and GAA by the kidney, we calculated fractional excretion rates of creatine and GAA. As shown in Fig.3 A, B, fractional excretion of both GAA and creatine were significantly higher in *Slc16a12* KO rats compared to WT or HET rats. Since renal handling of creatinine may directly be affected by SLC16A12 deficiency, we next measured GFR in WT and *Slc16a12* KO rats with the exogenous marker FITC-Sinistrin (9). As shown in Fig.3 C, D, plasma half-life of injected FITC-sinistrin and hence GFR were similar in WT and *Slc16a12* KO rats. Thus, SLC16A12 is critical for reabsorption of both creatine and its precursor GAA in the kidney.

Arteriovenous (RAV) differences for GAA, creatine and creatinine in *Slc16a12* KO rats.

To further dissect the role of the kidney in creatinine, creatine and GAA handling, we performed simultaneous renal artery and renal vein plasma sampling, determined GAA, creatine and creatinine concentrations and calculated renal arteriovenous (RAV) differences (Fig.4).

RAV differences were positive for creatinine, as expected for a solute secreted by the kidney. However, RAV differences for creatinine were similar between WT and *Slc16a12* KO rats, suggesting normal tubular secretion of creatinine upon loss of *Slc16a12*. RAV differences for creatine were not different between WT and *Slc16a12* KO rats, indicating that creatine is neither secreted nor produced in the kidney, regardless of the presence or absence of *Slc16a12*. In contrast, GAA RAV differences were negative in WT rats, supporting the notion that the kidney is an important site of GAA production. GAA RAV differences were similar between WT and *Slc16a12* KO rats. Hence, despite significantly reduced systemic GAA levels and ongoing urinary GAA losses, renal GAA output in *Slc16a12* KO rats is not increased as expected. In both WT and *Slc16a12* KO rats, the positive RAV difference for creatinine exceeded the negative RAV difference for GAA, revealing that not all creatine losses are replenished by renal synthesis. Extrarenal sites of production and/or dietary creatine sources likely account for the remainder.

Adaptions in kidney and liver upon Slc16a12 deletion

We next assessed the adaption in the kidney upon deletion of the *Slc16a12* gene. To this end, we quantified transcript expression of GATM, the rate limiting enzyme in creatine biosynthesis, the apical creatine and GAA uptake transporter SLC6A8 as well as of other transporter or enzymes implicated in creatine transport (15, 16)(13). As shown in Figure 5, mRNA expression of all the transcripts studied was unchanged in HET and *Slc16a12* KO rats compared to WT rats. Quantification of GATM protein expression by immunoblotting of kidney lysates, however, revealed significantly reduced GATM expression in kidneys of *Slc16a12* KO rats compared to WT or HET rats (Fig. 5B). The specificity of GATM antibody was confirmed using blocking peptide and similar finding were observed with two different antibodies (supporting data: 10.6084/m9.figshare.13376963). In the liver, GATM, GAMT and SLC6A8 transcript expression in HET and *Slc16a12* KO rats were not altered compared to

WT rats (Fig. S1 (https://figshare.com/s/6006f084b60069f527db). As in the kidney, we observed absence of SLC16A12 transcript in the livers of *Slc16a12* KO rats, and intermediary SLC16A12 transcript levels in HET rats (Fig. S1 (https://figshare.com/s/6006f084b60069f527db)).

DISCUSSION

SLC16A12 is a facilitative transporter, selective for creatine, expressed at the basolateral membrane of proximal tubular cells (1, 6, 17). Our studies with *Slc16a12* -deficient male rats reveal that SLC16A12 is critical for reclamation of filtered creatine and its precursor GAA in the proximal tubule. Fractional excretion rates for creatine and GAA are increased by ~7 fold in the absence of SLC16A12. *Slc16a12* KO rats did not exhibit other signs of tubular dysfunction, indicating that loss of SLC16A12 does not cause generalized proximal tubular dysfunction (Fanconi syndrome). Importantly, both HET and *Slc16a12* KO rats did not display increased urinary glucose excretion, as reported in the Swiss index family with the heterozygous c.643C.A; p.Q215X *SLC16A12* mutation, supporting our previous conclusion that the glucosuria in the index family was due to a simultaneous *SLC5A2* mutation (6). However, we cannot completely rule out at the moment a direct impact of the mutant p.Q215X SLC16A12 protein on glucose reabsorption in the proximal tubule. Additional studies with transgenic animals carrying a corresponding heterozygous c.643C.A; p.Q215X *SLC16A12* mutation are needed to definitively rule out this possibility. Another limitation of our work is the exclusive use of male rats. The intent of our study was to investigate the functional role of SLC16A12 in the kidney and not sex-specific differences in the metabolism of creatine or GAA, which should be addressed in future studies.

Reduced renal GAA reabsorption in *Slc16a12* KO rats cannot simply be explained by reduced SLC16A12-mediated GAA transport. We previously demonstrated that creatine transport by SLC16A12 is not inhibited by GAA even at high concentrations, suggesting that GAA is not a

substrate for SLC16A12 (6). This finding was recently independently confirmed (17). In the latter study, the authors also elegantly demonstrated that SLC16A12 mediates pH- and ion-independent, facilitative diffusion of creatine in mammalian cells, the direction of transport (influx or efflux) only depending on the concentration gradient across the plasma membrane. The proximal tubule reclaims filtered GAA and creatine by Na^+- and Cl^--dependent creatine transporter SLC6A8 (Fig. 6). In contrast to SLC16A12, however, SLC6A8 mediates transport of GAA. SLC6A8 mediated uptake of GAA and creatine from the glomerular filtrate is expected to result in a rise of the intracellular GAA and creatine concentration in the proximal tubular cell, which drives basolateral efflux of creatine through SLC16A12 and GAA through a hitherto unknown transport mechanism. In the absence of SLC16A12, the intracellular concentrations of creatine will rise. A high intracellular creatine concentration is expected to have two major consequences: inhibition of SLC6A8 transport activity (and thus reduced creatine and GAA uptake) and downregulation of GATM enzyme activity and thus reduced GAA biosynthesis (Fig. 6) (7, 12). Both GAA and creatine supplementation induce a downregulation of GATM activity. While the effect of GAA is believed to be indirect due to the conversion of GAA to creatine in the liver, creatine directly downregulates GATM expression, and probably also inhibits enzyme activity (7, 22). In the setting of significantly (~70 %) reduced circulating GAA and creatine levels, as observed in *Slc16a12* KO rats, increased GATM expression is expected. However, our data demonstrate that GATM protein levels in kidneys of *Slc16a12* KO rats were reduced, clearly representing an inadequate response to severe systemic creatine deficiency. These results align with our above stated hypothesis of creatine accumulation and secondary downregulation of GATM expression in proximal tubular cells due to SLC16A12 deficiency. Interestingly, GATM downregulation seems to occur at a posttranslational level since GATM transcript expression was unaltered in *Slc16a12* KO rats. Absolute 24 h urinary GAA excretion rates are increased ~2x in *Slc16a12* KO rats compared to WT rats, indicating that GAA synthesis is increased at extrarenal sites.

Similarly, 24 h urinary creatine excretion is increased ~3x in *Slc16a12* KO rats compared to WT rats, indicating that creatine synthesis is increased, as expected. Nevertheless, circulating creatine levels remain low in *Slc16a12* KO rats. Likely, both reduced creatine reclamation by the kidney, and inadequate GAA supply for creatine biosynthesis due to inhibition of GATM (as seen in chronic renal failure (20)) contribute to the incomplete compensation in *Slc16a12* KO rats. Creatine is converted to creatinine non-enzymatically at a constant rate and then excreted in the urine. Hence, the observed reductions of circulating and urinary creatinine in *Slc16a12* KO rats likely reflect a decrease in total body creatine content. However, given the reduced body weight found in *Slc16a12* KO rats, we cannot exclude the possibility that muscle mass (an important and large creatine storage site) is also reduced in *Slc16a12* KO rats, thereby contributing to attenuated creatinine production.

HET rats had a ~50 % reduction of renal SLC16A12 expression and exhibited a phenotype that was indistinguishable from WT rats. This finding contrasts to humans affected by the heterozygous (c.643C.A; p.Q215X) *SLC16A12* mutation which displayed reduced plasma GAA levels and an increased fractional excretion of GAA (6). Together, these results suggest a dominant-negative effect rather than haploinsufficiency of the mutant human SLC16A12 protein as the underlying mechanism of the phenotype observed in humans.

In summary, our results reveal that SLC16A12 is essential for the maintenance of physiological levels of creatine, the creatine precursor GAA and the creatine degradation product creatinine in blood and urine.

METHODS

Rats

All animal experiments were approved by the local Veterinary authorities of the Canton Bern, Switzerland (approval # BE 59/16) and conducted in agreement with the Swiss Animal Welfare Law. The mutant rat strain F344-Slc16a12$^{Tn(sb\text{-}T2/Bart3)2.298Mcwi}$ (strain #438) was obtained from the Rat Resource and Research Center (RRRC) at the University of Missouri, Columbia, MO (11). This Sleeping Beauty mutant rat strain was derived by crossing F344-Tg(T2/Bart3)2Ceb and F344-Tg(PGK2-SB11)Ceb. The mutation carried by this strain consists of an insertion of a Sleeping Beauty transposable element gene trap construct into the 1st intron of the *slc16a12* gene. All rats used in this study were in the F344/NHsd background and WT and KO rats were always bred from heterozygous matings. Rats were housed in a temperature- and humidity-controlled room with an automatic 12 hours light/dark cycle and had free access to food (standard diet # 2223, Provimi Kliba AG, containing 4.3mg of creatine per kg) and water. For urine collections, rats were housed in individual metabolic cages (Techniplast, Italy). Prior to collections, rats were allowed 2 days of adaptation. Twenty-four-hour urine samples were collected under mineral oil to avoid evaporation. Rats were sacrificed at 9 weeks. Genotyping was done by PCR using genomic DNA isolated from rat phalanges with a set of three primers; RRRC 438 A (5'-TTA TTC TGC TCA AGT ATT CCT GTC G-3'), RRRC 438 B (5'-CTG TTG ACA TTG AGT CAT AGG AGG T-3') located in the *slc16a12* gene on chromosome 1 and rat transposon A (5'-CCT AAC TGA CTT GCC AAA AC-3') located on the inserted transposon.

Real time PCR

Total RNA was extracted from kidney or liver using Trizol extraction method. cDNA was synthesized from 500 ng total tissue RNA by reverse transcriptase (PrimeScript RT Master

Mix; Takara) with oligo dT primers. Real-time PCR was performed with the primers and probes from Roche described in Suppl. Table S1 on a 7500 Fast Real-Time PCR System. Ct values for duplicate technical replicates were averaged, and the amount of mRNA relative to β-actin was calculated using the ΔCt method.

Blood and urine analyses

Blood and urinary electrolytes, glucose, uric acid and urea were determined at the core laboratory of the Bern University Hospital, Bern, Switzerland. All blood parameters were measured in plasma except osmolality that measured in serum and glucose in total blood. Analysis of guanidinoacetate and creatine in rat plasma and urine was performed using liquid chromatography-mass spectrometry (LC-MS/MS; Thermo UHPLC Ultimate 3000 XRS mounted with a Supelco Discovery HS F5 HPLC column and coupled to AB SCIEX 5500 TripleQuad) by the Division of Clinical Chemistry and Biochemistry of the University Children's Hospital Zurich, Zurich, Switzerland, as described (6). In short, one volume of the internal standards (guanidinoacetate-d2 and creatine-d3) in TCA (0.6 M) was added to one volume of plasma or urine sample diluted by a factor of 20 or 100, respectively. After short vortexing, the samples were placed on ice for 20 min and centrifuged at 15'000 g at 4 °C for 10 min. The supernatant was pipetted into an HPLC-vial and ten µL were injected into the LC-MS system. The analytes were detected by MRM in positive ionization mode using seven-point calibration curves for quantification. The fractional excretion of guanidinoacetate and creatine were calculated using the following formulas: $FEGAA = 100 * ((GAA_{urine} * Creatinine_{plasma})/(GAA_{plasma}/Creatinine_{urine}))$, $FECreatine = 100 * ((Creatine_{urine} * Creatinine_{plasma})/(Creatine_{plasma}/Creatinine_{urine}))$

Measurement of GFR in conscious mice

GFR was measured noninvasively by recording the decrease in transcutaneous fluorescence of FITC-sinistrin over time using a small fluorometer attached to the rat, as described previously(9). FITC-sinistrin (5 mg/kg) was injected intravenously via the tail. The fluorometer was programmed to make a transcutaneous measurement every 5 s, and up to 2 h of measurements were stored on the device. Each rat was measured twice.

Western Blot

Kidney tissue was homogenized with ceramic beads (Lysing Matrix D, MPBio) at 4 °C in RIPA buffer (Sigma, R0278) containing protease inhibitors (Roche, Mannheim, Germany). Homogenates were clarified by centrifugation at 30,000g for 10 minutes and subsequently used for protein quantification according to the kit instruction (BCA, Pierce, 23225), SDS–polyacrylamide gel electrophoresis and immunoblotting.

Antibodies were obtained from the following sources: rabbit polyclonal anti-GATM (NBP1-89211) (1:2000), GATM recombinant protein antigen (NBP1-89211PEP), rabbit polyclonal anti-GATM (MBS8245218), (1:2000) mouse monoclonal b-Actin (sc-69879) (1:1000), HRP-conjugated goat anti-rabbit IgG (1:10000) (sc-2004) and HRP-conjugated goat anti-mouse IgG (1:10000) (sc-516102). To quantify the expression of each of the proteins under study densitometry determination of the visualized bands was performed using the Image Lab software (Bio-Rad).

Statistical analysis

For comparisons between groups the unpaired Student's t test (two groups) or 1-way ANOVA with Tukey post-hoc test (multiple groups) was used. Data were analyzed using GraphPad Prism 8.2 (GraphPad Software, San Diego, CA, USA). All statistical tests were two-sided and $p < 0.05$ was considered statistically significant. Unless stated otherwise, data are shown as

means and error bars indicate SEM. The data supporting the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.c.5233718.

DISCLOSURES

The authors report no conflict of interest. DGF has served as a consultant for Otsuka Pharmaceuticals (no relation to submitted work), and received research funding from Novartis, Abbvie and Otsuka Pharmaceuticals (no relation to submitted work).

Author contributions

Sophia N. Verouti: substantial contributions to acquisition of data, analysis and interpretation of data and manuscript writing

Delphine Lambert: substantial contributions to acquisition of data.

Déborah Mathis: substantial contributions to acquisition of data.

Ganesh Pathare: substantial contributions to acquisition of data.

Geneviève Escher: substantial contributions to acquisition of data and interpretation of data

Bruno Vogt: substantial contributions to interpretation of data and manuscript writing

and Daniel G. Fuster: substantial contributions to conception and design, interpretation of data and manuscript writing

FIGURE LEGENDS

Figure 1 (A) SLC16A12 transcript expression in kidneys of male rats normalized to actin, (B) Body weight of male rats at 9 weeks, (C) food intake, (D) water intake, (E) liver weight, (F) kidney weight and (G) heart weight (n=8 per genotype). Data shown are means±SEM. Data were analyzed using one-way ANOVA and compared with Tukey post-hoc test. ***p<0.001, ****p<0.0001.

Figure 2 Plasma levels of (A) GAA, (B) creatine and (C) creatinine in male rats (n=5-6 per genotype). Twenty-four hour urinary levels of (D) GAA, (E) creatine and (F) creatinine excretion in male rats (n=5-6 per genotype). Data shown are means±SEM. Data were analyzed using one-way ANOVA and compared with Tukey post-hoc test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 3 (A) Fractional excretion of GAA and (B) creatine in male rats (n=5-6 per genotype), (C) half-life ($t^{1/2}$) of i.v. injected FITC-Sinistrin and (D) FITC-Sinistrin-based estimation of GFR in male rats (n=9-10 per genotype). Data shown are means±SEM. Data were analyzed using one-way ANOVA and compared with Tukey post-hoc test. ***p<0.001, ****p<0.0001.

Figure 4 Renal artery and vein plasma levels of (A) GAA, (B) creatine and (C) creatinine in male rats fed a standard chow (n=3-6 rats per genotype), (D) Renal artery-vein (RAV) concentration differences of creatinine, creatine and GAA in rats fed a standard chow. Data shown are means±SEM. Data were analyzed using one-way ANOVA with Tukey post-hoc test. *p<0.05, **p<0.01, ****p<0.0001 comparisons made between rats with different genotype; #p<0.05, ##p<0.01 comparisons made between artery and vein.

Figure 5 Transcript expression (A) and protein levels (B) of GATM, and transcript expression of (C) SLC6A8, (D) Sgk-1, (E) mTOR, (F) SLC6A6 and (G) SLC16A13 in kidney of WT, HET, KO male rats normalized to actin under standard diet (n=8 per genotype). Data shown are means±SEM. Data were analyzed using one-way ANOVA and compared with Tukey post-hoc test.

Figure 6 Models of proximal tubular handling of creatine and GAA in (A) the presence or (B) absence of SLC16A12.

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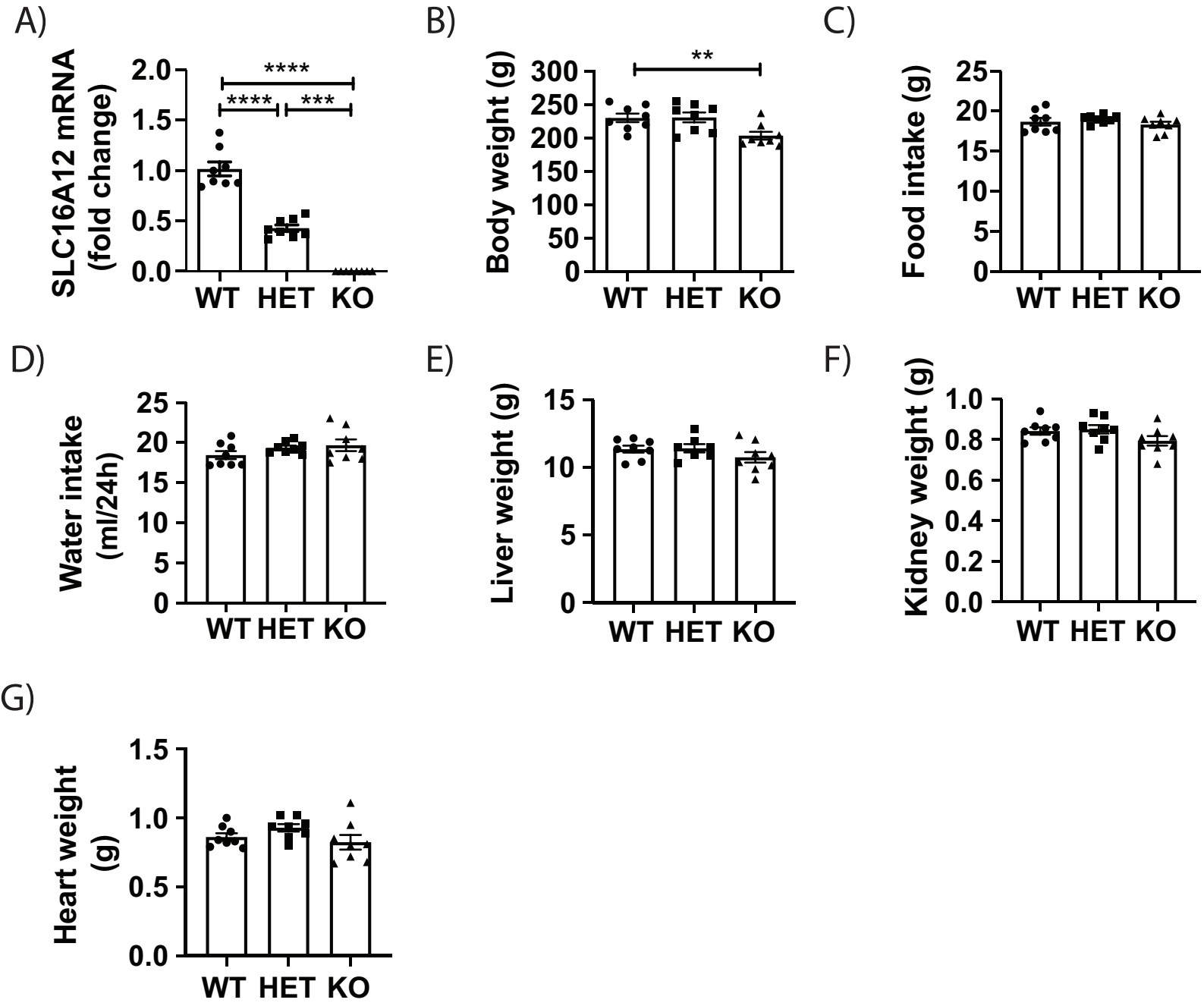

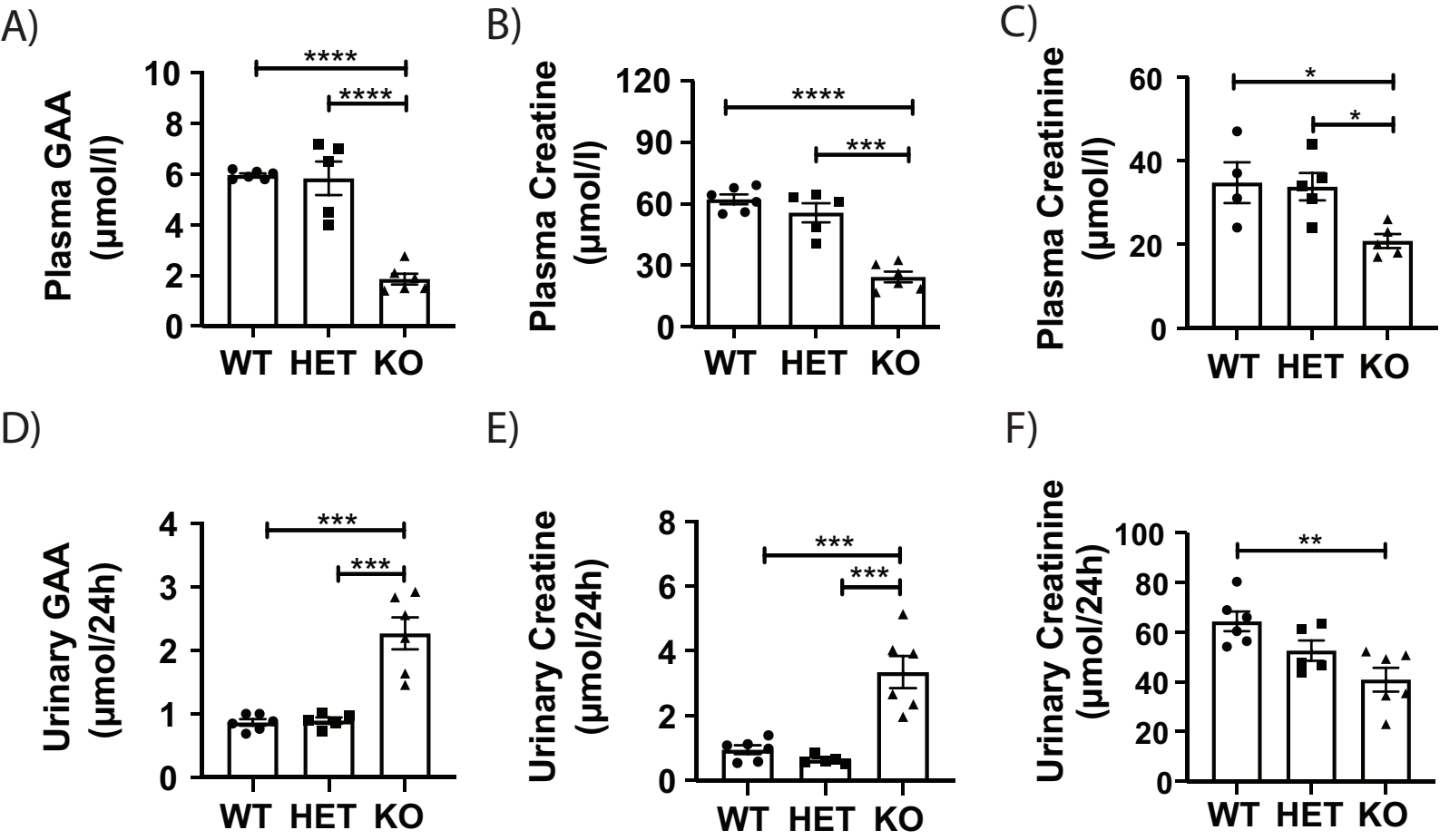

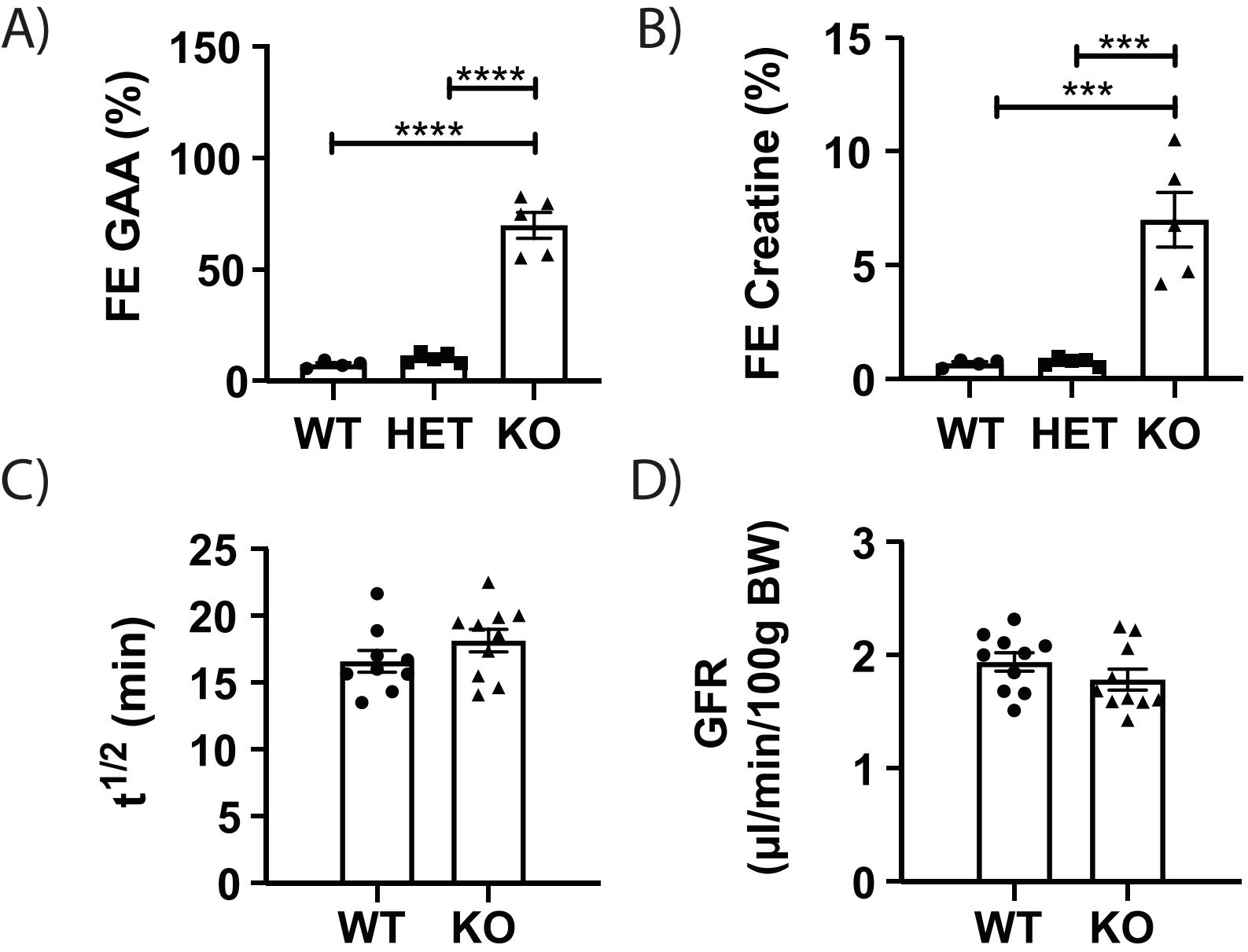

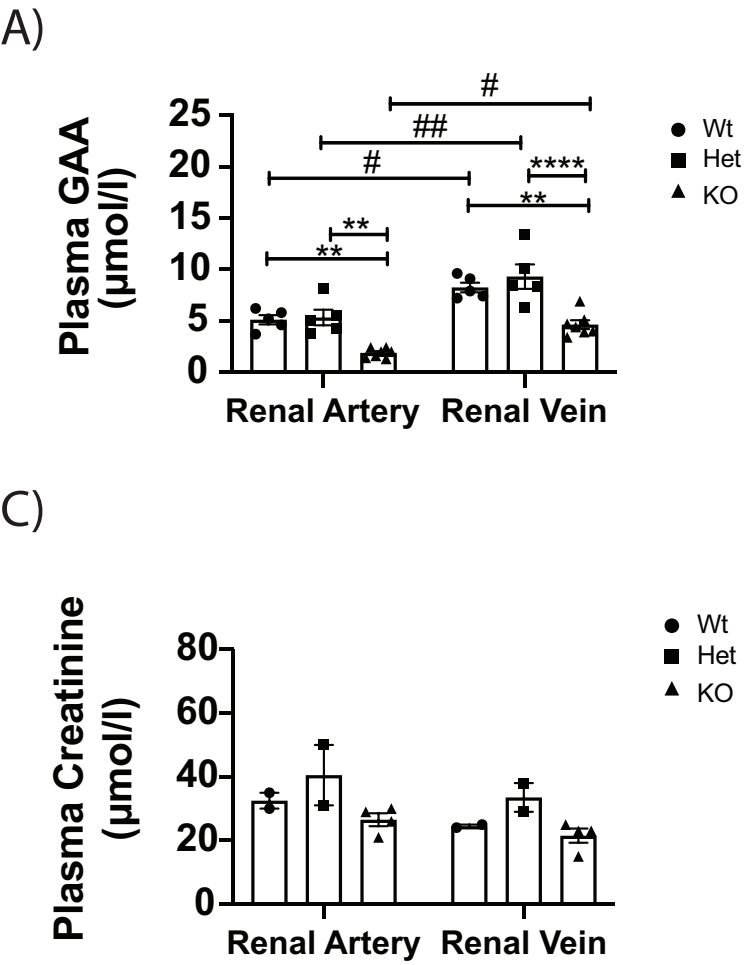

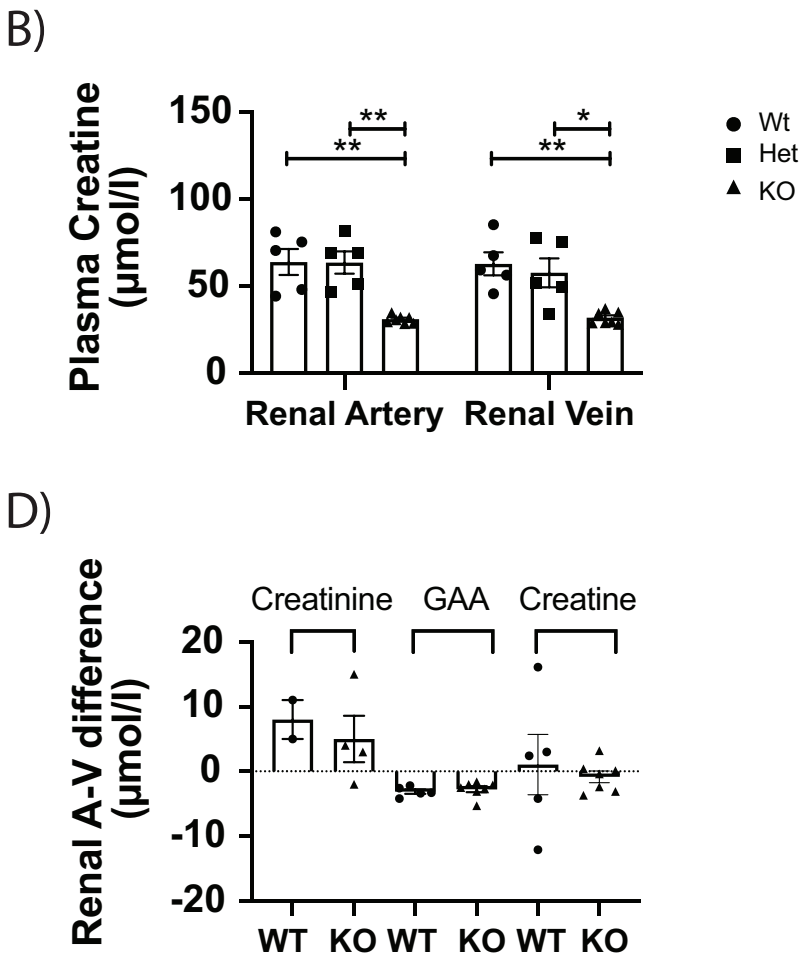

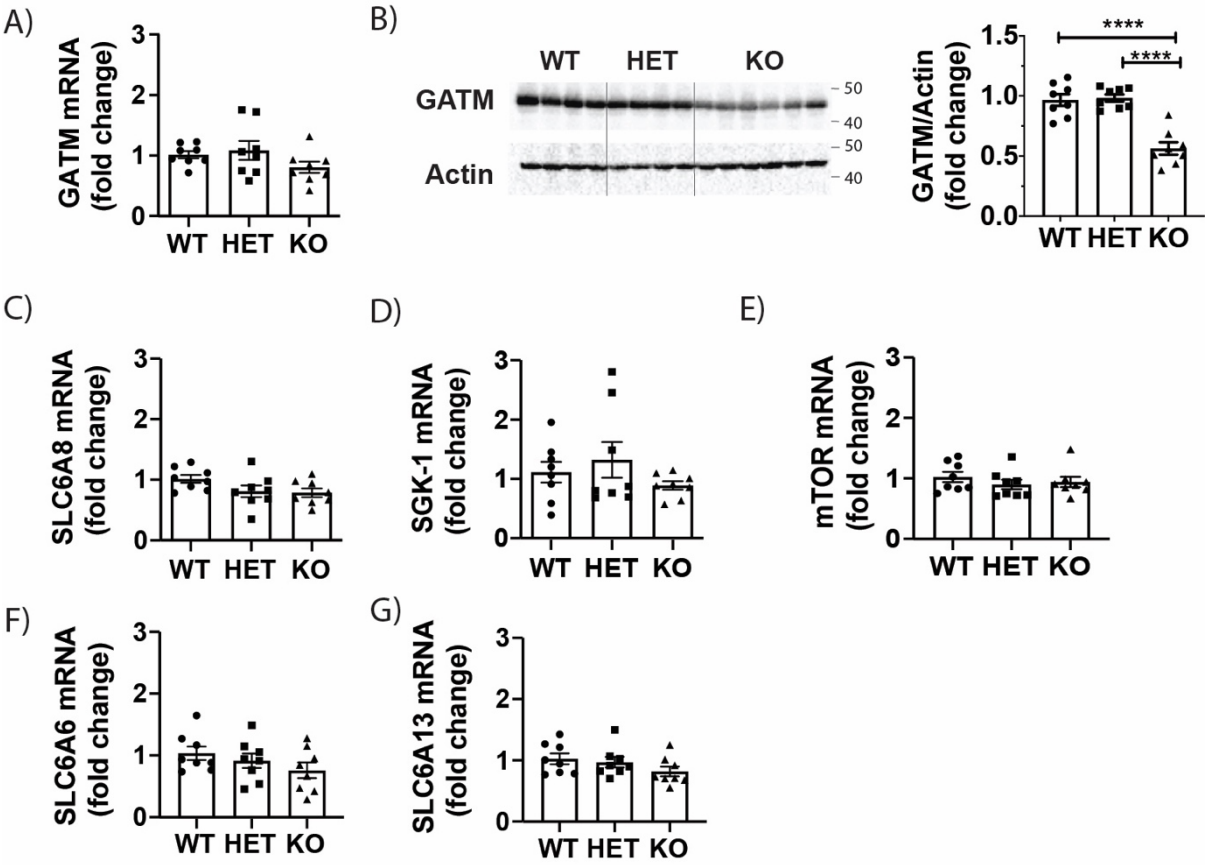

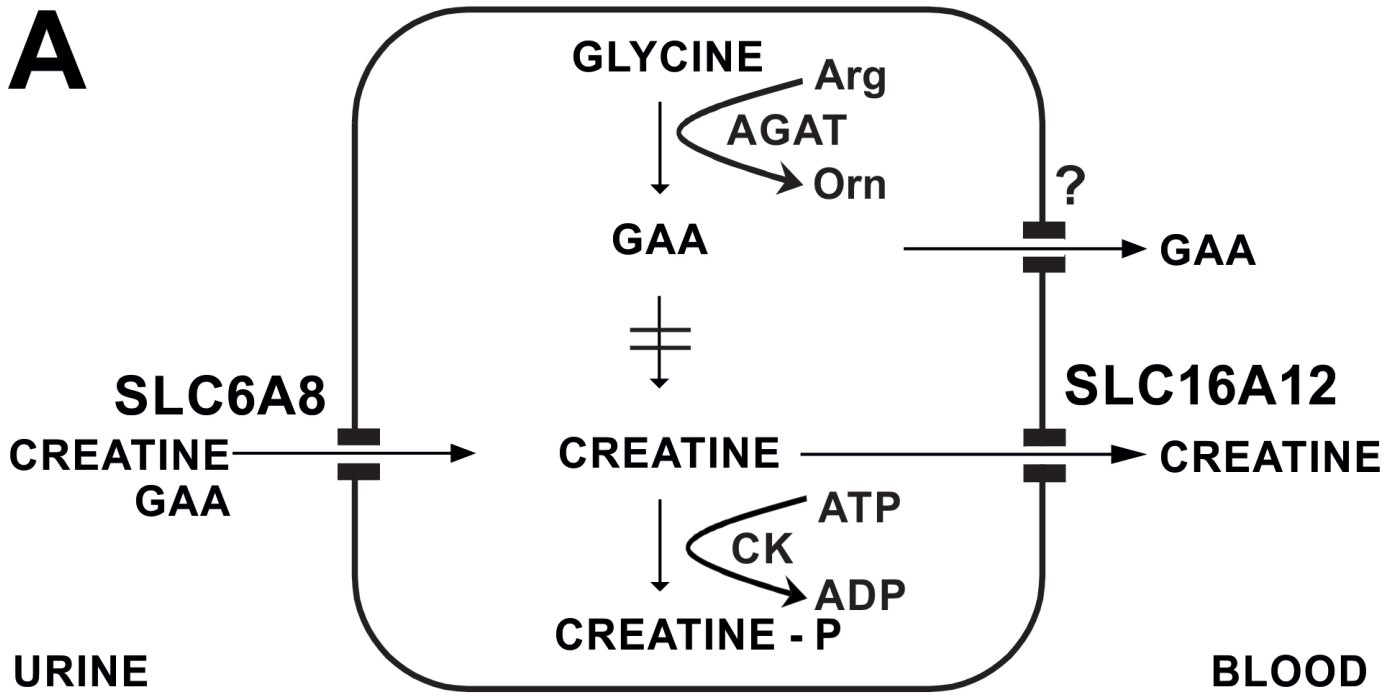

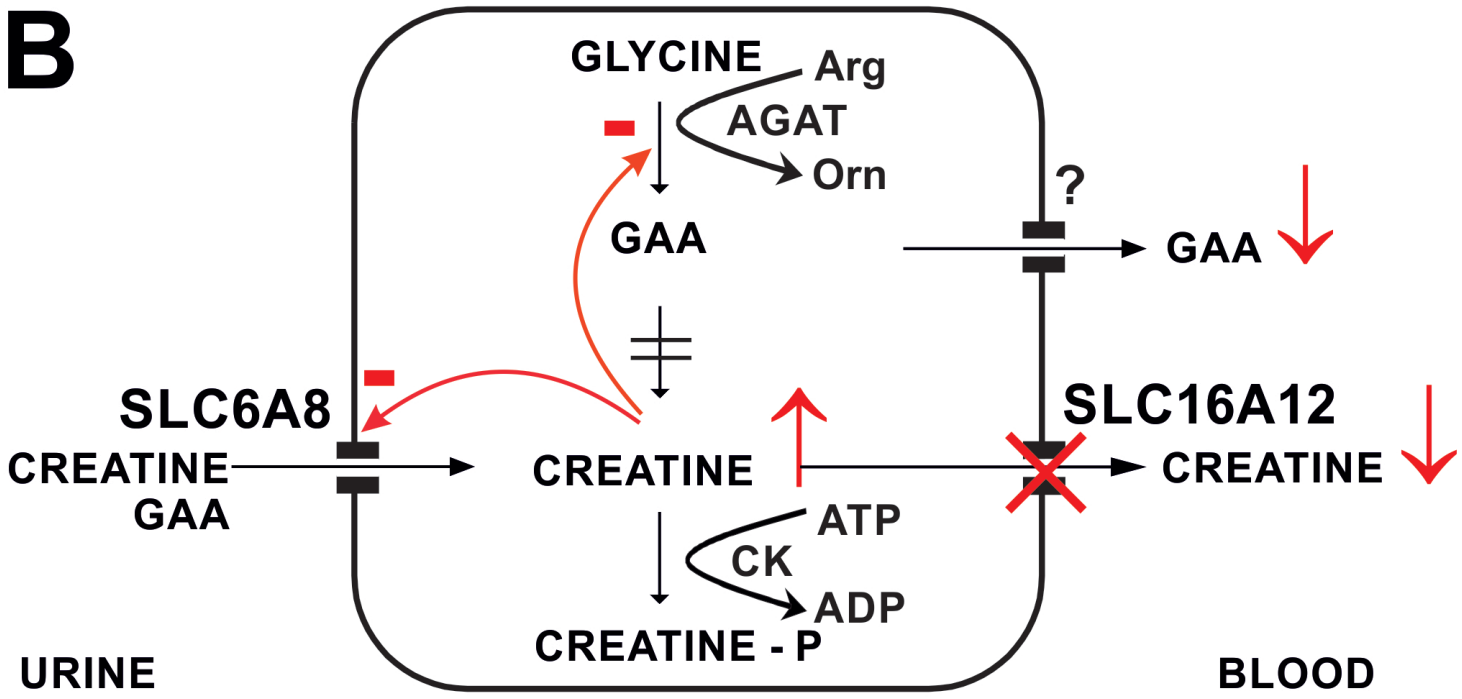

Table 1 Blood and urine parameters of male WT and *slc16a12* KO rats fed a standard chow (n=5-10 per genotype). Data shown are means±SEM. Data were analyzed by unpaired t-test.

	Blood		
	WT	**KO**	**P value**
Na (mmol/L)	153.8±11.4	148.8±3.5	0.25
K (mmol/L)	3.8±0.3	3.7±0.2	0.38
Cl (mmol/L)	100±8.5	96.8±3.5	0.28
Ca (mmol/L)	3.1±0.2	2.9±0.1	0.06
P (mmol/L)	2.5±0.5	2.5±0.2	0.96
Mg (mmol/L)	0.9±0.1	0.8±0.1	0.24
Glucose (mmol/L)	7.2±0.5	7.0±0.4	0.72
Osmolality (mOsmo/kg)	303.9±1.3	303.1±1.1	0.67
Urea (mmol/L)	4.8±0.24	5.1±0.2	0.35
Uric acid	32.4±10.53	21.4±0.8	0.40
	Urine		
	WT	**KO**	**P value**
Na (mmol/24 h)	0.8±0.1	0.8±0.2	0.77
K (mmol/24 h)	1.1±0.2	1.1±0.3	0.99
Cl (mmol/24 h)	1.0±0.1	0.9±0.2	0.59
Ca (mmol/24 h)	0.01±0.003	0.01±0.003	0.74
P (mmol/24 h)	0.4±0.06	0.4±0.05	0.64
Mg (mmol/24 h)	0.09±0.02	0.1±0.03	0.70
Glucose (mmol/24 h)	0.07±0.009	0.04±0.006	0.06
Osmolality (mmol/24 h)	1760±104.4	1426±220.9	0.15
pH	6.2±0.02	6.2±0.06	0.58
Urea (mmol/24 h)	6.2±0.4	5.7±0.7	0.55
Uric acid (mmol/24 h)	4.9±0.6	6.2±1.3	0.40

The solute carrier SLC16A12 is critical for creatine and guanidinoacetate handling in the kidney

Model

SLC16A12 KO rats

RESULTS

↓ plasma GAA, creatine
↑ absolute and fractional urinary excretion of GAA and creatine

↓ plasma and urinary excretion of creatinine but normal GFR

Similar renal artery-vein GAA differences in WT and Slc16a12 KO rats

CONCLUSIONS

Our results reveal that Slc16a12 in the basolateral membrane of the proximal tubule is critical for reabsorption of creatine and guanidinoacetate (GAA). Furthermore, in the absence of Slc16a12, urinary losses of GAA are not adequately compensated by increased tubular GAA synthesis due to downregulation of the rate limiting enzyme L-arginine:glycine amidinotransferase in the kidney.

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