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Accumulation of natriuretic peptides is associated with protein energy wasting and activation of browning in white adipose tissue in chronic kidney disease.

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Running title: Browning in chronic kidney disease

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Abstract (204 words)

Protein energy wasting is a common feature of patients with chronic kidney disease (CKD) and is associated with poor outcomes. Protein energy wasting and cachexia, a severe form of protein energy wasting, are characterized by increased resting energy expenditure but the underlying mechanisms are unclear. Browning corresponds to the activation of inducible brown adipocytes in white adipose tissue and occurs in states of cachexia associated with hypermetabolic disease such as cancer. Here we tested the hypothesis that CKD-associated protein energy wasting could result from browning activation as a direct effect of the uremic environment on adipocytes. In a murine model of CKD (5/6 nephrectomy), there was increased resting energy expenditure, expression of uncoupling protein 1 (a thermogenic protein uncoupling oxidative phosphorylation in mitochondria) and citrate synthase activity (a proxy of mitochondrial density in white adipose tissue). Mice with CKD also exhibited increased levels of atrial natriuretic peptide, a well known activator of browning. The incubation of primary adipose cells with plasma from patients receiving dialysis treatment and having signs of protein energy wasting led to an increased synthesis of uncoupling protein 1. Similarly, primary adipose cells exposed to atrial natriuretic peptide at concentrations relevant of CKD led to a significant increase of uncoupling protein 1 content. Thus, accumulation of cardiac natriuretic peptides during CKD could contribute to the browning of white adipose tissue and protein energy wasting.

Key words: Browning, protein energy wasting, chronic kidney disease, cardiac natriuretic peptides

Translational Statement

This paper describes the discovery, using both cellular and CKD mice models, of the role of the natriuretic peptides (NPs) to promote thermogenesis by activation of inducible brown adipocytes in white adipose tissue. With a deeper understanding of how NPs control energy balance in CKD, it may provide important clues to the underling new strategies to manage the PEW. In the next step, we will test if some specific inhibitors of browning process can improve PEW in CKD patients.

Introduction

Protein energy wasting (PEW) and cachexia that is the severe form of PEW are associated with poor survival in chronic kidney disease (CKD) and may occur in 20 to 80% of hemodialysis (HD) patients.[1,2] However, the mechanisms involved in uremic PEW are complex and remain poorly understood. PEW is different from malnutrition as it cannot be overcome by nutritional supplementation.[3] To date, there are few, if not any effective therapies to improve PEW associated with CKD.[4]

The increase of resting energy expenditure (REE) and its role in PEW during CKD remain discussed. If in CKD mice, an increase of REE has already been described[5,6,7], observations in CKD patients are more contrasted. Some studies reported a reduction of REE in non-dialysis CKD patients[8] based on indirect methods, but there is a strong evidence for increased REE in HD and peritoneal dialysis patients.[9,10] Many factors may be responsible for PEW such as systemic inflammation, acidosis, imbalance in appetite hormones (such as leptin) and insulin resistance.[11] Although significant progresses have been made towards understanding PEW, its determinants and cellular mechanisms are still to be discovered.

There are two types of adipose tissues (AT): brown (BAT) and white (WAT) adipose tissue that possess opposite physiological functions and different anatomical dispositions. WAT is the main site for energy storage in the form of triglycerides (stored as a lipid droplet into adipose cells); in contrast, BAT contributes to REE and thermogenesis.[12] The functional characteristics of BAT are driven by numerous mitochondria to enable β-oxidation of fatty acids and the production of heat by uncoupling of the electron transport chain by the uncoupling protein 1 (UCP1). BAT was first discovered in small adult hibernating mammals and human newborns as a non-shivering thermogenic system. Persistence of BAT in human adults was confirmed by Nedergaard et al. who highlighted symmetrical hypermetabolic areas during cold exposure, with positron emission tomography scan corresponding to BAT.[13] The persistence of BAT in adult humans is nowadays well established and extensively documented.[14]

In addition to classical brown adipocytes, inducible brown adipocytes were identified. They are located in dedicated interscapular deposits and around large vessels in WAT, this phenomenon being referred to as «browning» or beige fat. In response to various stimuli (e.g. thyroid hormones[15]; cardiac natriuretic peptides (NPs)[16,17], Fibroblast Growth Factor 21 (FGF21)[18]; adipokines[19],…) white adipocytes are able to overexpress thermogenic proteins

among which UCP1. The thermogenic activity of BAT and WAT browning significantly contributes to REE in rodents and humans.[20]

Several studies highlighted the potential adverse role of BAT in the development of PEW and cachexia in the context of hypermetabolic diseases such as cancers[5] and a similar phenomenon could also be present in CKD. Indeed, Zhao et al. reported in uninephrectomized rats morphological changes of intra-abdominal WAT with polygonal multilocular cells.[21] Histological analysis showed an increased abundance of mitochondria in WAT of CKD rats.[22] These morphological changes could correspond to the transformation of WAT into beige adipocytes as confirmed recently by Kir et al.[5] However, the mechanisms contributing to the activation of BAT and its role in PEW associated with CKD remain poorly described.

The uremic syndrome is characterized by the progressive retention of compounds known as uremic toxins (UTs) that are normally cleared by the kidneys.[23] The link between the accumulation of UTs, browning and PEW has never been studied, except for parathyroid hormone (PTH).[5] We previously observed in mice that a major uremic toxin p-cresyl sulfate (PCS), was a mediator of lipoatrophy suggesting its putative role in the activation of brown/beige fat.[24] Atrial natriuretic peptide (ANP) as well as its sequence homologue, brain natriuretic peptide (BNP) and the N-terminal pro-B-type natriuretic peptide (NT-proBNP), are retained in CKD and are associated with unfavorable outcomes[25,26]. BMI is inversely associated with these cardiac peptides in healthy adults as well as in the CKD population [27,28] suggesting that NPs could also play an important role in energy balance by browning activation as observed in experimental models.[16,17]

In the present study, we tested the hypothesis that CKD was associated with browning activation and PEW due to the direct action of UTs. Specifically, we answered the following questions: 1) is browning induced in a CKD mouse model, and in white adipocytes treated with uremic serum and 2) what are the roles of UTs (NPs and protein-bound UTs) in the induction of adipose tissue browning during CKD.

RESULTS

CKD mice exhibit PEW and browning

The uremic status of 5/6 nephrectomized mice was evidenced by blood urea nitrogen (**Figure 1a**). Mice biometric data and organ weights are presented in **Figure 1b-f**. CKD mice

exhibited a significant difference in total body weight (**Figure 1b**) and decreased epididymal WAT (a intraabdominal fat depot), inguinal WAT (a subcutaneous fat depot) as well as interscapular brown fat (iBAT) (**Figure 1c-f**). Mice weight loss was associated with an increase of energy expenditure in uremic animals as shown in **Figure 2**. Respiratory measurements indicated elevated O_2 consumption (VO_2) and decreased respiratory quotient (RQ) (i.e. the ratio between oxygen consumption and carbon dioxide production) in uremic mice during dark phases and during the last 24-hr period indicated that lipids were the preferential source of energy substrates (**Figure 2a-b and 2e-f)**. Heat production was increased in CKD mice (**Figure 2 c-d**) both during acting and resting phases. The weight loss was not due to increased physical activity, which was decreased in CKD mice (**Figure 2g**). Food diet and intakes were not different between the 2 groups (**Figure 2 h**).

We analyzed morphological, molecular, and functional evidence of browning of WAT in CKD mice. Brown or beige adipocytes can be distinguished from white adipocytes by their smaller size, brown-selective genes like UCP1, and numerous mitochondria. Compared to sham, eWAT (**Figure 3a),** we observed a decrease in cell size and an increase in UCP1 immunostaining. UCP1 mRNA expression was higher in WAT and iBAT from CKD mice (**Figure 3b-d**). In addition to UCP1, other markers of brown adipocytes were determined in WAT and iBAT samples. The expression of peroxisome proliferator activator receptor gamma coactivator-1alpha (PGC1α), Cell death-inducing DFFA-like effector A (CIDEA), PR domain containing 16 (PRPMD16) and peroxisome proliferator activated receptor-gamma (PPARγ), were significantly higher in WAT and iBAT (**Figure 3b-d**). Triglycerides content in iBAT from CKD mice was reduced suggesting an activation of lipolysis in BAT that could contribute to the increased energy expenditure (**Figure 3e**). Protein expression of UCP1, PGC1α and PPARγ were significantly higher in CKD mice in eWAT. (**Figure 3f-i**). We reported a negative correlation between body weight and Ucp1 expression levels (r_s=-0.63, $P<0.05$) (**Figure 3j**). To determine the functional significance of the alterations in eWAT morphology and UCP1 expression, we measured mitochondrial citrate synthase activity. We observed a very significant increase in citrate synthase (CS) activity (a proxy of mitochondrial density) in CKD mice. (**Figure 3k**) Since mitochondrial activity is rather low in eWAT of sham mice, an increase in mitochondrial activity is a mandatory component of browning of WAT. Collectively, these data provide strong evidence that eWAT gene expression is altered in uremia, toward a more thermogenic genotype.

Cold temperature challenge is well recognized to activate brown fat mechanism of thermogenesis. We investigated if browning activation still play a role in thermogenis

regulation in uremic context. CKD had no effect on body temperature of mice without cold stress. Unlike sham mice, cold exposure at 6°C for 7 hr resulted in a significant reduction of rectal temperature in CKD mice (**Figure 3l**). The biometry and renal parameters were presented in Supplementary **Table S1**. As shown in **Figure 3m-o**, sham mice at 6°C exhibited significantly increased levels of brown adipocyte markers in eWAT, but the induction of thermogenic genes was blunted in CKD mice. Taken together, these findings suggesting that uremia leads to cold intolerance, likely due to a defect in activation of the browning process in eWAT under cold conditions.

Uremic serum stimulates expression of UCP1 in primary adipocytes

Plasma serum from healthy volunteers (HV) and HD patients with PEW criteria[2] were collected. Clinical and biological characteristics of the subjects are summarized in **Supplementary Table S2**. There was no significant difference between the two groups, except for age and as expected parameters related to renal function (i.e. creatinine, urea, estimated glomerular filtration rate). Of note, no significant difference of PTH and CRP was noticed between the two groups but we observed an increase of BNP concentration in HD group. Uremic plasma induced an increase of UCP1 and PGC1α protein content in primary adipocytes (**Figure 4a-b**) compared to HV plasma. These results suggest that some compounds found in uremic plasma could induce an increased expression of UCP1 and PGC1α in primary adipocytes and therefore an activation of thermogenic functions through browning.

Given that we previously demonstrated that protein-bound UTs such as PCS induced an adipose tissue dysfunction[24], we incubated preadipocytes 3T3-L1 cells with indoxyl sulfate (IS) and PCS at the concentrations found in CKD patients. We failed to observe any increase of UCP1 abundance (**data not shown).** The effect of PCS and IS on 3T3-L1 cells differentiation was investigated by incubating the cells for 8 days with UTs after induction of adipocyte differentiation. Lipid accumulation estimated by red oil staining and triglycerides content in 3T3-L1 cells were similar after exposure to PCS and IS or control condition (**data not shown**). These data suggest that these two UTs did not trigger the browning phenomenon as was observed with uremic plasma. Cardiac NPs are good candidates, since they are hallmarks of both clinical[29] and experimental[16] heart-associated cachexia. In order to define their potential role in the pathogenesis of the WAT browning phenotype, primary adipocytes were incubated during 24 hr with ANP at concentrations found in CKD patients (280pM).

ANP exposure triggered a huge increase of UCP1 and PGC1α protein in primary adipocytes (**Figure 4c-d**).

ANP increase brown adipocyte characteristics in vivo

In CKD mice, plasma levels of ANP were significantly higher compare as sham mice (**Figure 5a**). In order to validate the specific role of ANP to promote browning in CKD mice model, mice were given a neprilysin inhibitor (sacubitril, in drinking water) for 2 weeks. Sacubitril inhibits the breakdown of NPs such as ANP and contribute to increase plasma levels of ANP. The mean sacubitril intake was 65.3 mg/day/kg body weight. As shown in **Figure 5b**, plasma levels of ANP were significantly higher in the mice receiving sacubitril, and were in the same range as those observed in CKD mice. Mice treated with sacubitril exhibited a lower fat accretion (**Supplementary Table S3**). Sacubitril treatment had a significant effect on the expression of brown adipocyte markers in adipose tissue like UCP1 and PGC1α (**Figure 5c-e**). Together, these results suggest that ANP could play an important role in cachexia in CKD through the activation of the browning of WAT.

DISCUSSION

This study aimed to determine whether browning could contribute to PEW associated with CKD and whether UTs accumulation could be the underlying cause of increased REE in CKD. We confirmed that the increase in REE in 5/6 nephrectomized mice is associated with activation of brown adipose tissue thermogenesis. We demonstrated for the first time that the uremic milieu can promote browning since HD plasma induced UCP1 and PGC1α expression in cultured primary murine adipocytes. In addition, we found that NPs are involved in browning during CKD.

Cachexia and PEW result from complex pathways. A better understanding of the underlying mechanisms is therefore a major issue to reduce morbidity and mortality associated with CKD. The central role of browning in PEW and cachexia has recently emerged particularly in cancer.[30] Several experimental studies suggested that a similar phenomenon could be observed in CKD. Upregulated UCP1 expression in iBAT was previously observed in 5/6 nephrectomized mice associated with an increased REE.[7,31] The development of “beige” adipocytes was suggested in different CKD mice model[21,22]. Here,

we confirmed that browning occurs in intra-abdominal WAT and is associated with PEW in CKD.

CKD is associated with numerous metabolic perturbations and accumulation of UTs. Some of these toxins could be involved in browning activation. CKD is characterized by a lack of renal clearance of leptin and treatment of CKD mice with a leptin receptor antagonist prevents PEW by decreasing UCP1 expression in iBAT.[7] Activation of BAT could be concomitant with the browning and therefore results from similar molecular determinants. Recently, Kir et al has suggested that parathyroid hormone (PTH) is involved in stimulating a thermogenic program in 5/6 nephrectomized mice. Fat-specific knockout of parathyroid hormone-receptor blocked adipose tissue browning and PEW in CKD mice.[5] PTH(1-34) administration triggered a significant increase of UPC1 and PGC1α expression in iWAT and iBAT suggesting that browning occurs.[5] It should however be observed that the concentration used (a single subcutaneous injection of 1mg/kg) was supraphysiologic namely much higher than the concentration commonly encountered during CKD. However, in clinical study, HD patients with hyperparathyroidism exhibited an increased REE that is reduced after parathyroidectomy.[32] Increase in inflammatory cytokines could also stimulate WAT browning[30] but anti-cytokine therapies proved to be ineffective in CKD.[33] In our study, uremic serum from HD patients was able to activate browning. Of note in our cohort of HD patients, PTH or CRP were not increased in malnourished patients. These data suggest that other mediators accumulated in CKD, in addition to PTH and cytokines could stimulate WAT browning.

Protein-bound toxins were first investigated because of their huge accumulation and deleterious impact in CKD. We previously showed that PCS administered to mice with normal kidney function for four weeks induced lipoatrophy-like features associated with CKD.[24] IS is a potent agonist of the nuclear receptor Aryl hydrocarbon Receptor (AhR) and is well known to interfere with adipocyte differentiation.[34] Recent data suggested that tryptophan, the precursor of IS, is involved in thermogenesis.[35] In our study, we failed to see any effect of IS and PCS on browning. Indeed, IS and PCS at levels found in CKD did not impair adipocyte differentiation in contrast to a previous report.[36] It should however be emphasized that in this study, adipocyte cells were incubated with the non-conjugated form of PCS (i.e. p-cresol) without any supplementation of bovine serum albumin (BSA) in the culture medium. Given that PCS and IS are tightly bound to albumin, and that only the unbound toxin fraction is biological active, the doses used were not representative of CKD conditions.

NPs are a group of peptide hormones mainly secreted by the heart, signaling via cGMP coupled receptors which are known for their natriuretic ability to lower blood pressure by stimulating renal sodium and water excretion. The physiological actions of ANP and BNP on target cells and organs are similar and commonly mediated via natriuretic peptide receptor-A (NPR-A).[37] ProBNP is cleaved to BNP and NT-proBNP, by the proteolytic enzyme furin, and the two are secreted from the heart in equimolar amount. Even if the impact of kidney function on NT-proBNP was much more pronounced than that on BNP, some data clearly demonstrated that NT-proBNP levels are significantly correlated with BNP levels at all stages of kidney dysfunction including HD patients.[38] In the literature, BNP and NT-pro-BNP were associated with CV diseases and mortality[25,26] but until now, they were rather considered as biomarkers of unfavorable outcomes, than as a cause.[39]

Novel physiological functions of NPs were recently discovered. Sarzani et al demonstrated that some NPs receptor were expressed in adipose tissue of rats and human.[40] In vitro, ANP and BNP can stimulate lipolysis of human adipose cells.[41] Bordicchia et al demonstrated a potent thermogenic effect of NPs with induction of brown-like adipocytes after treatment with beta-adrenergic receptor agonists and also an increase in UCP1 after treatment of HMADs cells (human multipotent adipose-derived stem) with ANP. Infusion of BNP in mice resulted in an increased REE and overexpression of Ucp1 in WAT mediated through p38 MAPK pathway.[16,17] However, the concentrations of ANP used in these in vitro studies were 70-fold higher than the concentrations observed in end-stage CKD patients. In general population, increased NPs are associated with reduction in WAT deposition.[42,43] One study reported a negative correlation between BMI and NT-proBNP levels in non-dialysis CKD patients[28] and other that NT-proBNP were significantly higher when the PEW component number was higher in HD patients[44]. It was also found that NT-proBNP is negatively correlated to albumin concentration in HD patients.[45] We found that ANP increases UCP1 synthesis by primary adipocyte at concentrations relevant for CKD and that NPs could therefore contribute to browning and PEW in CKD. Supporting this view, Zhao et al. showed that lisinopril treatment, an angiotensin-converting enzyme inhibitor (ACE) protected from lipid transformation from unilocular of multilocular cells suggesting a decrease of browning.[22] ACE is also a major cardio-protective treatment and could inhibit browning phenomenon by preventing cardiac remodeling and the increase of NPs. Using sacubitril treatment (i.e. a potent neprilysin inhibitor that increased the circulating concentration of ANP) in mice, we are able to activate browning in adipose tissue in the same rang that observed in CKD mice. The impact of sacubitril in human on adipose tissue remains

unclear. In a post-hoc analysis of the PARADIGM-HF trial that included 3778 patients with known diabetes without renal disease, BMI increased over the course of follow-up in patients randomly assigned to sacubitril/valsartan, compared with those receiving enalapril [46]. Also, in a small cohort (n=39) sacubitril/valsartan did not alter energy expenditure compare to amlodipine[47]. This suggests that activation of browning in this population does not appear to be clinically relevant, but we unfortunately no data are available in CKD patients.

The present study has however several limitations. First, *in vitro* the impact of only three uremic toxins (ANP, PCS and IS) was tested on UCP1 expression and we cannot exclude that other aspects of uremic environment could have an impact on energy balance and browning activation. Secondly, this hypothesis was not evaluated in a cohort of CKD patients. Further studies with a large and well phenotyped cohort with adipose tissue biopsies and measures of REE will be necessary to tease out these potential associations.

In conclusion, our study provides novel insights on the mechanisms of PEW in CKD using two independent models with consistent functional defects. We demonstrated that uremic environment induced browning activation *in vivo* and *in vitro* and we identified NPs as one of UTs involved in browning in CKD condition. With a deeper understanding of how NPs control energy balance in CKD, we may develop new strategies to manage PEW.

Materials and methods

Chemicals and reagents

Unless mentioned all chemicals were from Sigma Aldrich (Saint Quentin Fallavier, France) and all solvents were from Carlo Erba (Peypin, France). Primary antibodies were listed in **Supplemental Table S4.** Secondary goat anti rabbit IgG were from BioRad (Marne-la-Coquette, France).

Plasma collection and constitution of plasma pools

This research was approved by the local institutional review board (L16-196). All subjects involved in the research signed written informed consent forms prior to enrolment. Patients included were more than 18 years old, male or female, and HD patients were following HD sessions for at least 3 months and presents PEW criteria according to Fouque et al.[2] Patients with CKD were considered having PEW if they presented at least 3 of the following 4 criteria: BMI < 23 kg/m^2, albumin level < 38 g/L, prealbumin level < 300 mg/L, and normalized protein catabolic rate < 0.8 g/kg/d. Exclusion criteria were diabetes, systemic

inflammatory diseases, autoimmune diseases, cancer, oral antidiabetic treatments or insulin. Plasma were collected from HV, and HD patients and pooled in equal amounts. Plasma pools were sterile filtered (cut-off 0.22 µm), aliquoted, and stored at -80°C until use.

Culture of 3T3L1 adipose cells

Mouse 3T3-L1 fibroblasts were obtained from the American Type Culture Collection (CL-173; ATCC, LGC Standard SARL, Molsheim, France) and was differenced as previously reported. Exposure of UTs and adipose cells differentiation are detailed in **Supplementary Material.**

Animals models

All experimental procedures were approved by the local ethic committee (S**upplementary material).** Male C57Bl6 mice were purchased from Charles River laboratory or Janvier SA (Le Genest- Saint-Isle, France) and housed in an air-conditioned room with a controlled environment of 21 ± 0.5°C and 60-70 % humidity, under a 12h light/dark cycle (light on from 7 am to 7 pm) with free access to food and water.

Isolation, culture and differentiation of adipose stem cells

Mouse primary adipocytes were obtained from 10-12 weeks-old male C57BL/6J mice from subcutaneous inguinal adipose tissues. The isolation and amplification of adipose stem cells (ASC) have already been described.[48] Beiging induction and adipocytes treatment are described **in Supplementary material**. At the end of the incubation period, the culture mediums were removed and stored at -80°C until analysis

Uremic mice model.

Moderate kidney failure was induce mice by 5/6 nephrectomy as previously described[24] and mice were used after 3 weeks of uremia. Metabolic studies, euthanasia and necropsy are detailed in **Supplementary material**.

Cold challenge experiment

Sham or uremic mice were housed individually (with a free access to food or drinking water) in an environmental cabinet at a temperature of 6°C for 7 hours. Colonic temperature was measured hourly at a depth of 1 cm.

Sacubutril treatment

Two groups of 5 male C57BL/6J mice were used. Sacubitril (Santa Cruz) was dissolved in drinking water containing 0.5% (v/v) ethanol to a final concentration of 0.4g/L. Mice were given sacubitril in drinking water for 2 weeks. Control mice were given the vehicle of sacubitril namely water containing 0.5% (v/v) ethanol for 2 weeks. Water intake was monitored three times a week to estimate the daily intake of sacubitril. Note that no difference in water intake was observed between control or sacubitril mice.

Citrate synthetase activity:

Citrate synthetase activity have been measured in frozen eWAT tissues (approximately 40 mg) and procedure has been detailed in **Supplementary Materials.**

Protein extraction, Western Blotting and RT-PCR

Primary adipose cells or adipose tissues were lysed using lysis buffer. Protein concentrations were determined by Bradford assay (Bio-Rad, Marne la Coquette, France). mRNA levels of key genes of browning were measured by RT-PCR. The sequences information's of the mouse primer sets are summarized in **Supplementary Table S5**. Western Blot and RT-PCR procedures are detailed in **Supplementary Materials.**

Histology

eWAT samples were dehydrated, embedded in paraffin, and cut into 5-µm sections. Sections were stained with hematoxylin and eosin for histology. Immunohistochemistry was performed according to a standard protocol (**Supplementary Material**).

Statistical analysis

Data were analyzed using GraphPad Prism 6.0 (GraphPad softwares, La Jolla, USA) software The data are expressed as mean ± standard error of the mean (SEM, animal study), or as median (interquartile range – IQR) when variables were not normally distributed. Statistical analysis are detailed in **Supplementary Material.** A $P<0.05$ was considered as statistically significant in all analysis.

Supplementary Material

Supplementary information is available on Kidney International's website.

DISCLOSURE:

The authors have no conflict of interest to disclose in this work.

AUTHOR CONTRIBUTIONS

ML performed the study, researched data, analyzed the results, and wrote the manuscript. EN, AB, BB, EB, DR, AM, YD, MP, CB, SG, JCS, ML, WA, DF researched data and analyzed the results. CS and LK conceived the study, analyzed the results, and wrote the manuscript. LK is the guarantor of this work and, as such, take full responsibility for it.

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FIGURE LEGENDS

Figure 1: CKD mice present a protein energy wasting (PEW) phenotype.

a-Urea levels in CKD and sham mice after 3 weeks of uremia; **b**-Evolution of body weight; **c**-Representative macroscopic pictures of sham and CKD mice at necropsy. The arrowheads point epididymal white adipose tissue (eWAT) that is almost absent in CKD mice; **d**-Representative macroscopic pictures of interscapular brown adipose tissue (iBAT); **e-**Representative macroscopic pictures of eWAT; **f**-The weight of fat pads (eWAT: epididymal; iWAT: inguinal; and iBAT). n=7-12. The data are expressed as mean ± SEM. Statistical analysis were conducted using Student t-test. *$p < 0.05$, ***$p < 0.001$ compared with the sham group.

Figure 2: CKD mice exhibited an increased energy expenditure.

Sham and CKD mice were housed individually in metabolic cages for 3 days. **a**-Oxygen consumption (VO_2) at each time point and **b**-Average of VO_2 over the last 24 hours; **c**-Heat production at each time and **d**-average of heat production over the last 24 hours; **e**-Respiratory quotient (RQ) at each time point and **f**-average RQ over the last 24 hours; **g**-Locomotor activity; **h**-Food intake. n=6 per group. Data are presented as mean ± SEM. Statistical analysis was conducted using Student t-test. *$p < 0.05$; **$p < 0.01$.

Figure 3: CKD is associated with an activation of BAT and browning of WAT.

a- H&E staining and immunohistochemical of UCP1 in eWAT sections from sham and CKD mice. Gene expression in eWAT (**b**), iWAT (**c**) and iBAT (**d**) were determined by quantitative reverse transcription PCR (qRT–PCR), n=4-6; **e**-Triglyceride (TG) content in iBAT, n=4; Quantifications of (**f**) UCP1, (**g**) PGC-1α, and (**h**) PPARγ by western blotting, normalized to tubulin and expressed in arbitrary units (AU),and **i**- representative blot of UCP1, PGC1α and PPARγ in eWAT of sham and Nx5/6 mice. n=4-5; **j**-Relation between final body weight (BW) and Ucp1 expression in eWAT. N=4-5. **k-**Citrate synthase activity in eWAT. n=7-9. **l**- Effect of uremia on colonic temperature following acute cold exposure at 6°C for 7h (n = 5). Quantifications of (**m**) UCP1 and (**n**) PGC1α by western blotting, normalized to tubulin and expressed in arbitrary units (AU) and **o**- representative blot of UCP1 and PGC1α in eWAT of sham and Nx5/6 mice after acute cold exposure. n=4. Data are expressed as mean ± SEM. Statistical analysis was conducted using Student t-test or

Mann & Whitney U tests. *p < 0.05; **p < 0.01, *** p <0.001, compared with the sham group, and $ p<0.05 compared to sham control exposed à 6°C.

Abbreviations: eWAT, epididymal WAT, iWAT, inguinal WAT, iBAT, interscapular BAT, TG, Trigycerides, BW, body weight, CIDEA, cell death-inducing DFFA-like effector A, PGC1α, peroxisome proliferator activator receptor gamma coactivator-1alpha, PPARγ, peroxisome proliferator activated receptor-gamma, PRMD16, PR domain containing 16, UCP1, uncoupling protein 1.

Figure 4: Uremic plasma and ANP increases UCP1 protein content in primary cultures of inguinal adipocytes

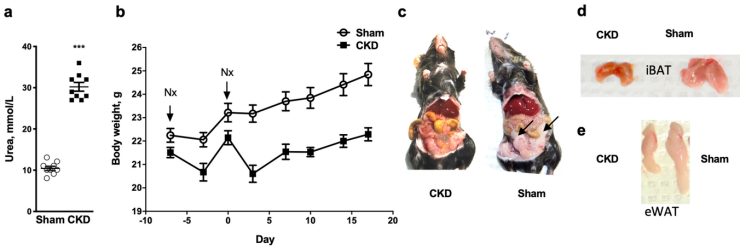
a-UCP1 and **b**-PGC1α protein content in primary adipocytes incubated for 24h with either, 17% (v/v) Healthy Volunteers (HV) plasma pool, or 17% (v/v) hemodialysis (HD) plasma pool (n=5-6) **c**-UCP1 and **d**-PGC1α protein content in primary adipocytes incubated for 24h with either 10% (v/v) FCS (Control) or ANP (280 pM), n=3. Quantifications of UCP1 and PGC1α were performed by western blotting, normalized to tubulin and expressed in arbitrary units (AU). Data are expressed as mean ± SEM. Statistical analysis was conducted using Student t-test. *p < 0.05; ***p < 0.001, compared with the HV or FCS condition.

Abbreviations: FCS, Fetal calf serum, HV, Healthy volunteers, HD, Hemodialysis.

Figure 5: Mice treated with Sacubitril exhibit increased ANP concentration and increased expression of brown adipocyte markers in WAT.

a-ANP levels in CKD and sham mice at 3 weeks (n=10). **b-** ANP levels in mice treated with vehicle or sacubitril at 2 weeks (n=5). Note that the concentration of ANP observed under chronic administration of sacubitril match the concentrations observed in CKD mice Quantifications of (**c**) UCP1 and (**d**) PGC1α, and **e**- representative blot of UCP1 and PGC1α in mice treated with vehicle or sacubitril. Quantifications of UCP1 and PGC1α were performed by western blotting, normalized to tubulin and expressed in arbitrary units (AU), n=4-5. The data are expressed as mean ± SEM. Statistical analysis was conducted using Student t-test. **p < 0.01, ***p<0.001, compared with sham or vehicle mice.

Figure 1


a
Urea, mmol/L
***
Sham CKD
b
Body weight, g
Sham
CKD
Nx
Nx
Day
c
CKD
Sham
d
CKD
Sham
iBAT
e
CKD
Sham
eWAT

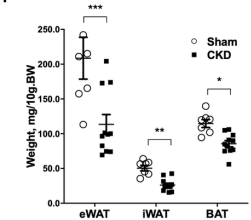

f
Weight, mg/10g.BW
***
**
*
Sham
CKD
eWAT
iWAT
BAT

Figure 2

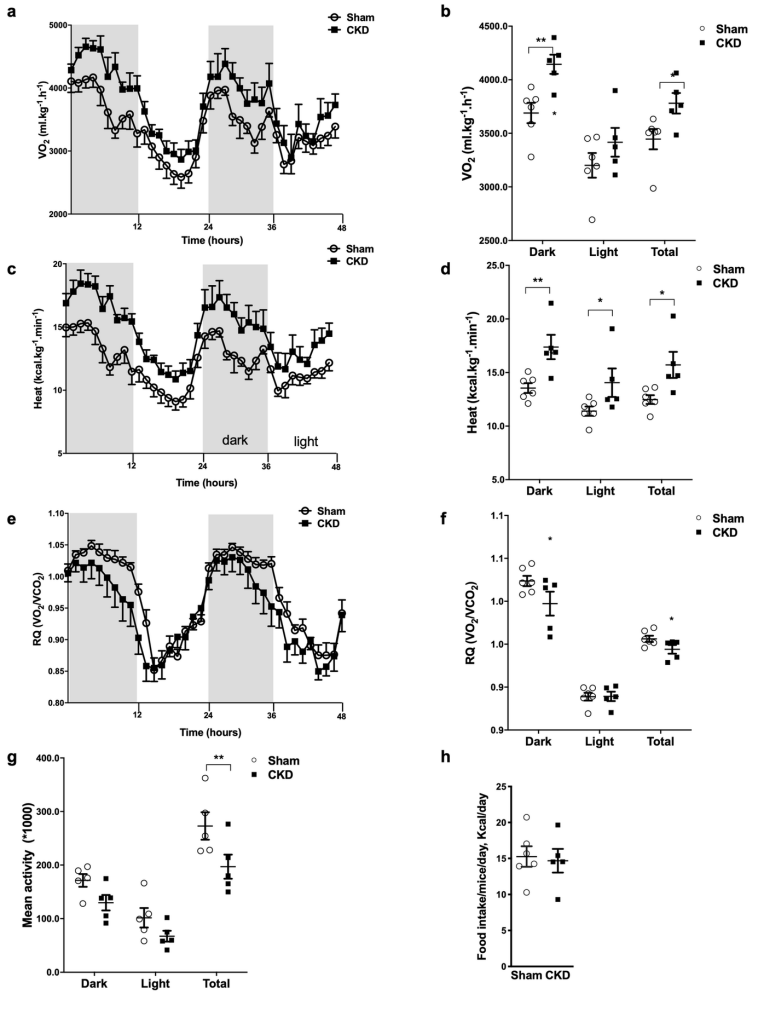

a
Sham
CKD
VO2 (mL.kg-1.h-1)
Time (hours)
b
Sham
CKD
VO2 (mL.kg-1.h-1)
Dark
Light
Total
c
Sham
CKD
Heat (kcal.kg-1.min-1)
dark
light
Time (hours)
d
Sham
CKD
Heat (kcal.kg-1.min-1)
Dark
Light
Total
e
Sham
CKD
RQ (VO2/VCO2)
Time (hours)
f
Sham
CKD
RQ (VO2/VCO2)
Dark
Light
Total
g
Sham
CKD
Mean activity (*1000)
Dark
Light
Total
h
Food intake/mice/day, Kcal/day
Sham
CKD

Figure 3

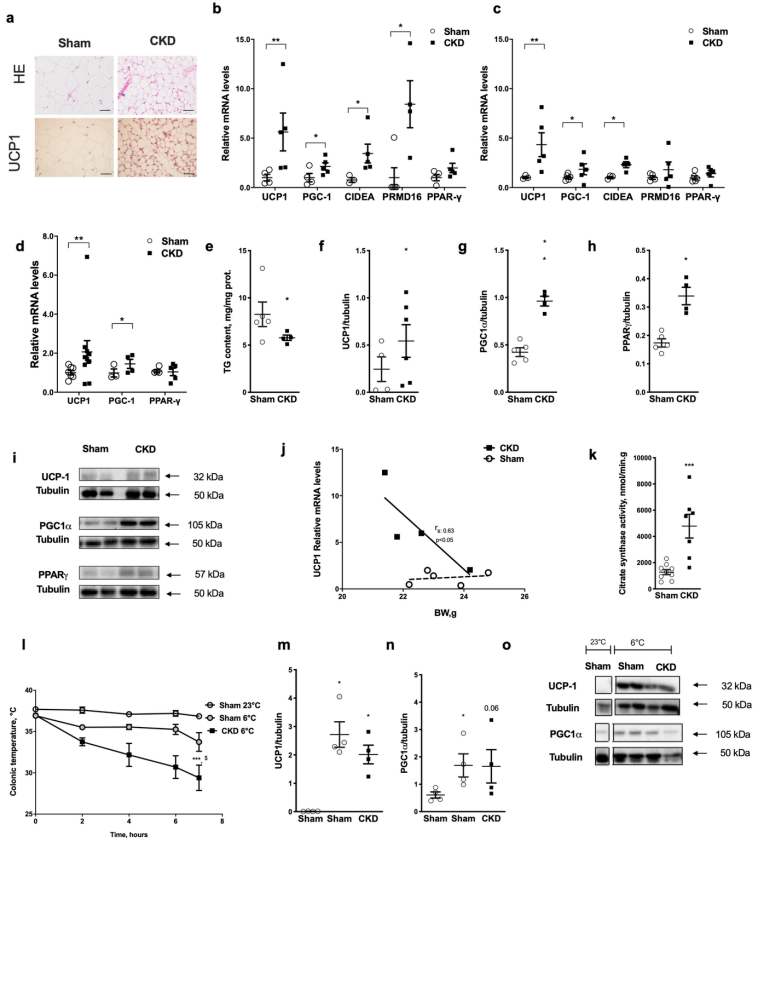

a
Sham
CKD
HE
UCP1
b
Relative mRNA levels
Sham
CKD
UCP1
PGC-1
CIDEA
PRMD16
PPAR-γ
c
Relative mRNA levels
Sham
CKD
UCP1
PGC-1
CIDEA
PRMD16
PPAR-γ
d
Relative mRNA levels
Sham
CKD
UCP1
PGC-1
PPAR-γ
e
TG content, mg/mg prot.
Sham CKD
f
UCP1/tubulin
Sham CKD
g
PGC1α/tubulin
Sham CKD
h
PPARγ/tubulin
Sham CKD
i
Sham
CKD
UCP-1
Tubulin
32 kDa
50 kDa
PGC1α
Tubulin
105 kDa
50 kDa
PPARγ
Tubulin
57 kDa
50 kDa
j
CKD
Sham
UCP1 Relative mRNA levels
BW,g
k
Citrate synthase activity, nmol/min.g
Sham CKD
l
Colonic temperature, °C
Sham 23°C
Sham 6°C
CKD 6°C
Time, hours
m
UCP1/tubulin
Sham Sham CKD
n
PGC1α/tubulin
0.06
Sham Sham CKD
o
23°C
6°C
Sham Sham CKD
UCP-1
Tubulin
32 kDa
50 kDa
PGC1α
Tubulin
105 kDa
50 kDa

Figure 4

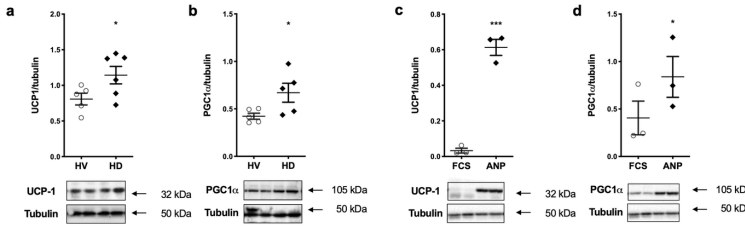
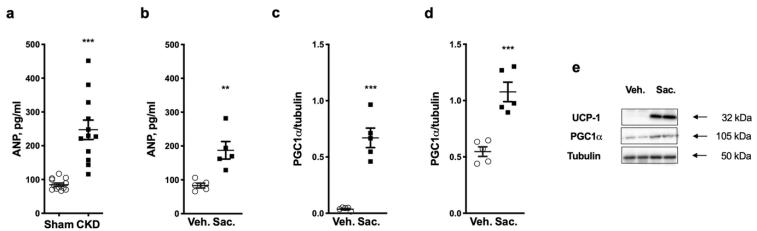
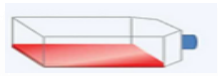

a
UCP1/tubulin
2.0
1.5
1.0
0.5
0.0
*
HV
HD
UCP-1
Tubulin
32 kDa
50 kDa
b
PGC1α/tubulin
1.5
1.0
0.5
0.0
*
HV
HD
PGC1α
Tubulin
105 kDa
50 kDa
c
UCP1/tubulin
0.8
0.6
0.4
0.2
0.0
***
FCS
ANP
UCP-1
Tubulin
32 kDa
50 kDa
d
PGC1α/tubulin
1.5
1.0
0.5
0.0
*
FCS
ANP
PGC1α
Tubulin
105 kDa
50 kDa

Figure 5


a
ANP, pg/ml
500
400
300
200
100
0
***
Sham CKD
b
ANP, pg/ml
500
400
300
200
100
0
**
Veh. Sac.
c
PGC1α/tubulin
1.5
1.0
0.5
0.0
***
Veh. Sac.
d
PGC1α/tubulin
1.5
1.0
0.5
0.0
***
Veh. Sac.
e
Veh.
Sac.
UCP-1
PGC1α
Tubulin
32 kDa
105 kDa
50 kDa

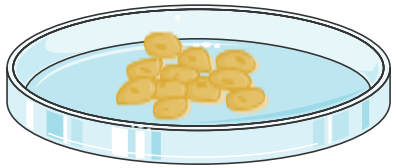
Accumulation of natriuretic peptides is associated with protein energy wasting and activation of browning in white adipose tissue in chronic kidney disease.

In vitro



Human uremic serum

Atrial natriuretic peptide (ANP)



↑ **Uncoupling protein-1 (UCP-1)**

Primary murine adipocytes

In vivo

Mice
5/6 nephrectomy

↑**ANP**

White adipose tissue browning

↑ **UCP-1**

↑ **Citrate synthetase activity**

↑ Thermogenesis
↑ Resting energy expenditure

PEW-cachexia

In vivo

Mice

Sacubitril (65.3 mg/day/kg)
Three weeks

↑**ANP**

White adipose tissue browning

↑ **UCP-1**

CONCLUSION:

Natriuretic peptides accumulation during CKD could contribute to the browning of WAT and PEW-cachexia

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