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

Mathilde Luce[1,2], Christophe Barba[1,2], Dan Yi[1], Anne Mey[2], Damien Roussel[3], Emilie Bres[1,2], Bérengère Benoit[2], Myriam Pastural[4], Samuel Granjon[5], Jean Christophe Szelag[4], Maurice Laville[1], Walid Arkouche[4], Anais Bouchara[1], Elsa Nyam[6,7], Denis Fouque[1,2], Christophe O. Soulage[2,8] and Laetitia Koppe[1,2,8]

[1]Department of Nephrology, Civil Hospices of Lyon, Lyon Sud Hospital Center, Pierre Benite, France; [2]University of Lyon, CarMeN lab, National Institute of Applied Sciences of Lyon (INSA-Lyon), French National Institute of Health and Medical Research (INSERM) U1060, National Institute of Agricultural Research (INRA), Claude Bernard University Lyon 1, Villeurbanne, France; [3]Natural and Anthropic Hydrosystems Ecology Laboratory, University of Lyon, Claude Bernard Lyon University 1, National School of Public Works of the State (ENTPE), Villeurbanne, France; [4]Association pour l'Utilisation du Rein Artificiel dans la région Lyonnaise (AURAL), Lyon, France; [5]Laboratoire d'Analyse Médicale Cerballiance Rhône alpes, Lyon, France; [6]Montreal Diabetes Research Center, CRCHUM, Montréal, Quebec, Canada; and [7]Department of Medicine, University of Montreal, Montreal, Quebec, Canada

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.

Kidney International (2020) **98,** 663–672; https://doi.org/10.1016/j.kint.2020.03.027

KEYWORDS: browning; cardiac natriuretic peptides; chronic kidney disease; protein energy wasting

Translational Statement

This paper describes the discovery, using both cellular and chronic kidney disease (CKD) mice models, of the role of natriuretic peptides (NPs) in promoting thermogenesis by activation of inducible brown adipocytes in white adipose tissue. A deeper understanding of how NPs control energy balance in CKD may provide important clues for developing new strategies to manage protein-energy wasting (PEW). As a next step, we will test whether specific inhibitors of the browning process can improve PEW in CKD patients.

Protein-energy wasting (PEW) and cachexia, a 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 any, effective therapies to improve PEW associated with CKD.[4]

Whether there is an increase in resting energy expenditure (REE), and its role in PEW during CKD, remain topics of

Correspondence: *Laetitia Koppe, Department of Nephrology and Nutrition, Lyon Sud Hospital Center, Chemin du Grand Revoyet, 69495, Pierre Bénite, France. E-mail: Laetitia.koppe@chu-lyon.fr*

[8]COS and LK contributed equally to this work.

Received 21 March 2019; revised 26 February 2020; accepted 9 March 2020; published online 23 April 2020

discussion. In CKD mice, an increase of REE has been described,[5–7] but observations made in CKD patients are conflicting . Some studies reported a reduction of REE in nondialysis CKD patients[8] based on indirect methods, but strong evidence shows 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 progress has been made toward understanding of PEW, its determinants and cellular mechanisms remain to be discovered.

There are 2 types of adipose tissues (AT)—brown (BAT) and white (WAT)— and they possess opposite physiological functions and different anatomic dispositions. WAT is the main site for energy storage in the form of triglycerides (stored as a lipid droplet in 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 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, using positron emission tomography scanning corresponding to BAT.[13] The persistence of BAT in adult humans is currently 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, a phenomenon 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] and adipokines[19]), white adipocytes are able to overexpress thermogenic proteins, including UCP1. The thermogenic activity of BAT and WAT browning contributes significantly to REE in rodents and humans.[20]

Several studies highlighted the potential contributing role of BAT in the development of PEW and cachexia in the context of hypermetabolic diseases such as cancers,[5] and a similar process may be occurring in CKD. Zhao *et al.* reported in uninephrectomized rats morphologic changes of intra-abdominal WAT in polygonal multilocular cells.[21] Histologic analysis showed an increased abundance of mitochondria in the WAT of CKD rats.[22] These morphologic changes 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.

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 the role of 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] Body mass index 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 via 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, owing to the direct action of UTs. Specifically, we answered the following questions: (i) Is browning induced in a CKD mouse model, and in white adipocytes treated with uremic serum; and (ii) 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 determined by measures of 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 (an intra-abdominal fat depot), inguinal WAT (a subcutaneous fat depot), and interscapular brown fat (iBAT; Figure 1c–f). Mice weight loss was associated with an increase in energy expenditure in uremic animals (Figure 2). Respiratory measurements showing elevated O_2 consumption (VO_2) and a decreased respiratory quotient (i.e., the ratio between oxygen consumption and carbon dioxide production) in uremic mice during both dark phases and the last 24-hour period indicated that lipids were the preferred source of energy substrates (Figure 2a, b, e, and f). Heat production was increased in CKD mice (Figure 2c and d) during both acting and resting phases. The weight loss was not due to increased physical activity, which was decreased in CKD mice (Figure 2g). Diet and food intake did not differ between the 2 groups (Figure 2h).

We analyzed morphologic, 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, in epididymal (e)WAT (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-activated receptor-gamma coactivator 1-alpha (PGC1α), cell death–inducing DNA fragmentation factor (DFF)A-like effector A (CIDEA), PR

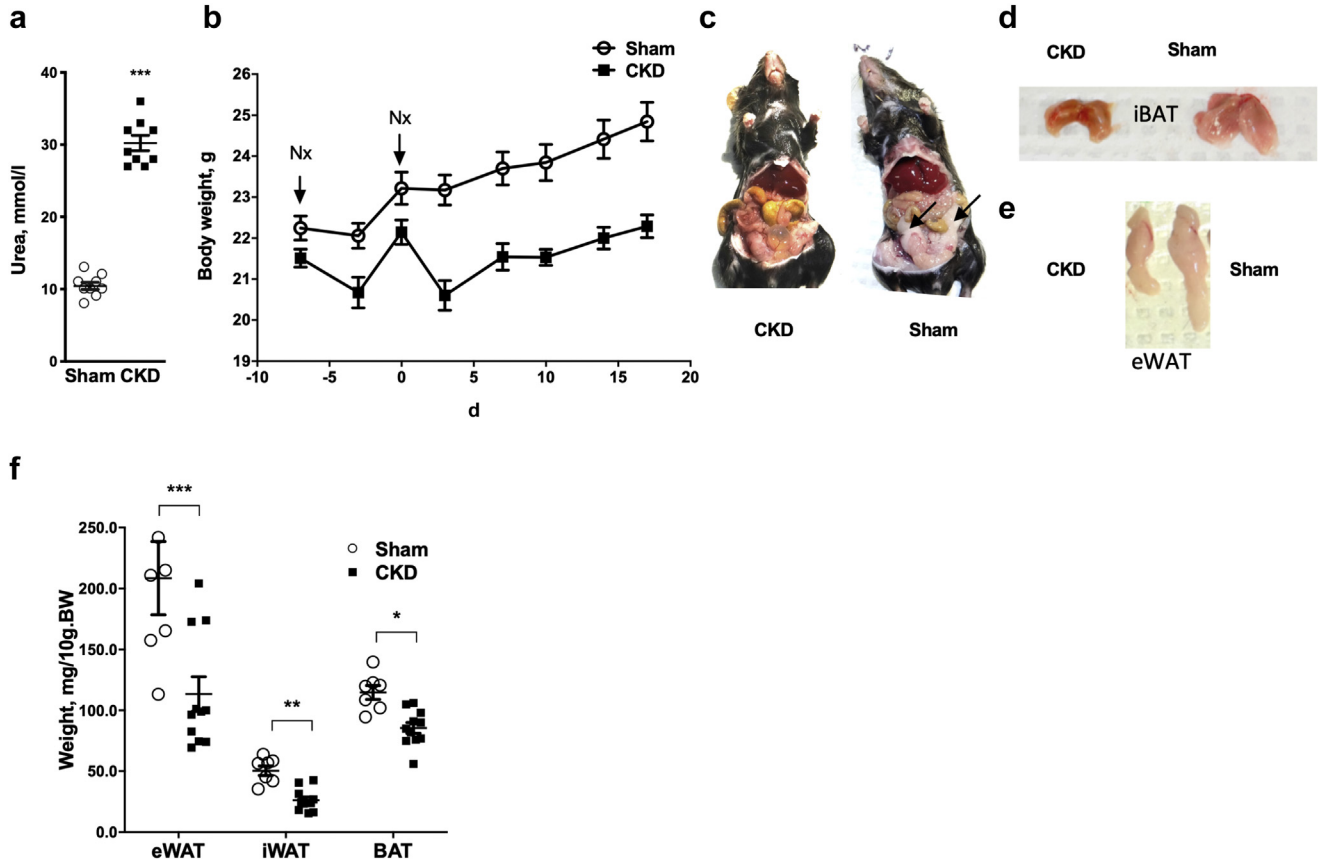

Figure 1 | Mice with chronic kidney disease (CKD) present a protein energy–wasting (PEW) phenotype. (**a**) Urea levels in CKD and sham mice after 3 weeks of uremia. (**b**) Evolution of body weight (BW). (**c**) Representative macroscopic pictures of sham and CKD mice at necropsy. The arrows point to 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, inguinal white adipose tissue [iWAT], and iBAT). n = 7–12. Data are expressed as mean ± SEM. Statistical analyses were conducted using Student's *t* test. *$P <$ 0.05, ***$P <$ 0.001 compared with the sham group.

domain containing 16 (PRPMD16), and peroxisome proliferator activated receptor-gamma (PPARγ) were significantly higher in WAT and iBAT (Figure 3b–d). Triglyceride 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 activity (a proxy of mitochondrial density) in CKD mice (Figure 3k). Given that mitochondrial activity is rather low in the 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.

Stress from cold temperature is well recognized to activate the brown fat mechanism of thermogenesis. We investigated whether browning activation still plays a role in thermogenesis regulation in the uremic context. CKD had no effect on the body temperature of mice without cold stress. Unlike in sham mice, cold exposure at 6 °C for 7 hours resulted in a significant reduction of rectal temperature in CKD mice (Figure 3l). The biometry and renal parameters are 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 suggest 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 was collected from healthy volunteers and HD patients who met PEW criteria.[2] Clinical and biological characteristics of the subjects are summarized in Supplementary Table S2. There was no significant difference between the 2 groups, except for age and, as expected, parameters related to renal function (i.e., creatinine, urea,

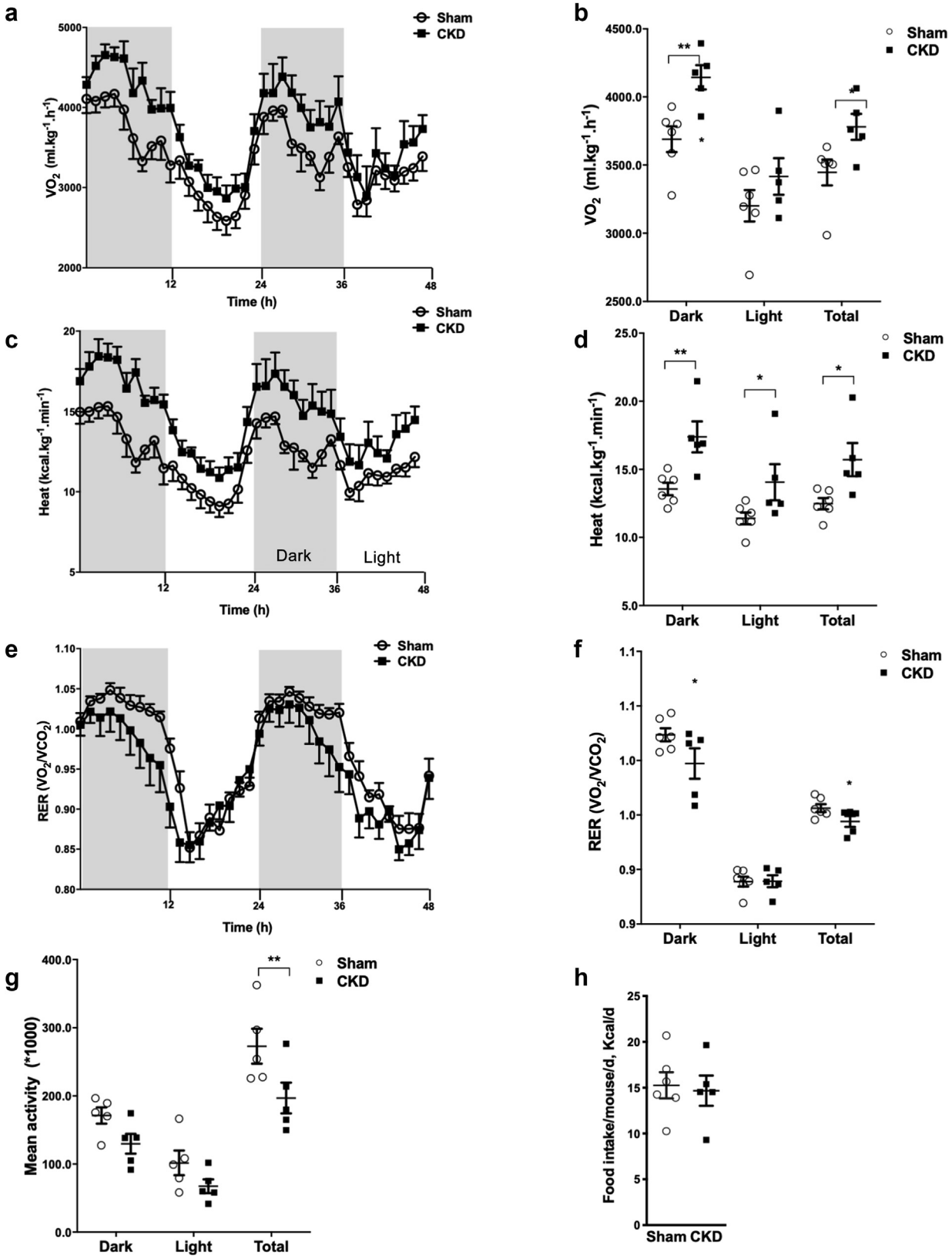

Figure 2 | Mice with chronic kidney disease (CKD) exhibited increased energy expenditure. Sham and CKD mice were housed individually in metabolic cages for 3 days. (**a**) Oxygen consumption (VO_2) at each time point. (**b**) Average of VO_2 over the preceding 24 hours. (**c**) Heat production at each time point. (**d**) Average of heat production over the preceding 24 hours. (**e**) Respiratory quotient (RQ) at each time point. (**f**) Average RQ over the preceding 24 hours. (**g**) Locomotor activity. (**h**) Food intake. n = 6 per group. Data are presented as mean ± SEM. Statistical analysis was conducted using Student's *t* test. *$P < 0.05$; **$P < 0.01$. VCO_2, carbon dioxide consumption.

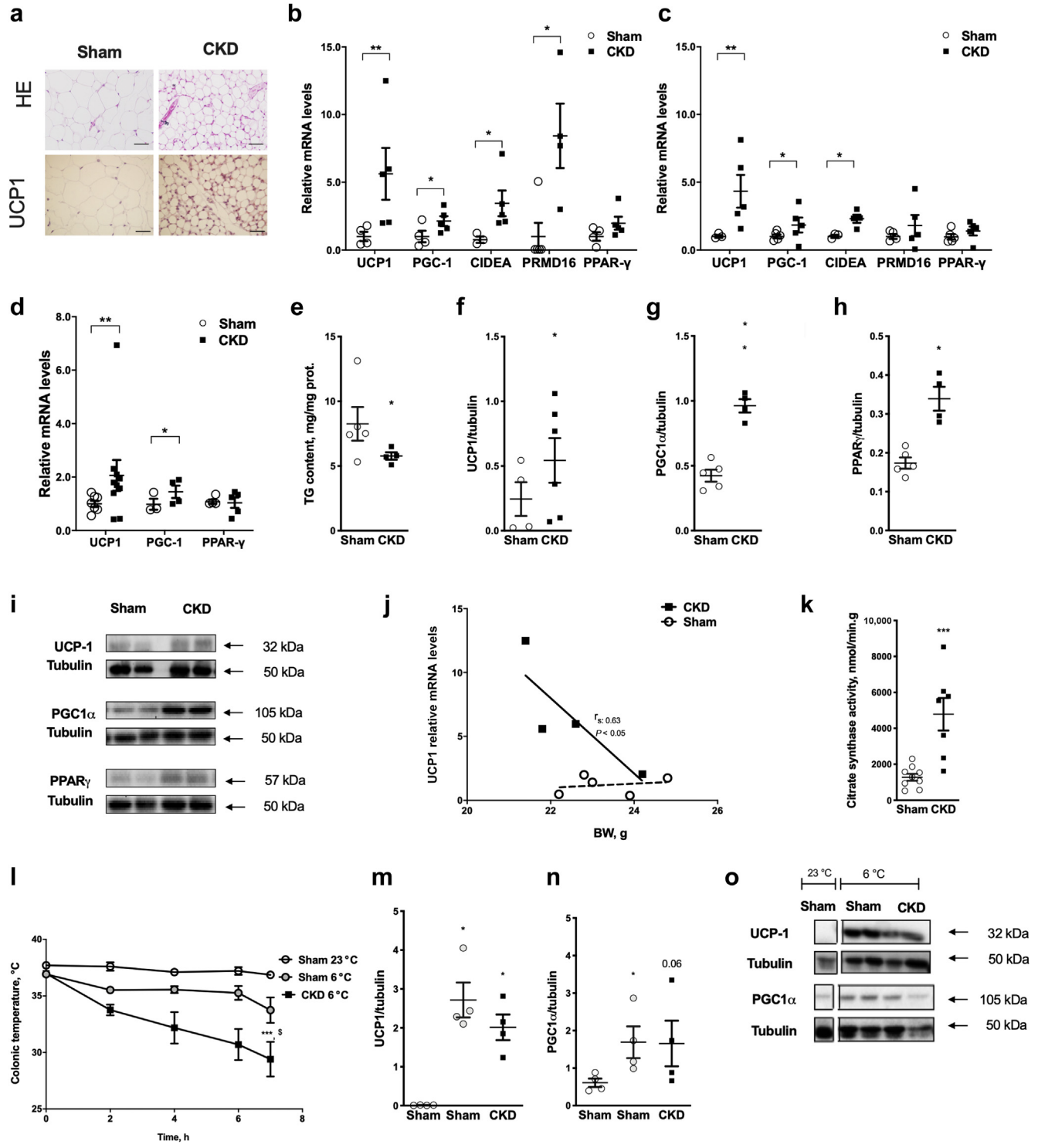

Figure 3 | Chronic kidney disease (CKD) is associated with an activation of brown adipose tissue (BAT) and browning of white adipose tissue (WAT). (**a**) Hematoxylin and eosin (HE) staining and immunohistochemical analysis of uncoupling protein 1 (UCP1) in epididymal white adipose tissue (eWAT) sections from sham and CKD mice. Gene expression in (**b**) eWAT, (**c**) inguinal white adipose tissue (iWAT), and (**d**) interscapular brown adipose tissue (iBAT) was determined by quantitative reverse transcription polymerase chain reaction; n = 4–6. (**e**) Triglyceride (TG) content in iBAT; n = 4. Quantifications of (**f**) UCP1, (**g**) peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC1α), and (**h**) peroxisome proliferator activated receptor-gamma (PPARγ) by Western blotting, normalized to tubulin and expressed in arbitrary units (AU). (**i**) Representative blot of UCP1, PGC1α, and PPARγ in the eWAT of sham and 5/6 nephrectomized (Nx 5/6) mice; n = 4–5. (**j**) The relationship 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 7 hours; n = 5. Quantifications of (**m**) UCP1 and (**n**) PGC1α by Western blotting, normalized to tubulin and expressed in AU. (**o**) Representative blot of UCP1 and (continued)

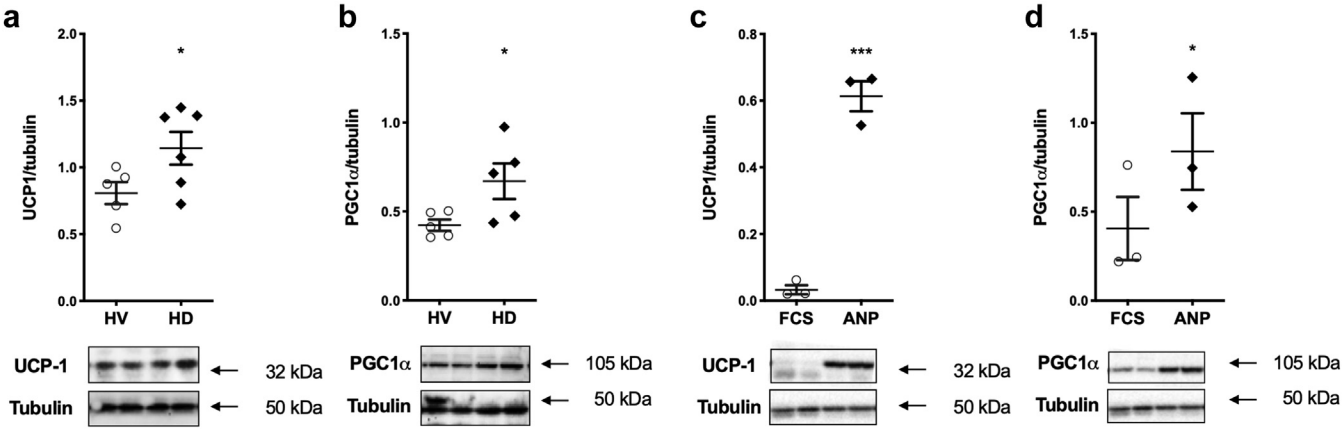

Figure 4 | Uremic plasma and atrial natriuretic peptide (ANP) increases uncoupling protein 1 (UCP1) protein content in primary cultures of inguinal adipocytes. (**a**) UCP1 and (**b**) peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC1α) protein content in primary adipocytes incubated for 24 hours with either the 17% (v/v) healthy volunteers (HV) plasma pool or the 17% (v/v) hemodialysis (HD) plasma pool (n = 5–6). (**c**) UCP1 and (**d**) PGC1α protein content in primary adipocytes incubated for 24 hours with either 10% (v/v) fetal calf serum (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's *t* test. *$P < 0.05$; ***$P < 0.001$, compared with the HV or FCS condition.

estimated glomerular filtration rate). Of note, no significant difference in PTH or C-reactive protein was seen between the 2 groups, but we observed an increase of BNP concentration in the HD group. Uremic plasma induced an increase of UCP1 and PGC1α protein content in primary adipocytes (Figure 4a and b), compared to plasma from healthy volunteers. These results suggest that some compounds found in uremic plasma could induce increased expression of UCP1 and PGC1α in primary adipocytes and therefore activate 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 cell 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 triglyceride content in 3T3-L1 cells were similar after exposure to PCS and IS versus control (data not shown). These data suggest that these 2 UTs did not trigger the browning phenomenon observed with uremic plasma. Cardiac NPs are good candidates, as 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 for 24 hours with ANP at concentrations found in CKD patients (280 pM). ANP exposure triggered a huge increase of UCP1 and PGC1α protein in primary adipocytes (Figure 4c and d).

ANP increases brown adipocyte characteristics *in vivo*

In CKD mice, plasma levels of ANP were significantly higher compared to those in sham mice (Figure 5a). In order to validate the specific role of ANP in promoting browning in the 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 contributes to the increased plasma levels of ANP. The mean sacubitril intake was 65.3 mg/day per kg body weight. As shown in Figure 5b, plasma levels of ANP were significantly higher in the mice that received sacubitril, and they 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 activation of the browning of WAT.

DISCUSSION

This study aimed to determine whether browning could contribute to PEW associated with CKD and whether UT 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 BAT thermogenesis. We demonstrated for the first time that the uremic milieu can promote browning, as HD plasma induced UCP1 and PGC1α expression in cultured primary murine adipocytes. In addition, we found that NPs are involved in browning during CKD.

Figure 3 | (continued) PGC1α in eWAT of sham and Nx 5/6 mice after acute cold exposure; n = 4. Data are expressed as mean ± SEM. Statistical analysis was conducted using Student's *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 the sham control exposed at 6 °C. CIDEA, cell death–inducing DNA fragmentation factor A–like effector; PRMD16, PR domain containing 16; prot., protein. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

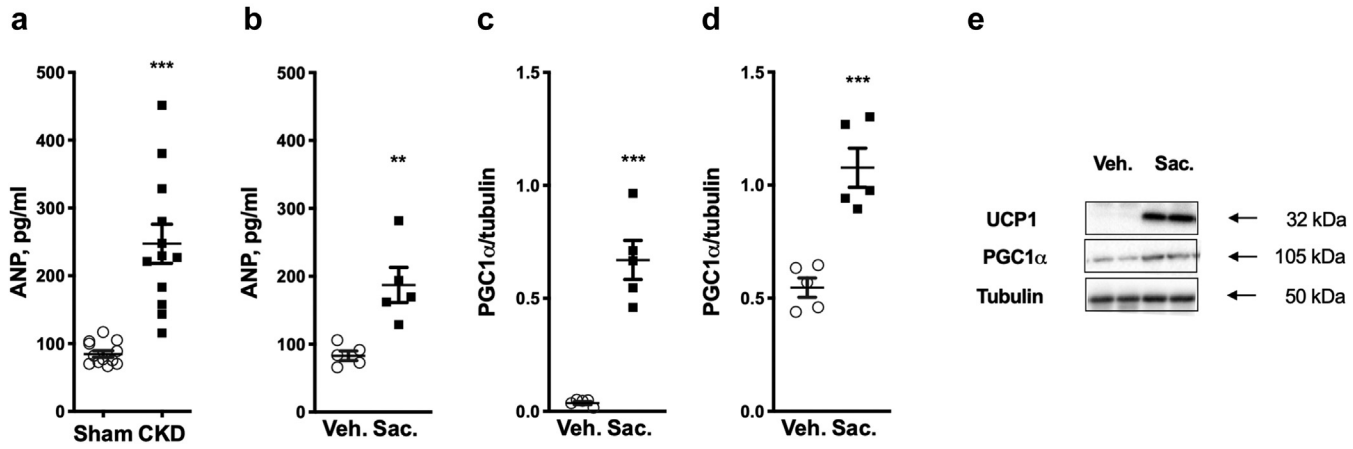

Figure 5 | Mice treated with sacubitril (Sac.) exhibit increased atrial natriuretic peptide (ANP) concentration and increased expression of brown adipocyte markers in white adipose tissue. (**a**) ANP levels in chronic kidney disease (CKD) and sham mice at 3 weeks (n = 10). (**b**) ANP levels in mice treated with vehicle (Veh.) or Sac. at 2 weeks (n = 5). Note that the concentration of ANP observed under the chronic administration of Sac. matches the concentrations observed in CKD mice. Quantifications of (**c**) uncoupling protein 1 (UCP1) and (**d**) peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC1α). (**e**) Representative blot of UCP1 and PGC1α in mice treated with Veh. or Sac. Quantifications of UCP1 and PGC1α were performed by Western blotting, normalized to tubulin, and expressed in arbitrary units (AU); n = 4–5. Data are expressed as mean ± SEM. Statistical analysis was conducted using Student's *t* test. $^{**}P < 0.01$, $^{***}P < 0.001$, compared with sham or Veh. mice.

Cachexia and PEW result from complex pathways. A better understanding of the underlying mechanisms is therefore critical to reducing morbidity and mortality associated with CKD. The central role of browning in PEW and cachexia, particularly in cancer, has recently emerged.[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 a different CKD mouse 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 can be concomitant with the browning and therefore results from similar molecular determinants. Recently, Kir *et al.* have suggested that PTH is involved in stimulating a thermogenic program in 5/6 nephrectomized mice. Fat-specific knockout of PTH-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 be noted, however, that the concentration used (a single subcutaneous injection of 1 mg/kg) was supraphysiologic, that is, much higher than the concentration commonly encountered during CKD. However, in a clinical study, HD patients with hyperparathyroidism exhibited an increased REE that is reduced after parathyroidectomy.[32] An increase in inflammatory cytokines may 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 and C-reactive protein were not increased in malnourished patients. These data suggest that other mediators that accumulate 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 4 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 suggest that tryptophan, the precursor of IS, is involved in thermogenesis.[35] In our study, we failed to see any effect of IS or PCS on browning. Indeed, IS and PCS at levels found in CKD did not impair adipocyte differentiation, in contrast to findings in a previous report.[36] It should be emphasized, however, that in this study, adipocyte cells were incubated with the nonconjugated 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 biologically active, the doses used were not representative of CKD conditions.

NPs are a group of peptide hormones secreted mainly by the heart, signaling via cyclic guanosine monophosphate–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 are 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 2 are secreted from the heart in equimolar amounts. Even if the impact of kidney function on NT-proBNP was much more pronounced than that on BNP, some data clearly demonstrate that NT-proBNP levels are significantly correlated with BNP levels at all stages of kidney dysfunction, including in HD patients.[38] In the literature, BNP and NT-pro-BNP were found to be associated with cardiovascular diseases and mortality,[25,26] but until now they were considered to be biomarkers, rather than causes, of unfavorable outcomes.[39]

Novel physiological functions of NPs were recently discovered. Sarzani *et al.* demonstrated that some NP receptors were expressed in adipose tissue of rats and humans.[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 an increase in UCP1 after treatment of human multipotent adipose-derived stem cells with ANP. Infusion of BNP in mice resulted in an increased REE and overexpression of Ucp1 in WAT mediated through the p38 mitogen-activated protein kinase 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 the general population, increased NPs are associated with reduction in WAT deposition.[42,43] One study reported a negative correlation between body mass index and NT-proBNP levels in nondialysis CKD patients[28]; another found that levels of NT-proBNPs 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 adipocytes 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, protected against lipid transformation in unilocular to multilocular cells, suggesting a decrease of browning.[22] Angiotensin-converting enzyme inhibitor is also a major cardio-protective treatment and could inhibit the browning phenomenon by preventing cardiac remodeling and the increase of NPs. Using treatment with sacubitril (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 range as that observed in CKD mice. The impact of sacubitril on human adipose tissue remains unclear. In a *post hoc* analysis of the PARADIGM-HF trial, which included 3778 patients with known diabetes without renal disease, body mass index 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 compared to amlodipine.[47] This suggests that activation of browning in this population is not clinically relevant, but unfortunately, no data for CKD patients are available.

The present study has several limitations. First, *in vitro,* the impact of only 3 uremic toxins (ANP, PCS, and IS) on UCP1 expression was tested, and we cannot exclude the possibility that other aspects of the uremic environment could have an impact on energy balance and browning activation. Second, 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 are necessary to tease out these potential associations.

In conclusion, our study provides novel insights on the mechanisms of PEW in CKD using 2 independent models with consistent functional defects. We demonstrated that the uremic environment induced browning activation *in vivo* and *in vitro,* and we identified NPs as one of the UTs involved in browning in CKD. 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 otherwise indicated, all chemicals were from Sigma Aldrich (Saint Quentin Fallavier, France), and all solvents were from Carlo Erba (Peypin, France). Primary antibodies are listed in Supplementary 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 enrollment. Patients included were >18 years old, male and female. HD patients were following HD sessions for at least 3 months, and presented with PEW criteria according to Fouque *et al.*[2] Patients with CKD were considered to have PEW if they presented at least 3 of the following 4 criteria: body mass index <23 kg/m^2, albumin level <38 g/l, prealbumin level <300 mg/l, and normalized protein catabolic rate <0.8 g/kg per day. Exclusion criteria were diabetes, systemic inflammatory diseases, autoimmune diseases, cancer, oral antidiabetic treatments, and use of insulin. Plasma were collected from healthy volunteers and HD patients and were 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 were differentiated as previously reported. Exposure of UTs and adipose cell differentiation are detailed in the Supplementary Methods.

Animal models

All experimental procedures were approved by the local ethics committee (Supplementary Methods). Male C57Bl6 mice were purchased from Charles River Laboratories (Saint-Constant, Quebec, Canada) 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 12-hour 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-week-old male C57BL/6J mice from subcutaneous inguinal adipose tissues. The isolation and amplification of adipose stem cells have already been described.[48] Beiging induction and adipocyte treatment are described in the Supplementary Methods. At the end of the incubation period, the culture media were removed and stored at –80 °C until analysis.

Uremic mouse model

Moderate kidney failure was induced in 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 the Supplementary Methods.

Cold challenge experiment

Sham or uremic mice were housed individually (with free access to food and 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 Biotechnology, Santa Cruz, CA) was dissolved in drinking water containing 0.5% (v/v) ethanol to a final concentration of 0.4 g/l. Mice were given sacubitril in drinking water for 2 weeks. Control mice were given water containing 0.5% (v/v) ethanol for 2 weeks. Water intake was monitored 3 times a week to estimate the daily intake of sacubitril. Note that no difference in water intake was observed between control and sacubitril mice.

Citrate synthetase activity

Citrate synthetase activity has been measured in frozen eWAT tissues (approximately 40 mg), and the procedure has been detailed in the Supplementary Methods.

Protein extraction, Western blotting, and real-time polymerase chain reaction

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 real-time polymerase chain reaction. Sequence information for the mouse primer sets is summarized in Supplementary Table S5. Western Blot and real-time polymerase chain reaction procedures are detailed in the Supplementary Methods.

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 Methods).

Statistical analysis

Data were analyzed using GraphPad Prism 6.0 software (GraphPad Software, La Jolla, CA). The data are expressed as mean ± SEM (animal study) or as median (interquartile range) when variables were not normally distributed. Statistical analyses are detailed in the Supplementary Methods. $P < 0.05$ was considered statistically significant in all analyses.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

This study was supported by the Fondation du Rein (Prix Jeune Chercheur 2016 FdR/FRM to LK), the Hospices Civils de Lyon, INSERM (Institut National de la Santé et de la Recherche Médicale), and INSA-Lyon (Institut National des Sciences Appliquées de Lyon). MLu and EB were supported by a grant—«Année Recherche»—from Hospices Civils de Lyon and Agence Régionale de Santé (ARS). CB was supported by a grant from Fondation Recherche Medicale. We thank all patients for their participation in this study.

AUTHOR CONTRIBUTIONS

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

SUPPLEMENTARY MATERIAL

Supplementary File (Word)

Table S1. Biometry and renal parameters of sham and CKD mice exposed to cold (6 °C for 7 hours).

Table S2. Baseline characteristics of healthy volunteers and hemodialysis patients for primary adipocyte experiments.

Table S3. Biometry of mice treated with vehicle or sacubitril for 2 weeks.

Table S4. Antibodies.

Table S5. Sequence information for mice primers.

Supplementary Methods.

REFERENCES

1. Kalantar-Zadeh K, Ikizler TA, Block G, et al. Malnutrition-inflammation complex syndrome in dialysis patients: causes and consequences. *Am J Kidney Dis*. 2003;42:864–881.
2. Fouque D, Kalantar-Zadeh K, Kopple J, et al. A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. *Kidney Int*. 2008;73:391–398.
3. Reid J, Noble H, Davenport A, et al. Defining cachexia in a renal population. *J Ren Care*. 2015;41:79–80.
4. Ikizler TA, Cano NJ, Franch H, et al. Prevention and treatment of protein energy wasting in chronic kidney disease patients: a consensus statement by the International Society of Renal Nutrition and Metabolism. *Kidney Int*. 2013;84:1096–1107.
5. Kir S, Komaba H, Garcia AP, et al. PTH/PTHrP receptor mediates cachexia in models of kidney failure and cancer. *Cell Metab*. 2016;23:315–323.
6. Cheung WW, Rosengren S, Boyle DL, Mak RH. Modulation of melanocortin signaling ameliorates uremic cachexia. *Kidney Int*. 2008;74:180–186.
7. Cheung WW, Kuo H-J, Markison S, et al. Peripheral administration of the melanocortin-4 receptor antagonist NBI-12i ameliorates uremia-associated cachexia in mice. *J Am Soc Nephrol*. 2007;18:2517–2524.
8. Avesani CM, Draibe SA, Kamimura MA, et al. Decreased resting energy expenditure in non-dialysed chronic kidney disease patients. *Nephrol Dial Transplant*. 2004;19:3091–3097.
9. Ikizler TA, Wingard RL, Sun M, et al. Increased energy expenditure in hemodialysis patients. *J Am Soc Nephrol*. 1996;7:2646–2653.
10. Wang AY-M, Sea MM-M, Tang N, et al. Resting energy expenditure and subsequent mortality risk in peritoneal dialysis patients. *J Am Soc Nephrol*. 2004;15:3134–3143.
11. Carrero JJ, Stenvinkel P, Cuppari L, et al. Etiology of the protein-energy wasting syndrome in chronic kidney disease: a consensus statement from the International Society of Renal Nutrition and Metabolism (ISRNM). *J Ren Nutr*. 2013;23:77–90.
12. Peirce V, Carobbio S, Vidal-Puig A. The different shades of fat. *Nature*. 2014;510:76–83.

13. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab*. 2007;293:E444–E452.
14. Cypess AM, Lehman S, Williams G, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med*. 2009;360:1509–1517.
15. Weiner J, Hankir M, Heiker JT, et al. Thyroid hormones and browning of adipose tissue. *Mol Cell Endocrinol*. 2017;458:156–159.
16. Bordicchia M, Liu D, Amri E-Z, et al. Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. *J Clin Invest*. 2012;122:1022–1036.
17. Coué M, Moro C. Natriuretic peptide control of energy balance and glucose homeostasis. *Biochimie*. 2016;124:84–91.
18. Hondares E, Iglesias R, Giralt A, et al. Thermogenic activation induces FGF21 expression and release in brown adipose tissue. *J Biol Chem*. 2011;286:12983–12990.
19. Montanari T, Pošćić N, Colitti M. Factors involved in white-to-brown adipose tissue conversion and in thermogenesis: a review. *Obes Rev*. 2017;18:495–513.
20. Cypess AM, White AP, Vernochet C, et al. Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. *Nat Med*. 2013;19:635–639.
21. Zhao H-L, Sui Y, Guan J, et al. Fat redistribution and adipocyte transformation in uninephrectomized rats. *Kidney Int*. 2008;74:467–477.
22. Zhao H-L, Sui Y, He L, et al. Lipid partitioning after uninephrectomy. *Acta Diabetol*. 2011;48:317–328.
23. Duranton F, Cohen G, De Smet R, et al. Normal and pathologic concentrations of uremic toxins. *J Am Soc Nephrol*. 2012;23:1258–1270.
24. Koppe L, Pillon NJ, Vella RE, et al. p-Cresyl sulfate promotes insulin resistance associated with CKD. *J Am Soc Nephrol*. 2013;24:88–99.
25. Wang AY-M, Lai K-N. Use of cardiac biomarkers in end-stage renal disease. *J Am Soc Nephrol*. 2008;19:1643–1652.
26. Madsen LH, Ladefoged S, Corell P, et al. N-terminal pro brain natriuretic peptide predicts mortality in patients with end-stage renal disease in hemodialysis. *Kidney Int*. 2007;71:548–554.
27. Wang TJ, Larson MG, Levy D, et al. Impact of obesity on plasma natriuretic peptide levels. *Circulation*. 2004;109:594–600.
28. Yi S, Contreras G, Miller ER, et al. Correlates of N-terminal prohormone brain natriuretic peptides in African Americans with hypertensive chronic kidney disease: the African American Study of Kidney Disease and Hypertension. *Am J Nephrol*. 2009;29:292–298.
29. Szabó T, Postrach E, Mähler A, et al. Increased catabolic activity in adipose tissue of patients with chronic heart failure. *Eur J Heart Fail*. 2013;15:1131–1137.
30. Petruzzelli M, Schweiger M, Schreiber R, et al. A switch from white to brown fat increases energy expenditure in cancer-associated cachexia. *Cell Metab*. 2014;20:433–447.
31. Cheung WW, Ding W, Gunta SS, et al. A pegylated leptin antagonist ameliorates CKD-associated cachexia in mice. *J Am Soc Nephrol*. 2014;25:119–128.
32. Cuppari L, de Carvalho AB, Avesani CM, et al. Increased resting energy expenditure in hemodialysis patients with severe hyperparathyroidism. *J Am Soc Nephrol*. 2004;15:2933–2939.
33. Nowak KL, Hung A, Ikizler TA, et al. Interleukin-1 inhibition, chronic kidney disease-mineral and bone disorder, and physical function. *Clin Nephrol*. 2017;88:132–143.
34. Minakuchi H, Wakino S, Hosoya K, et al. The role of adipose tissue asymmetric dimethylarginine/dimethylarginine dimethylaminohydrolase pathway in adipose tissue phenotype and metabolic abnormalities in subtotally nephrectomized rats. *Nephrol Dial Transplant*. 2016;31:413–423.
35. Agudelo LZ, Ferreira DMS, Cervenka I, et al. Kynurenic acid and Gpr35 regulate adipose tissue energy homeostasis and inflammation. *Cell Metab*. 2018;27:378–392.e5.
36. Tanaka S, Yano S, Sheikh AM, et al. Effects of uremic toxin p-cresol on proliferation, apoptosis, differentiation, and glucose uptake in 3T3-L1 cells. *Artif Organs*. 2014;38:566–571.
37. Nakagawa Y, Nishikimi T, Kuwahara K. Atrial and brain natriuretic peptides: Hormones secreted from the heart. *Peptides*. 2019;111:18–25.
38. Takase H, Dohi Y. Kidney function crucially affects B-type natriuretic peptide (BNP), N-terminal proBNP and their relationship. *Eur J Clin Invest*. 2014;44:303–308.
39. Vanholder R, Pletinck A, Schepers E, Glorieux G. Biochemical and clinical impact of organic uremic retention solutes: a comprehensive update. *Toxins*. 2018;10:33.
40. Sarzani R, Dessì-Fulgheri P, Paci VM, et al. Expression of natriuretic peptide receptors in human adipose and other tissues. *J Endocrinol Invest*. 1996;19:581–585.
41. Sengenès C, Berlan M, De Glisezinski I, et al. Natriuretic peptides: a new lipolytic pathway in human adipocytes. *FASEB J*. 2000;14:1345–1351.
42. Sarzani R, Strazzullo P, Salvi F, et al. Natriuretic peptide clearance receptor alleles and susceptibility to abdominal adiposity. *Obes Res*. 2004;12:351–356.
43. Neeland IJ, Winders BR, Ayers CR, et al. Higher natriuretic peptide levels associate with a favorable adipose tissue distribution profile. *J Am Coll Cardiol*. 2013;62:752–760.
44. Ducros J, Larifla L, Merault H, Foucan L. NT-proBNP, cardiometabolic risk factors, and nutritional status in hemodialysis patients. *Int J Nephrol*. 2017;2017:1312547.
45. Helal I, Belhadj R, Mohseni A, et al. Clinical significance of N-terminal Pro-B-type natriuretic peptide (NT-proBNP) in hemodialysis patients. *Saudi J Kidney Dis Transplant*. 2010;21:262–268.
46. Seferovic JP, Claggett B, Seidelmann SB, et al. Effect of sacubitril/valsartan versus enalapril on glycaemic control in patients with heart failure and diabetes: a post-hoc analysis from the PARADIGM-HF trial. *Lancet Diabetes Endocrinol*. 2017;5:333–340.
47. Engeli S, Stinkens R, Heise T, et al. Effect of Sacubitril/Valsartan on exercise-induced lipid metabolism in patients with obesity and hypertension. *Hypertension*. 2018;71:70–77.
48. Lefevre C, Panthu B, Naville D, et al. Metabolic phenotyping of adipose-derived stem cells reveals a unique signature and intrinsic differences between fat pads. *Stem Cells Int*. 2019;2019:9323864.