SUPPLEMENTARY MATERIALS – Koppe et al., 2012

Complete methods

Chemicals and antibodies

All chemicals, reagents and culture media were obtained from Sigma Aldrich (Saint-Quentin Fallavier, France) when no other origin is specified. Recombinant Human insulin (100 UI/ml, Actrapid®) was from Novo Nordisk, (La Défense, France). Anti-Phospho-Akt 1/2/3 (ref 7985R), anti-Akt 1/2/3 (ref 8312), anti-IRS 1 (ref SC-559, SC 560), phospho-tyrosine (pY99, ref SC-7020), anti-ser 636 IRS-1 (ref SC-101711) and phospho-ERK (E-4, ref SC-7383) antibodies were purchased from Santa Cruz Biotechnology (Heidelberg, Germany). Anti-p85 (ref 06-497) antibodies were from Millipore (Molsheim, France), anti phospho-JNK from Cell Signaling (Ozyme, Saint-Quentin, France) and anti-tubulin antibodies were from Sigma Aldrich. Anti-mouse IgG and anti-rabbit IgG antibodies were from BioRad (Marnes-la-Coquette, France). Super Signal® West Pico Chemiluminescent Substrate and RestoreTM Western Blot Stripping Buffer were obtained from Thermo Scientific (Perbio, Brebières, France).

p-cresyl sulfate (PCS)

All *in vitro* experiments were performed according to the standard approach and the recommendations for handling uremic retention solutes published by the European Uremic Toxin Work Group (EUTox, http://*www.uremic-toxins.org)* and reviewed in reference. [1] The potassium salt of p-cresyl sulfate was synthesized as described by Feigenbaum and Neuberg. [2] In the *in vitro* study, concentration of PCS was 40 μg/ml (212 μM), which is the concentration found in humans in ESRD. [3-5] Since PCS was synthesized as a potassium salt, a solution of 35 μg/ml (200 μM) K_2SO_4 in saline was chosen as control to equal the potassium concentration in the K-salt of PCS. Since PCS is mainly protein-bound in biological systems, all *in vitro* experiments were performed in medium supplemented with 35g/L bovine serum albumin (BSA) according to the recommendations of EUTox. [1]

Animal experiments.

Animal experiments were performed under the authorization n°69-266-0501 (CarMeN lab, Direction Départementale des Services Vétérinaires du Rhône), All experiments were carried out according to the guidelines laid down by the French Ministère de l'Agriculture (n° 87-848) and the E.U. Council Directive for the Care and Use of Laboratory Animals of November 24th, 1986 (86/609/EEC). CD1 Swiss and C57BL/6J mice were purchased from 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 07:00h to 19:00h) with free access to food (13.4 kJ/g, 65% carbohydrates, 11% fat, 24% proteins (w/w), AO3, SAFE, Augy, France) and water.

Moderate chronic kidney disease was induced by 5/6 nephrectomy with a 2-step surgical procedure. Mice were anesthetized with ketamine (60 mg/kg) and xylazine (5 mg/kg). In brief, the upper and lower poles of the right kidney were resected by electro-coagulation. One week later, the left kidney was removed after ligation of the renal blood vessels and the ureter. Special care was taken to avoid damage to the adrenals. The control mice were subjected to a sham operation. All mice were given morphine chlorhydrate (5mg/kg ip, 3-times a day) for 2 days to prevent post surgical pain. Mice were housed by groups of five and food intake was estimated by measuring food weight. $NaHCO_3$ (80mM) was added to the drinking water of CKD mice to prevent metabolic acidosis [6]

PCS treatment

CD1 swiss mice were randomly assigned to receive twice daily (8:00 AM and 6:00 PM) intra-peritoneal injections of PCS (10 mg/kg) or vehicle for control mice, sham mice and CKD mice, for 4 weeks. Food intake and body weight were measured twice weekly. Food consumption was calculated as the difference between the amount given and that removed from the cage.

Prebiotic treatment

Eight week old C57BL/6J mice (30g) underwent a 5/6 nephrectomy as described above and were randomly divided into 2 groups: group 1 (CKD, n=11) was fed with control diet (AO4, SAFE, Augy, France) and group 2 (CKD + preB, n=13) with an

AO4 diet supplemented 5% (w/w) with a prebiotic (Arabino-xylo-oigosaccharide, OptiFlor ® (AXOS), Witaxos, Dury, France) for 4 weeks. At the end of the protocol, animals were anesthetized with sodium pentobarbital (35mg/kg), and blood and tissues were collected as described below.

Metabolic studies

Glucose tolerance test (GTT): After an overnight fast, animals were injected intraperitoneally with 1g/kg body weight of D-glucose in sterile water. Blood glucose was measured before and 15, 30, 60 and 120 min after injection. Blood glucose values were determined from a drop of blood sampled from the terminal portion of the tail, using an automatic glucose monitor (*Accu-Check Performa*, Roche, Meylan, France). Results were expressed as percentage of initial blood glucose concentration. The area under the curve (AUC) of glucose was calculated using GraphPad Prism software.

Insulin tolerance test (ITT): After an overnight fast, animals were injected intraperitoneally with 0.50 UI/kg body weight of recombinant human insulin (Actrapid®, Novo Nordisk). Blood glucose was measured before and 15, 30, 60 and 120 min after injection. The glucose disappearance rate for ITT (Kitt; $\%.min^{-1}$) was calculated using the formula given by Lundbeak. [7] $Kitt = 0.693 \times 100 / t_{1/2}$ where $t_{1/2}$ was calculated from the slope of the plasma glucose concentration, considering an exponential decrement of glucose concentration during the 30 min after insulin administration. [8]

Urine collection

Animals were placed in metabolic cages and after one day of habituation, 24-hour urine was collected and urine volume was determined gravimetrically. Urine protein concentration was measured with Bradford protein assay (BioRad, Marne-la-Coquette, France) using bovine serumalbumin (BSA) as standard.

Sacrifice and tissue dissection

To study insulin signaling in skeletal muscle, mice were injected with insulin (Actrapid® 0.75 UI/kg, ip) or saline solution 60 min after the last administration of

PCS or vehicle. 30 min after insulin injection mice were anesthetized with sodium pentobarbital (35mg/kg ip). The body weight (BW) and body length were measured and Lee index calculated as the cubic root of BW divided by naso-anal length. Blood (750μL) was collected through cardiac puncture in heparinized tubes, and centrifuged 2 min at 3500 g to separate plasma. Plasma samples were snap frozen in liquid nitrogen and stored at -80°C until analysis. Liver, heart, kidneys, gastrocnemius muscle, and epididymal, retroperitoneal and subcutaneous inguinal white adipose tissue (WAT) were dissected according to anatomical landmarks, weighed to the nearest milligram, snap frozen in liquid nitrogen and stored at -80°C.

Renal histology

Kidneys were fixed in 10% formalin, paraffin-embedded, and stained with hematoxylin phloxine saffron using standard histological techniques. The histologic changes were assessed in a blinded fashion by a skilled pathologist .

Biochemical measurements

Free and total PCS were quantified in serum by using reverse-HPLC coupled to a fluorescence detector as previously described. [9-10] The limit of detection (LOD) of the HPLC-fluorescence method used for measurement of serum PCS was 0.008 mg/dl. Many PCS concentrations, especially in baseline conditions, were below the LOD and thus replaced by $LOD^{1/2}$ (i.e. 0.0057 mg/dl) in statistical analyses. The plasma concentration of urea (UREA-Kit S180, bioMérieux, Marcy l'Etoile, France), total cholesterol and triacylglycerols was determined enzymatically, using a cholesterol assay kit RTU (bioMérieux) and triglycerides PAP (bioMérieux) according to manufacturer's recommendations. Creatinine assay was performed by enzymatic method (Roche, Meylan, France). Plasma adiponectin, insulin, TNF-alpha and MCP1 concentrations were determined with enzyme immuno assays (Cayman Chemicals, SpiBio, Montigny le Bretonneux, France) according to the manufacturer's recommendations. All assays were performed at least in duplicate. Muscle (gastrocnemius) and liver lipids were extracted using Chloroform-Methanol (2:1, v/v) [11] and total lipid content was estimated gravimetrically. Plasma malondialdehyde

(MDA) was measured using HPLC with visible detection as described by Grotto et al (2007).[12]

Cellularity study: Measurement of adipocyte size and number

Preparation of adipose tissue for determination of cell size was performed essentially as described by Etherton et al. [13] Briefly, 30-40 mg of epididymal white adipose tissue (WAT) was immediately fixed in osmium tetroxide, incubated at room temperature for 96h and then adipose cell size was determined by a Beckman Coulter Multisizer IV (Beckman Coulter) with a 400 μm aperture. The range of cell sizes that can effectively be measured using this aperture is 20–240 μm. The instrument was set to count 1,000 particles, and the fixed-cell suspension was diluted so that coincident counting was <10%. Cell-size distributions were drawn from measurement of at least 12,000 cell diameters per animal. The mean fat cell volume was measured and the fat cell number was calculated by dividing the tissue lipid content (estimated by lipid extraction) by the mean adipocyte weight (calculated by multiplying the mean adipocyte volume by the triacylglycerol density, namely 0.915). DNA content in epididymal WAT was measured, after delipidation of the samples, by a standard fluorimetric method using bisbenzimide and calf thymus DNA as standard.

Cell cultures

C2C12 myoblasts (*Mus musculus,* reference *CRL-1772 ATCC, LGC Standard,* Molsheim, France*)* were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % heat-inactivated Fetal Bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. When reaching 80% confluence, differentiation was induced by shifting to DMEM supplemented with 2% heat-inactivated Horse serum. Experiments were performed when more than 80% of cells had formed myotubes and after stabilization for 4h in serum-free medium supplemented with 35 g/l of BSA. Mouse 3T3-L1 fibroblasts were obtained from the American Type Culture Collection (reference CL-173). Cells were grown in DMEM and differentiation was induced with insulin (Actrapid®, 5 μM), IMBX (0.5 mM), dexamethasone (25nM) and rosiglitazone (10. μM) for 48 h then rosiglitazone (10. μM) and insulin (Actrapid®, 5 μM) for 48 h. Adipocytes were studied 10-12 days after

differentiation. Cultures were maintained at 37°C in a water-saturated atmosphere containing 5% (v/v) CO_2. The cells were used between the 7th and 15th passage. To test PCS toxicity effect on differentiated C2C12 myotubes, cell toxicity was determined using a 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium (MTT) (Cell Proliferation Kit I, Roche) assay and LDH activity (In Vitro Toxicology Assay Kit, Lactic Dehydrogenase based, Sigma Aldrich).

Insulin stimulation

C2C12 myotubes were incubated in DMEM supplemented with 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin in the presence or absence of PCS 40 μg/ml in bovine serum albumin (BSA, 35 g/L) for 30 min or K_2SO_4 (35 μg/ml) in BSA as control. Cells were stimulated with 100 nM insulin (Actrapid®, 100 nM) for 20 min.

Glucose Uptake Assay.

C2C12 cells grown in 12-well plates were treated with PCS for 30 min. Cells were incubated for 20 min with 100 nM insulin or 20 μM cytochalasin B. Glucose uptake was initiated by the addition of 2-deoxy-D-[^{3}H]-glucose (747 GBq/mmol, Perkin Elmer, Courtaboeuf, France) to a final concentration of 0.1 mM ($1.85.10^4$Bq/ml) for 5 min at 37°C. Uptake was terminated by removal of the assay buffer, followed by three washes in ice-cold PBS. Cells were solubilized with 0.1% SDS, and tritium was detected by liquid scintillation counting. Results were normalized by Bradford protein assay, and non-specific uptake measured in presence of cytochalasin B was subtracted from each determination.

Insulin signaling, gel electrophoresis and western blotting.

C2C12 cells were scraped on ice in Standard Lysis Buffer (SLB: 20mM Tris, 138mM NaCl, 2,7mM KCl, 1mM MgCl2, 5% Glycerol, 1%, NP40 and supplemented extemporaneously with 5mM EDTA, 1mM Na_3VO_4, 20mM NaF, 1mM DTT and a Protease inhibitor cocktail) and centrifuged (13,000 ×g, 15 min, 4°C). Gastrocnemius muscles were homogenized in SLB. Protein concentration of cell supernatants was determined with Bradford protein assay. Forty micrograms of protein lysate from

skeletal muscle or twenty micrograms of protein lysate from C2C12 cells were separated in SDS-polyacrylamide gels (10 %) and transferred onto a nitrocellulose membrane (Hybond-ECL TM, GE healthcare, Meylan, France). For, immunoprecipitation, the supernatant (800 μg protein) was incubated with 4 μg anti–IRS-1 antibody, for 30 minutes and then with Protein A magnetic beads (Millipore, Billerica, USA), overnight at 4°C under agitation. The beads were rinsed three times in SLB. After heat-denaturation (95° C, 10 min), the supernatant was loaded on an SDS-polyacrylamide gel. After transfer, the membranes were blocked with 5 % BSA in TBS-Tween for 2 h. Blots were probed overnight with specific primary antibodies at 4°C followed by 1-h incubation at room temperature with secondary antibodies. Protein bands were detected with the enhanced chemiluminescence substrate kit (Supersignal ® West Pico, Perbio, Brebières, France) using a camera Image Master VDS-CL (Amersham Pharmacia, Orsay, France) and quantified by Quantity One (Biorad, Marne-la-Coquette, France) and open-source Image-J (http://rsbweb.nih.gov/ij/index.html) softwares. Protein phosphorylation levels were normalized to the matching densitometric values of non-phosphorylated proteins (except for ERK and JNK which were normalized to tubulin concentration). Results were expressed as a percentage of control values.

MAPK/ERK Kinase (MEK)1/2 and Jun Kinase Inhibition studies

Cells were pretreated with ERK1/2 inhibitor U0126 (10 μM) or JUN Kinase inhibitor SP600125 (10 μM) for one hour prior to the incubation with PCS.

PCS transmembrane transport inhibition studies

Cells were pretreated with Probenecid (1 mM), a potent inhibitor of organic anion transporter (OAT) for one hour prior to the incubation with PCS.

Measurement of ROS production in C2C12 myotubes

The cells were incubated with the fluorescent probe 5-6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (10 μM) (Carboxy-H2 DCFDA, Invitrogen) for 30 min in the dark at 37 °C. After washing with phosphate buffered saline (PBS), cells were treated in a serum-free medium with 35 g/l of BSA and PCS (40μg/ml) in Krebs-

Ringer at 37 ºC or K_2SO_4 (35μg/ml) as a negative control. After 30 min, 1 h or 2 hours, cells were lysed with 2 ml of ultrapure water per well. The fluorescence was measured with a spectrofluorimeter (Photon Technology International, Bioritech, Chamarande, France), with a wavelength of 495 nm for excitation and 529 nm for emission. Results were normalized to the amount of protein in lysates, assayed by the Bradford method (Biorad). MDA was measured in C2C12 myotube lysate as an index of oxidative damages using HPLC with visible detection.[12] MDA concentrations were normalized to the amount of protein in lysates, assayed by the Bradford method.

Lipolysis and lipogenesis assay in 3T3-L1 adipocytes

3T3-L1 adipocytes grown in six-well dishes were incubated for 1h with PCS and subsequently for 3 hours with or without 10 μM isoproterenol hydrochloride. At the end of the incubation, the medium was collected and glycerol was assayed colorimetrically (Glycerol assay, R-biopharm, Saint Didier, France). Lipogenesis was measured as the incorporation of [^{14}C]-acetate into total neutral lipids in presence or absence of PCS (40 μg/ml). 3T3-L1 cells were incubated in DMEM medium containing 1% fatty acid-free BSA and 1 μCi of [^{14}C]-acetate (2.079 GBq/mmol, Perkin Elmer) for 4 h at 37°C. Total neutral lipids were extracted using Dole's extraction fluid (isopropanol/heptane/H_2SO_4 1N, 40/10/1, v/v/v) and [^{14}C] was detected by liquid scintillation counting. Lipolysis and lipogenesis data were normalized by protein concentration estimated using Bradford assay.

Adipose tissue collection

Human adipose tissue was obtained from an ongoing study approved by the Ethical Committee (CPP Lyon Sud-Est IV) of Lyon University Hospital (MODAIR study, ref D-09-17). Subcutaneous abdominal adipose tissue (1–2 g) was collected from 4 non obese men undergoing elective urologic surgery (radical prostatectomy). Their mean age was 66.3 ± 4.2 years and their mean body mass index was 24.7 ± 1.5 kg/m^2. All subjects gave written informed consent to the study and all procedures were in accordance with the principles of the Declaration of Helsinki.

Isolation of human adipocytes

Adipose tissue was weighed, minced and digested in 10 ml of Krebs Ringer Bicarbonate (KRB, pH 7.40) buffer containing 6 mM glucose, 1% (w/v) fatty acid-free bovine serum albumin (BSA) and 1.5 mg/ml of collagenase (type II, C6885, Sigma Aldrich). The vial was shaken (40 cycles.min^{-1}) at 37°C for 60 min. The resulting cell suspension was filtered through a nylon mesh (250 μm) and washed three times with KRB buffer containing 4% fatty acid-free BSA. Then adipocytes were resuspended in KRB containing 4% fatty acid-free BSA and counted in a hemacytometer.

Lipogenesis assay

Lipogenesis was measured in human isolated adipocytes as the incorporation of [^{14}C]-acetate into total neutral lipids in presence or absence of PCS (40 μg/ml). Briefly, 100,000 adipocytes were incubated for 4h at 37°C with gentle shaking (30 cycles.min^{-1}) in KRB buffer containing 1% fatty acid-free bovine serum albumin and 1 μCi of [^{14}C]-acetate. Incubations were run in triplicate and the results are the mean of four independent experiments. Total lipids were extracted using Dole's extraction fluid and [^{14}C] was detected by liquid scintillation counting. The results were expressed as percent of the control untreated adipocytes.

Statistics.

Data are expressed as means ± SEM. All data were analyzed using GraphPad Prism v5.0 software (GraphPad software, La Jolla, USA). Multiple comparisons were performed using ANOVA followed when appropriate by posthoc Fisher PLSD tests. Results of GTT and ITT tests were compared by two-way analysis of variance (time, treatment). Simple comparisons were performed using Student's *t*-test. When appropriate, Welch's correction for inequality of variances was applied. Adipocyte volume distribution curves were compared with the Kolmogorov-Smirnov two-sample test using "R" software (www.R-project.org). Differences were considered significant at the $P<0.05$ level.

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Supplementary Figure legends

Supplementary Figure 1: Chemical structure of p-cresol (A) and p-cresyl-sulfate (B).

Supplementary Figure 2: Renal ultrastructure in PCS and saline treated mice. A standard hematoxylin phloxine saffron staining was performed on kidney tissue sections. Note that no difference was observed between saline and PCS treated mice. Original magnification, x 200.

Supplementary Figure 3: Acute administration of PCS decreases insulin sensitivity in mice.

Mice were given a single injection of PCS (10mg.kg^{-1}ip) and insulin sensitivity was evaluated through insulin tolerance test and the measurement of insulin induced PKB/Akt phosphorylation in gastrocnemius muscle. **(A,B)** Administration of PCS (10 mg/kg) one hour before administration of insulin (0.5 UI/kg) significantly decreased the hypoglyceamic activity of insulin. Baseline glucose concentrations were 80 ± 9, 89 ± 5 and 91 ± 8 mg/dl for saline, PCS-1h and PCS-4h mice, respectively. Note that this effect was transient since 4 hour after PCS administration, insulin sensitivity did not differ anymore from saline treated mice. **(C)** Administration of PCS (10 mg kg^{-1}ip) one hour before administration of insulin (0.75 UI/kg) impaired insulin-induced phosphorylation of PKB/Akt in gastrocnemius muscle.

Supplementary Figure 4: p-cresyl sulfate, from 10 mg /ml to 80 mg/ml, interfere with insulin signaling pathways in C2C12 myotubes

C2C12 myotubes were incubated with PCS for 30 min and stimulated by 100 nM insulin for 20 min. The insulin signaling pathway was explored by Western Blotting through serine 473 phosphorylation of PKB/Akt. Data are mean ± SEM for n=3-5. Different letters indicate a difference significant at $p < 0.05$.

Supplementary Figure 5: PCS effects on C2C12 myotubes are not mediated by an increase in intracellular ROS production

Data are mean ± SEM for n=3-4 independent experiments. **(A)** intracellular ROS production: After incubation in Krebs buffer during 30 min, 10 μM of CM-H2DCFDA was added to the medium for another 30 min. Then cells were washed and treated with 50 μM 4-HNE for 30 min. Cells were incubated with H2O2 as a positive control. After lysis in water, fluorescence was read using a fluorimeter. **(B)** Cell malondialdehyde (MDA) content. All results were normalized to the protein concentration.

Supplementary Tables

Supplementary Table 1.
Serum concentration of PCS in mice chronically administered with PCS (10mg.kg^{-1}, twice a day) for 4 weeks

Treatment	Mouse	PCS, mg/dl	
		Total	Free
Saline	S1	0.012	<LOD
	S2	<LOD	<LOD
	S3	<LOD	<LOD
	S4	<LOD	<LOD
PCS (10mg/kg, 4wk)	P1	0.065	0.009
	P2	0.019	<LOD
	P3	<LOD	<LOD
	P4	<LOD	<LOD

Limit of detection (LOD) for PCS was 0.008 mg/dl

Supplementary Table 2
In vivo binding of PCS to plasma proteins in mouse

Mouse	Free PCS	Total PCS	Protein-bound PCS	% binding
1	0.153	1.411	1.258	87.8%
2	0.051	1.020	0.969	94.7%
3	0.052	1.392	1.340	96.1%
4	0.108	1.618	1.510	92.8%
5	0.098	1.591	1.493	93.4%
6	0.011	0.305	0.294	96.3%
7	0.017	0.459	0.442	96.2%
8	0.012	0.316	0.304	96.1%
9	0.013	0.318	0.305	95.7%
10	0.465	3.765	3.300	85.9%
11	0.429	3.800	3.371	87.3%
12	0.889	5.386	4.497	80.2%
13	1.495	7.117	5.622	73.4%
Mean				**90.5%**
SD				**7.2%**
SEM				**2.0%**

Note : The HPLC measurement allowed quantification of both total PCS and free PCS in mouse sera. Protein bound PCS was calculated as (total PCS – free PCS) and the percentage of protein binding as (total PCS – free PCS)/total PCS x 100. In mice, the in vivo plasma protein binding of PCS was ***90.5 ± 2.0%*** *(from n=13 mice). These data are in good agreement with the works par Meijers et al (2009) and Watanabe et al (2012). Indeed, Watanabe et al (2012) estimated in vitro the binding percentage of PCS to human serumalbumine (under conditions chosen to mimic the CKD stage 4/5) to be 90%, which corresponds to the percentage (91.4%) found in the serum of CKD patients (stage 5) previously reported by Meijers et al. (2009).*

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Supplementary Table 3:

Effect of treatment with PCS on C2C12 myotube viability

PCS (μg/ml)	**10**	**20**	**40**	**80**
Cell viability[‡] (% control)	95.7 ± 1.7	94.7 ± 2.8	102.9 ± 1.4	101.2 ± 1.1
LDH activity[£] (% control)	98 ± 1.7	98.6 ± 2	90.1 ± 2.3	99.4 ± 2.5

C2C12 muscle cells were treated for 16 hours with increasing concentrations of PCS in serum-free condition. [‡] *cell viability was measured by a MTT assay as described in methods.* [£]*Level of extracellular lactate dehydrogenase (LDH) activity. Data are means ± SEM from 5-6 independent experiments Data are expressed as percent of the control.*

Supplementary Table 4 :

Diet composition

	Control diet	Prebiotic diet
Ingredient (g/100g)		
AXOS (Opti'Flor, Witaxos)	-	5
A04 diet (SAFE)	100	95
Nutrient composition (g/100g)		
Carbohydrates	47.7	45.3
Proteins	16	15.2
Lipids	3.0	2.9
Energy (kJ/g)	11.8	11.2
From (%)		
Carbohydrates	67.7	67.7
Proteins	22.7	22.7
Lipids	9.6	9.6

Mean composition of the control and prebiotic diets. Data were obtained from SAFE (Augy, France). Abbreviation: AXOS, arabinoxylan oligosaccharides.

Supplementary Table 5

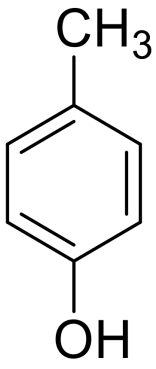
Biometry and organ weights in CKD mice fed with standard or prebiotic diet

	Nx5/6		
Diet	**Standard**	**Prebiotic (5% w/w)**	**p value**
Biometry			
Total energy intake,kJ	164.4 ± 0,02	163.7 ± 0.78	0.45
Body weight, g	24.5 ± 0.6	25.2± 0.8	0.57
Body length, cm	9.4 ± 0.1	9.4± 0.1	0.73
Lee index, $x10^3$	308.1 ± 2.5	311.8± 3.6	0.46
Organ weights			
Liver, mg/10g BW	449.5 ± 35.8	448.7± 13.8	0.98
Heart, mg/10g BW	59.2 ± 3.8	56.6± 3.4	0.63
Kidney, mg/10g BW	58.8 ± 2.8	56.6± 2.2	0.54
Gastrocnemius,mg/10g BW	58.4 ± 3.2	56.9± 2.7	0.94
Tibialis, mg/10g BW	23.7 ± 2.0	23.5± 2.0	0.74
WAT weights			
eWAT, mg/10g BW	70.4 ± 6.9	82.1± 6.0	0.24
scWAT, mg/10g BW	41.8 ± 6.2	54.9± 2.8	0.06
rWAT, mg/10g BW	8.0 ± 1.9	14.8± 1.3	0.01**
total WAT, 10g BW	129.3 ± 13.0	172.4± 9.9	0.02*

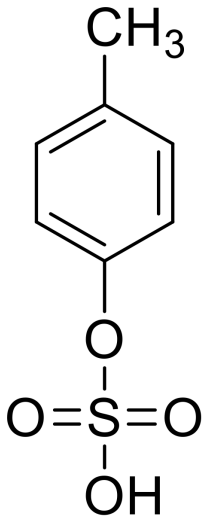
Data are mean ± SEM for n=8-9. Lee index was calculated as the cubic square of BW divided by naso-anal length. Abbreviations: BW, body weight, WAT, white adipose tissue, eWAT, epididymal WAT, scWAT, subcutaneous WAT, rWAT, retroperitoneal WAT. Differences were considered significant at P<0.05.

Supplementary Figure 1

A



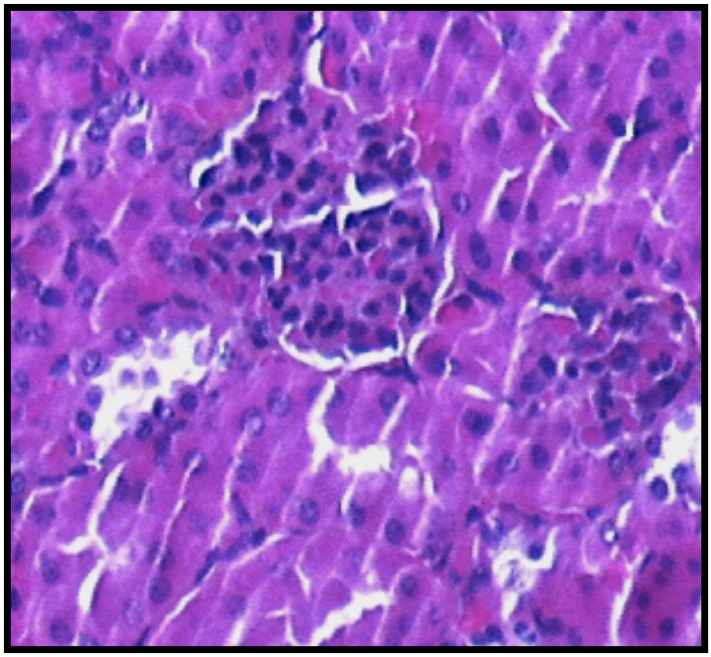
B

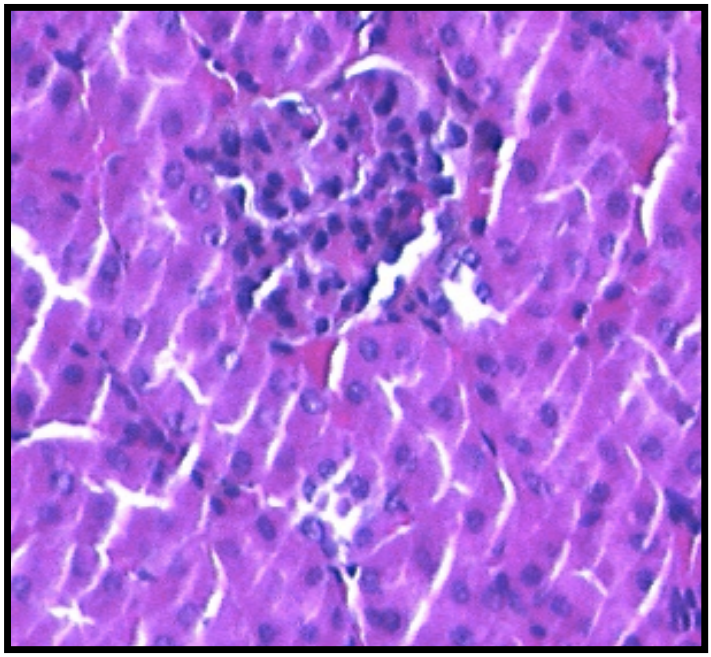


Supplementary Figure 2

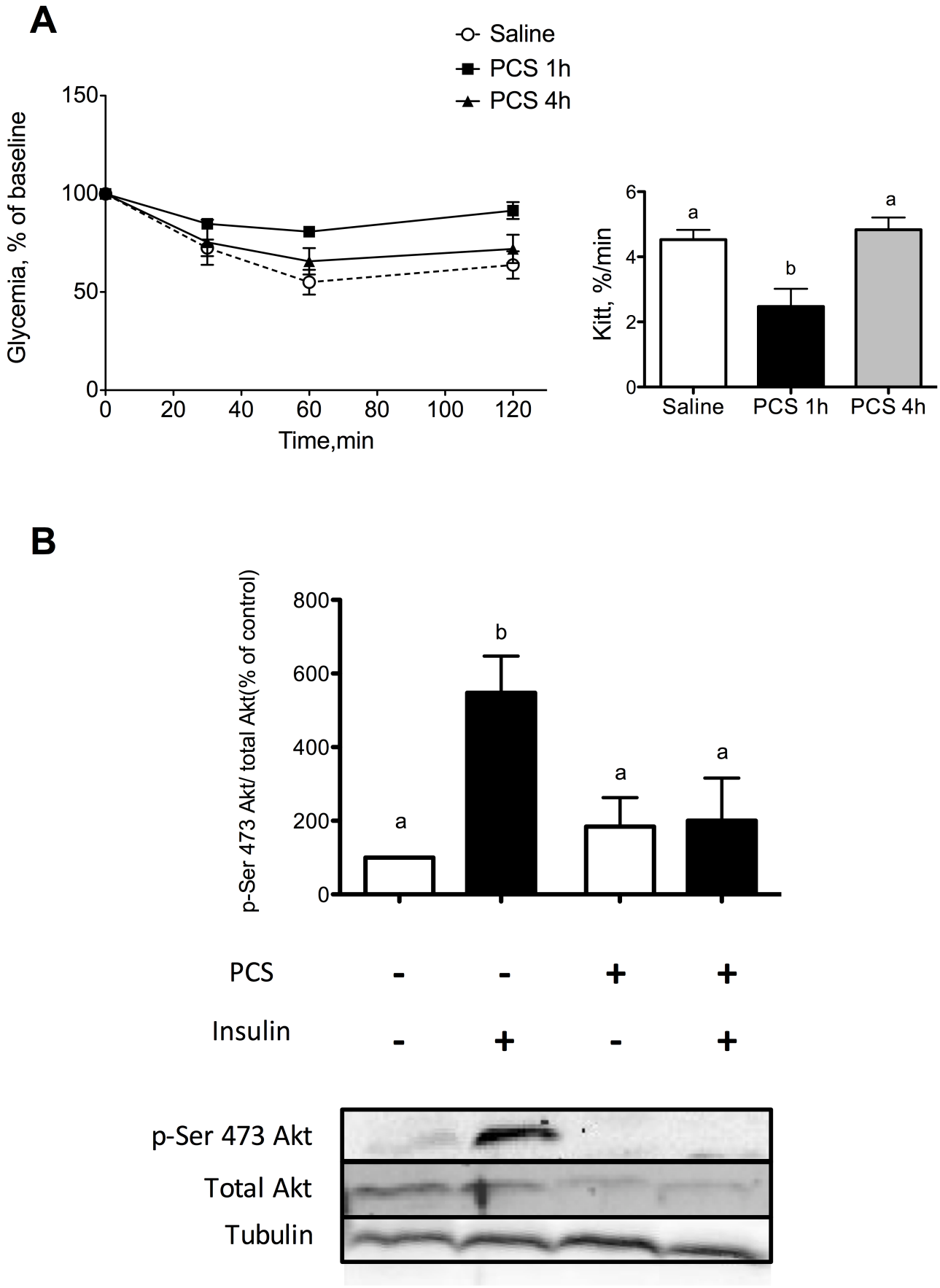
Saline

PCS 10 mg/kg

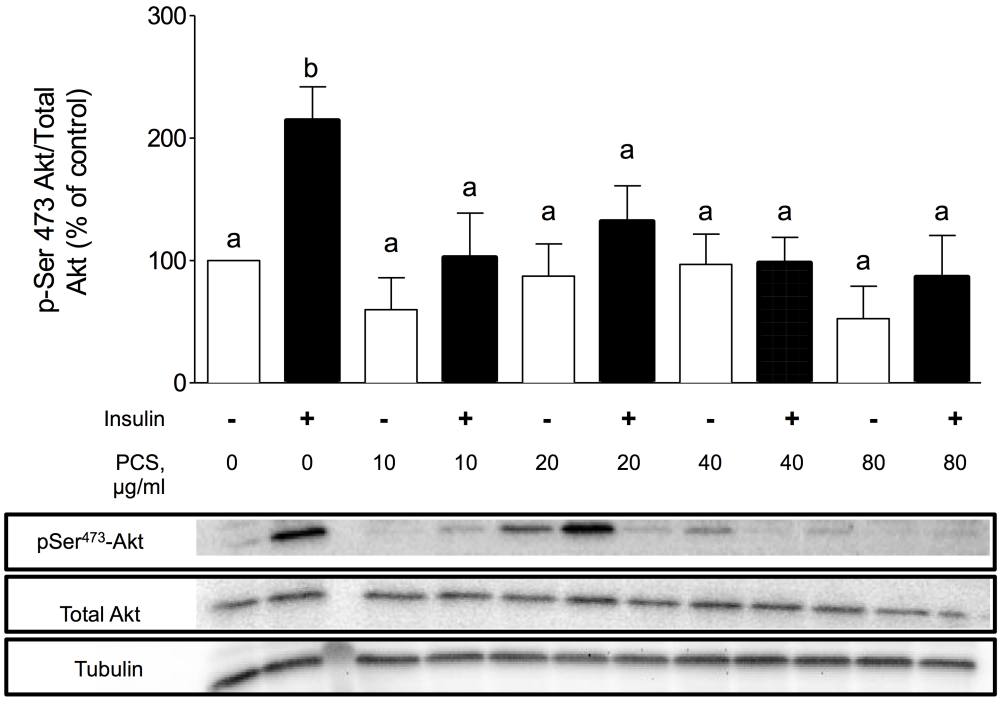




Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5