

Syndrome urémique et complications de la Maladie Rénale Chronique: sur la piste des toxines urémiques...

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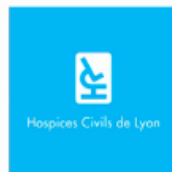
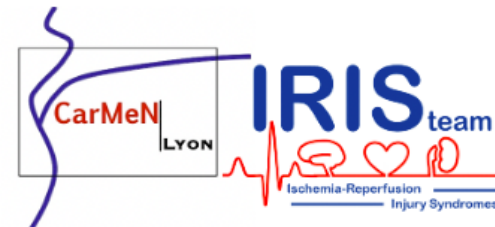
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Inserm

Institut national
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Université Claude Bernard Lyon 1



Au programme:

I- Maladie rénale et insuffisance rénale chronique

II- Syndrome urémique et toxines urémiques

III- L'intestin et le microbiote intestinal: acteurs centraux dans la production de toxines urémiques

IV- Exemples de toxicité des toxines urémiques liées aux protéines

V- Quelle(s) stratégie(s) thérapeutique(s) pour limiter la toxicité urémique ?

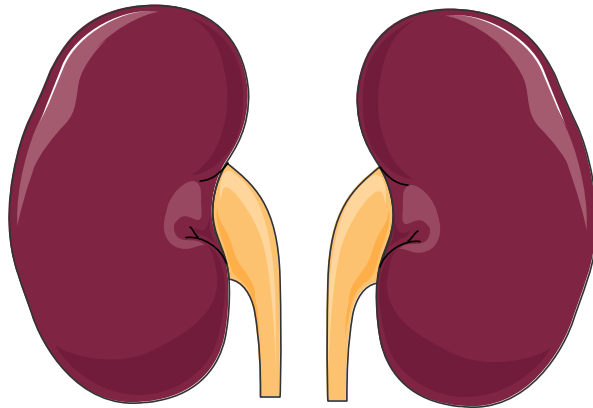
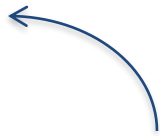
A quoi servent nos reins ?

Sécrétions endocrines

1,25OH D3

EPO

Rénine

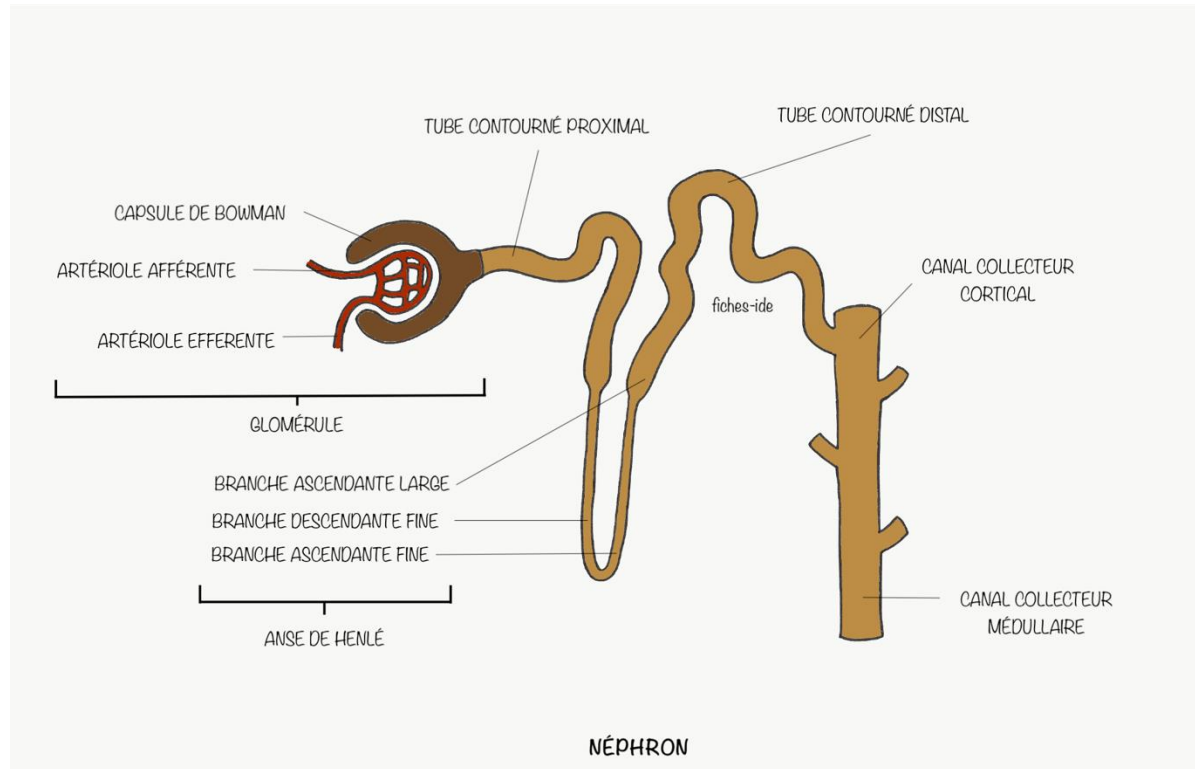


Urine
Eau
Electrolytes
Déchets +++

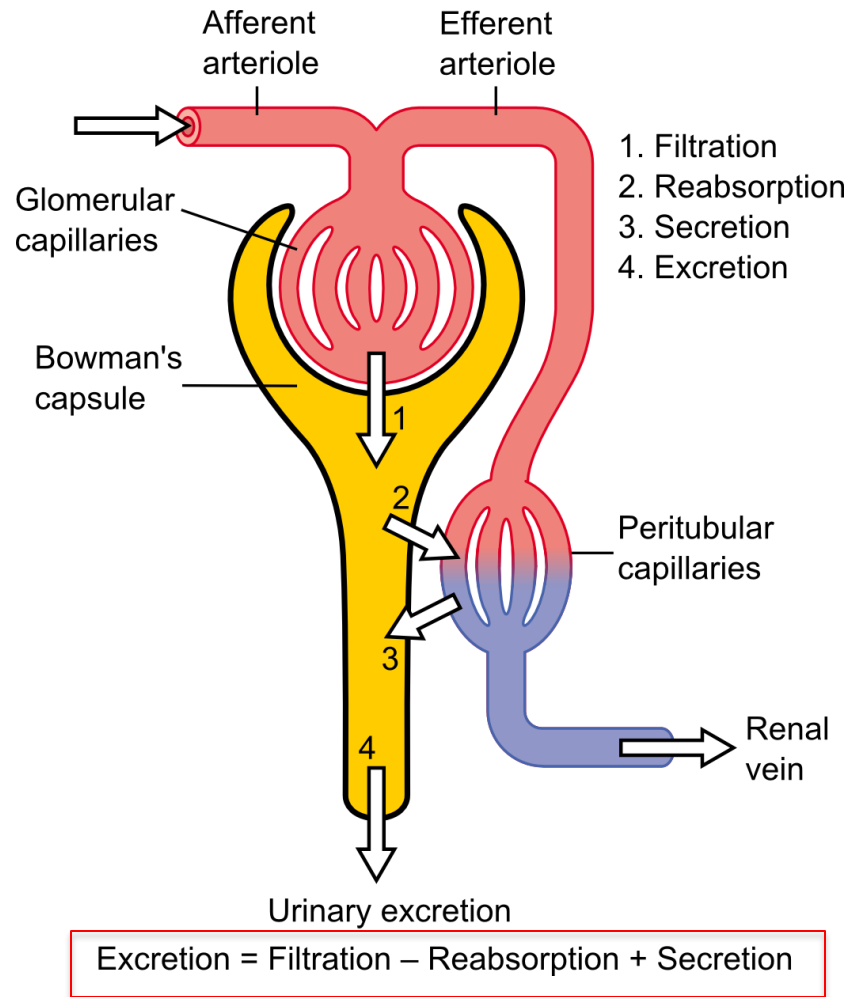
- Nos reins **filtrent** en permanence notre sang pour éliminer **les déchets non volatils** issus du fonctionnement de notre organisme (urée, acide urique, créatinine, oxalate,...). Excrétion des toxines et métabolites (médicaments).
- Régulation de la balance hydro-électrolytique (volume & composition) et de l'équilibre acido-basique
- Régulation de la pression artérielle (Rénine, volémie)
- Stimulation de l'érythropoïèse (sécrétion d'EPO)
- Métabolisme phospho-calcique (production de calcitriol= vit D active)

L'unité fonctionnelle rénale: le néphron

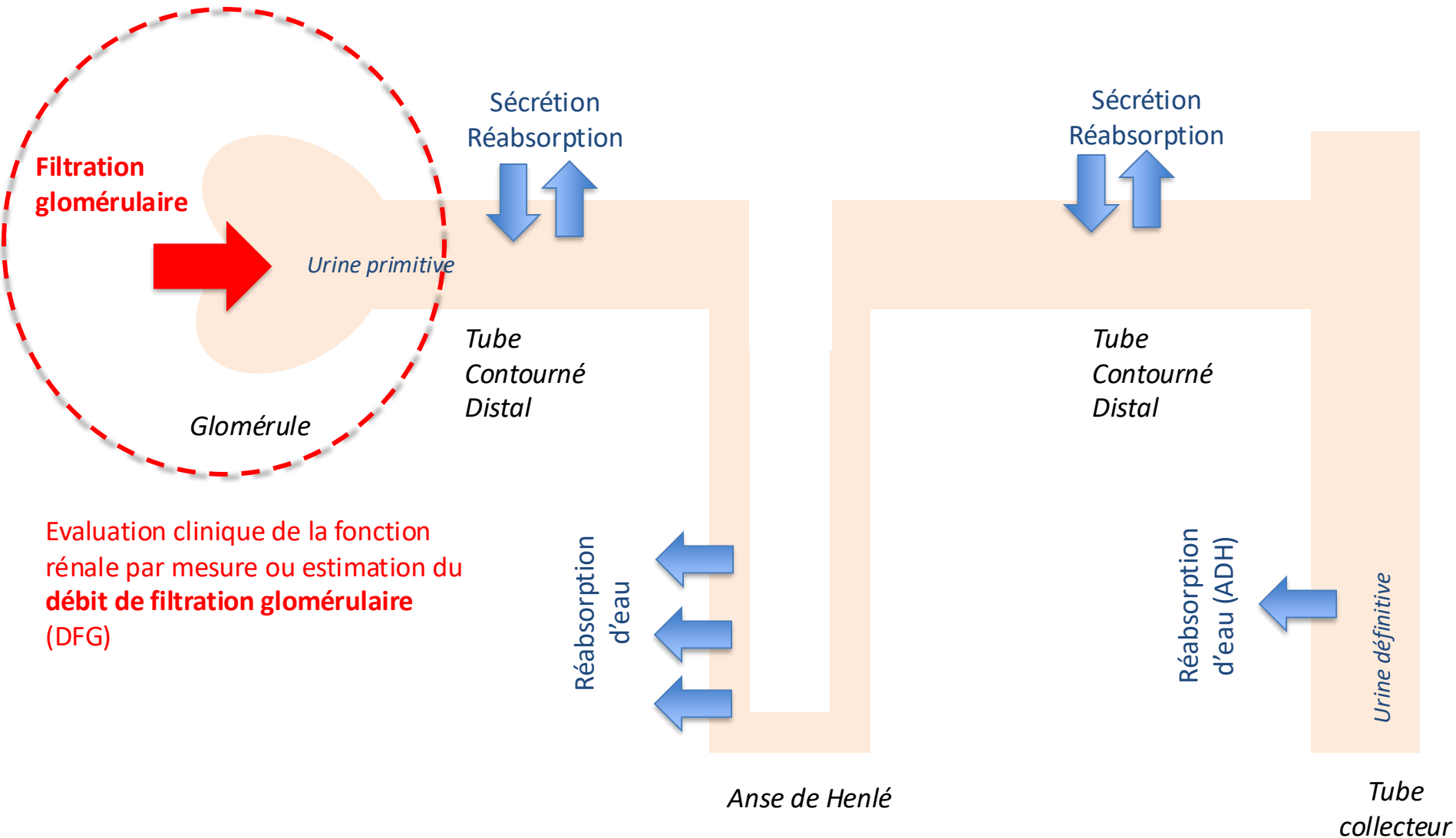
Environ 10^6 néphrons / reins



Les processus de production de l'urine



En pratique, comment évaluer la fonction rénale ?



Mesure du débit de filtration glomérulaire

= mesure de la clairance d'une substance

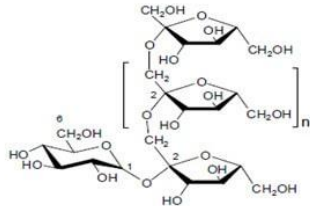
Clairance = **Volume de plasma** complètement épuré d'une substance par unité de temps

La substance choisie doit idéalement répondre à ces 5 critères:

- Pas de liaison aux protéines plasmatiques
- Filtrée mais ni réabsorbée ni sécrétée au niveau tubulaire
- Pas de métabolisme ou synthèse tubulaire
- Pas d'effet sur la filtration glomérulaire
- Non toxique

Mesure du débit de filtration glomérulaire

= mesure de la clairance de l'inuline (« gold standard »)

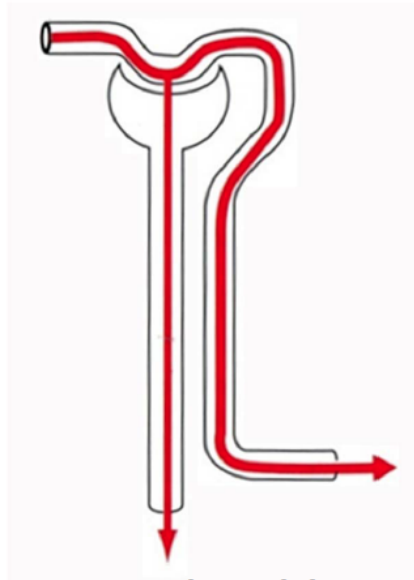


Inuline (MW: 6179 g/mol) librement filtrée, ni réabsorbée ni sécrétée

Quantité filtrée

$$1 \text{ mg/mL} \times 125 \text{ mL} = 125 \text{ mg d'inuline (dans 125 ml)}$$

(P_{inuline}) (FG)



Quantité excrétée

$$125 \text{ mg/mL} \times 1 \text{ mL} = 125 \text{ mg d'inuline (dans 1 ml)}$$

(U_{inuline}) (V)

quantité filtrée = quantité excrétée

Clairance (ml/min) = Débit de filtration glomérulaire

$$\text{Filtration glomérulaire} = \frac{U_{\text{inuline}} \times V}{P_{\text{inuline}}}$$

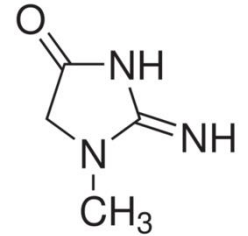
$$\text{Débit de filtration glomérulaire ou Clairance de l'inuline} = \frac{U_{\text{inuline}} \times DU}{P_{\text{inuline}}}$$

U : concentration urinaire
 P : concentration plasmatique
 DU : débit urinaire (ml/min)

Mesure du débit de filtration glomérulaire

Mesure de la clairance de la créatinine

- **Créatinine** = déchet du métabolisme musculaire
- Production et concentration assez stable d'un jour à l'autre
- Librement filtrée (MW:113 g/mol), légère sécrétion tubulaire
- Bémol: dépend de la masse musculaire (\pm activité physique \pm niveau d'hydratation)
- Permet une **estimation fiable** du DFG en clinique: 85-125mL/min chez l'adulte
- Nécessite dosage plasmatique + collecte de la diurèse des 24h (débit urinaire)



$$\begin{array}{l} \text{Débit de filtration glomérulaire} \\ \text{ou} \\ \text{Clairance de la créatinine} \end{array} = \frac{[\text{Créatinine}]_{\text{urinaire}} \times \text{Volume urine}_{24\text{h}}}{[\text{Créatinine}]_{\text{plasmatique}}}$$

Estimation de la clairance de la créatinine par la formule de Cockroft & Gault



Permet l'estimation de la clairance de la créatinine à partir de son seul dosage sanguin:

- $P_{\text{créatinine}}$ mesurée sur un échantillon de plasma
- $U_{\text{créatinine}} \times \text{Diurèse 24h}$ A l'équilibre, débit d'extraction urinaire de la créatinine = débit d'apport plasmatique qui dépend de sa production musculaire, elle-même fonction du **sexe, de l'âge et du poids**.

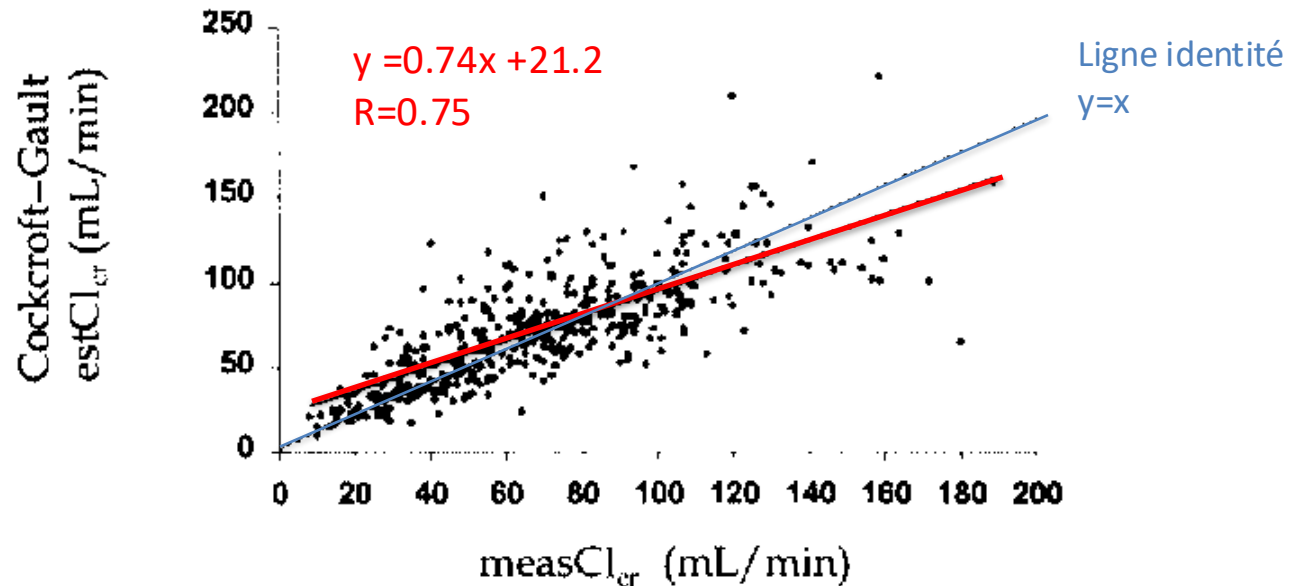
La formule de Cockroft & Gault estime $U_{\text{créatinine}} \times \text{Diurèse 24h}$ à partir de ces 3 paramètres

$$\begin{array}{l} \text{Débit de filtration glomérulaire} \\ \text{ou} \\ \text{Clairance de la créatinine} \end{array} = \frac{K \times \text{poids} \times (140 - \text{âge})}{P_{\text{créatinine}}}$$

où $K = 1,23$ chez l'homme et $1,04$ chez la femme

Clairance mesurée vs clairance estimée par la formule de Cockroft & Gault

Measured vs. Estimated Cl_{cr} *Entire Group (n = 420)*



Bonne corrélation pour la majorité des individus....

Autres formules disponibles: - MDRD

- CKD-EPI

The new CKD-EPI equation

ARTICLE

Annals of Internal Medicine

A New Equation to Estimate Glomerular Filtration Rate

Andrew S. Levey, MD; Lesley A. Stevens, MD, MS; Christopher H. Schmid, PhD; Yaping (Lucy) Zhang, MS; Alejandro F. Castro III, MPH; Harold I. Feldman, MD, MSCE; John W. Kusek, PhD; Paul Eggers, PhD; Frederick Van Lente, PhD; Tom Greene, PhD; and Josef Coresh, MD, PhD, MHS, for the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration)*

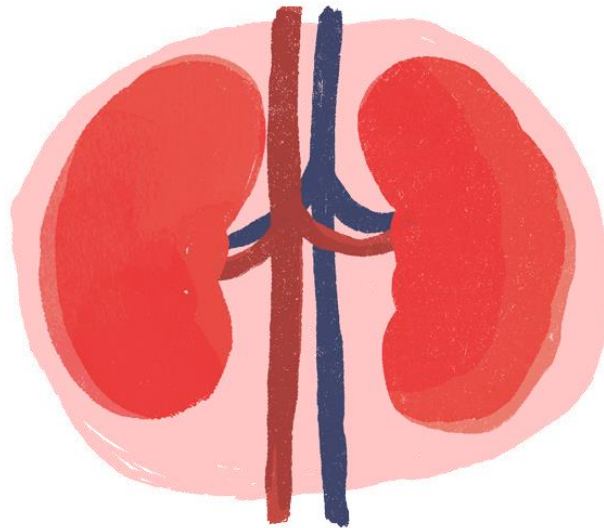
Ann Intern Med. 2009;150:604-612.

Table 2. The CKD-EPI Equation for Estimating GFR on the Natural Scale*

- Development dataset: n=5504
- Internal validation: n=2750
- External validation: n=3896
- Median GFR in the development = 68 mL/min/1.73 m²

Race and Sex	Serum Creatinine Level, $\mu\text{mol/L}$ (mg/dL)	Equation
Black		
Female	≤ 62 (≤ 0.7)	$\text{GFR} = 166 \times (\text{Scr}/0.7)^{-0.329} \times (0.993)^{\text{Age}}$
	> 62 (> 0.7)	$\text{GFR} = 166 \times (\text{Scr}/0.7)^{-1.209} \times (0.993)^{\text{Age}}$
Male	≤ 80 (≤ 0.9)	$\text{GFR} = 163 \times (\text{Scr}/0.9)^{-0.411} \times (0.993)^{\text{Age}}$
	> 80 (> 0.9)	$\text{GFR} = 163 \times (\text{Scr}/0.9)^{-1.209} \times (0.993)^{\text{Age}}$
White or other		
Female	≤ 62 (≤ 0.7)	$\text{GFR} = 144 \times (\text{Scr}/0.7)^{-0.329} \times (0.993)^{\text{Age}}$
	> 62 (> 0.7)	$\text{GFR} = 144 \times (\text{Scr}/0.7)^{-1.209} \times (0.993)^{\text{Age}}$
Male	≤ 80 (≤ 0.9)	$\text{GFR} = 141 \times (\text{Scr}/0.9)^{-0.411} \times (0.993)^{\text{Age}}$
	> 80 (> 0.9)	$\text{GFR} = 141 \times (\text{Scr}/0.9)^{-1.209} \times (0.993)^{\text{Age}}$

L'insuffisance rénale chronique



Insuffisance rénale aigüe

vs

Insuffisance rénale chronique (> 3 mois)

La maladie rénale chronique

Définition clinique:

Débit de Filtration Glomérulaire (DFG) < 60 mL/min/1.73m² pendant plus de 3 mois

Stades	Description	DFG (mL/min/1.73m ²)
1	Maladie rénale* avec DFG normal ou augmenté	≥ 90
2	Maladie rénale* avec faible baisse du DFG	60-89
3	Baisse modérée du DFG	30-59
4	Baisse sévère du DFG	15-29
5	Insuffisance rénale terminale	< 15

**Insuffisance
rénale**

* *Maladie rénale : anomalie morphologique ou histologique ou marqueur biologique d'atteinte rénale (sédiment urinaire, atteinte tubulaire) depuis > 3 mois*

La maladie rénale chronique

Définition clinique:

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* *Maladie rénale : anomalie morphologique ou histologique ou marqueur biologique d'atteinte rénale (sédiment urinaire, atteinte tubulaire) depuis > 3 mois*

Mise en place des stratégies de suppléance rénale

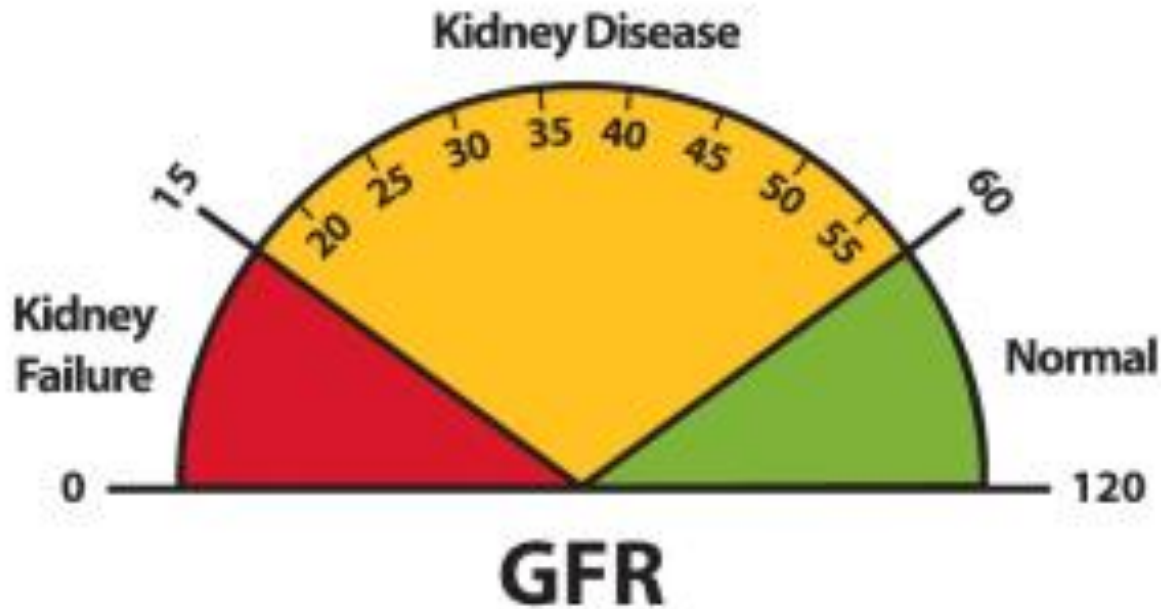
La maladie rénale chronique

Insuffisance rénale

Insuffisance
rénale
terminale



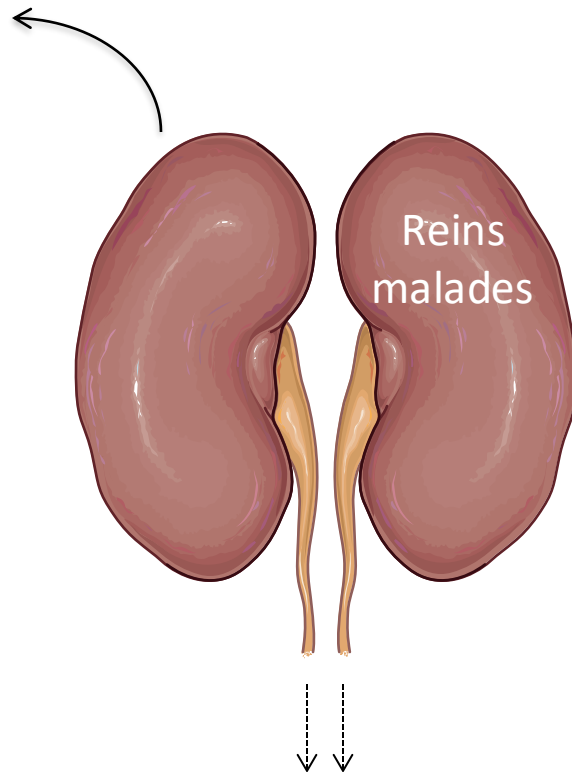
Suppléance
rénale



Que se passe t'il quand les reins sont malades?

L'insuffisance rénale chronique résulte de la **destruction progressive et irréversible des reins** et **l'altération de toutes leurs fonctions**.

Défaut de sécrétion



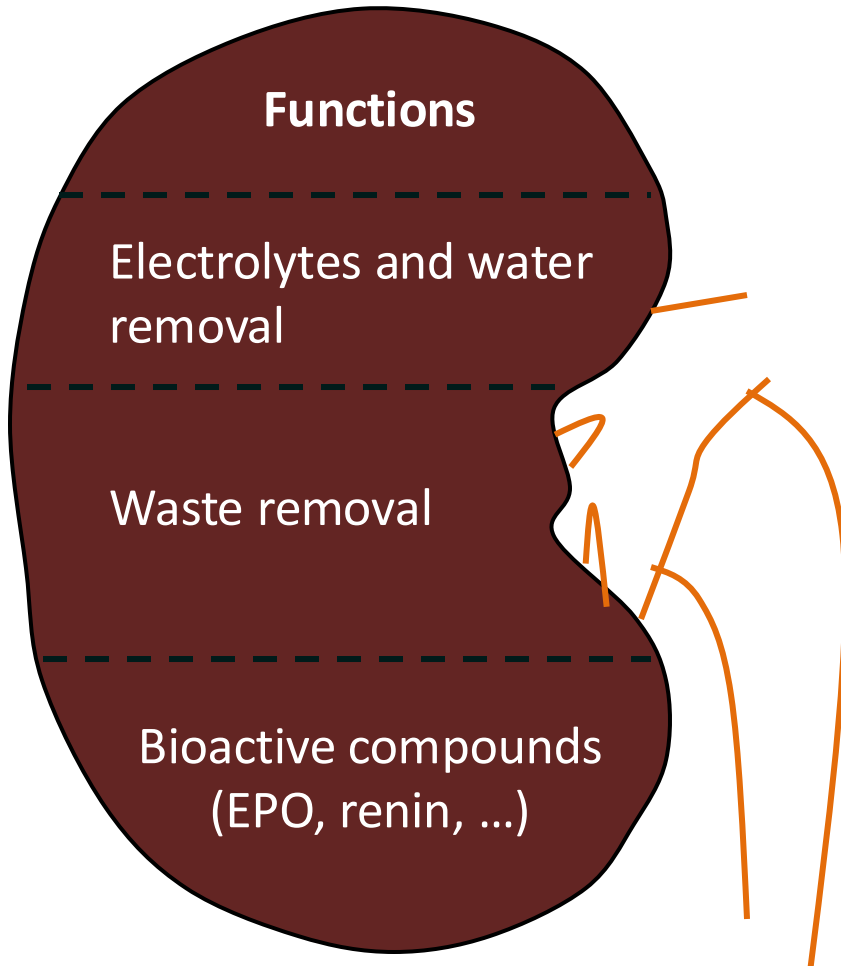
Plus d'urine !!!
(anurie/oligurie)

Quand les reins ne fonctionnent plus...

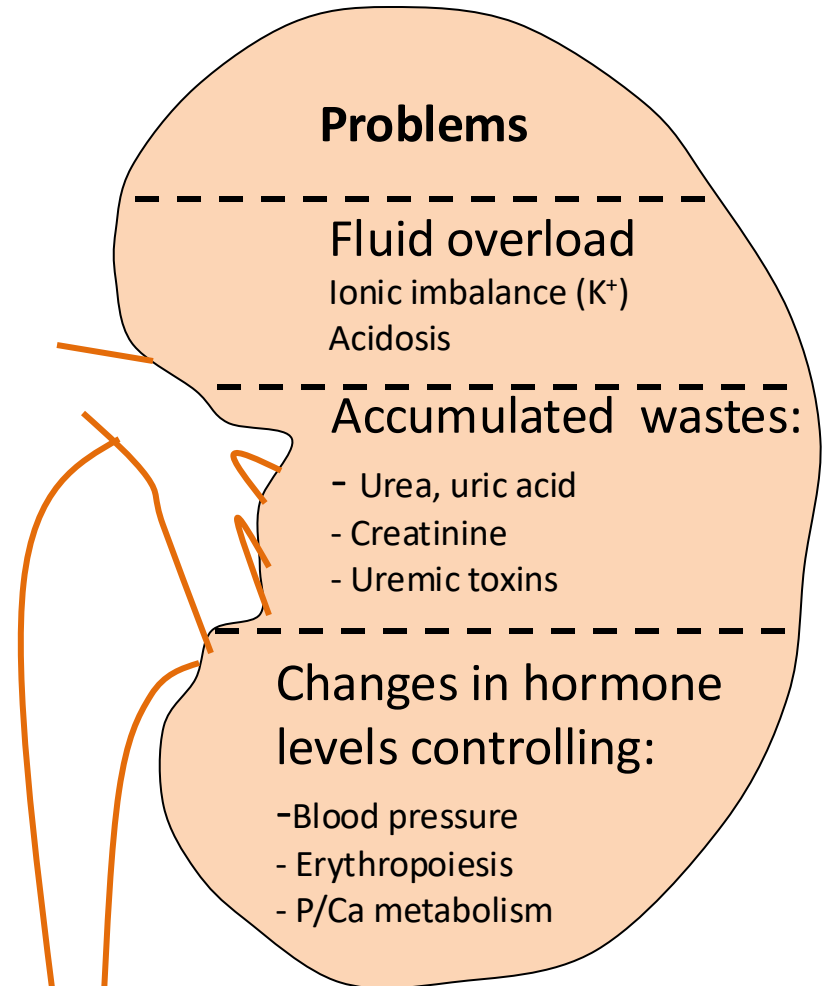
- Déséquilibre hydro-électrolytiques et acido-basique (acidose)
- Anémie
- Troubles du métabolisme phospho-calcique
- Dérégulation pression artérielle
- les déchets s'accumulent et l'organisme s'intoxique...

Au stades finaux de la maladie, cela se solde par **la mort du patient** si aucun traitement de suppléance rénale n'est appliqué.

Healthy kidney



Chronic Kidney Disease



Le « givre » urémique



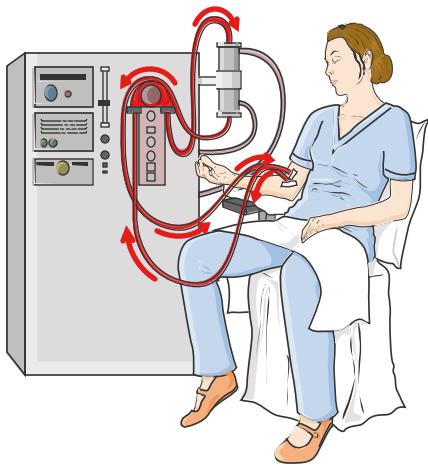
- Dépôts cristallins blanchâtres au niveau de la peau
- Signe d'une importante hyperazotémie
- Accumulation de **l'urée et d'autres déchets azotés** dans la sueur
- Cristallisation par évaporation
- Il était fréquent de faire cette observation avant l'utilisation généralisée des techniques de dialyse.
- Rarement observé dans les pays développés...

Le « *Givre Urémique* » peut être vu dans les stades avancés d'insuffisance rénale chronique
=> Traduit **l'accumulation dans les fluides biologiques de déchets azotés**

Que faire quand les reins ne fonctionnent plus du tout ?

Les techniques de dialyse

Hémodialyse



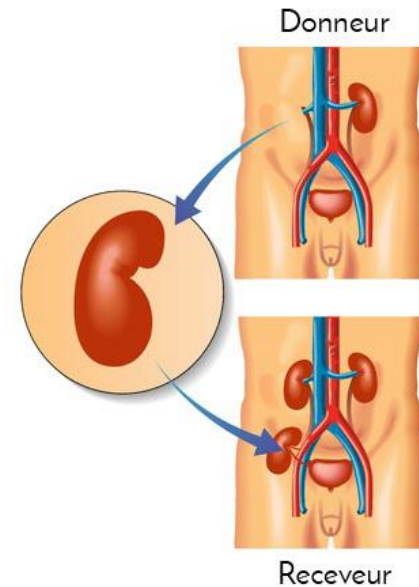
Coût 89 000 €/an

Dialyse péritonéale



Coût 64 000 €/an

La transplantation rénale



Coût 86000 € +20 000€/an

L'insuffisance rénale terminale en France

5-10% de la population souffrirait d'une maladie rénale pouvant conduire à un insuffisance rénale



89692 patients traités pour insuffisance rénale terminale
Prévalence : 1328 pmh



55%

49271 patients traités en dialyse
Dont 2956 en dialyse péritonéale (6%)
Prévalence : 729 pmh
Age médian: 71 ans

45%



40421 patients porteur d'un greffon rénal
Dont 12% issus d'un donneur vivant
Prévalence : 598 pmh
Age médian: 58 ans

Source : Rapport 2018 du réseau REIN (Réseau Epidémiologique et information en Néphrologie)
https://www.agence-biomedecine.fr/IMG/pdf/rapport_rein_2018_v2.pdf

L'insuffisance rénale terminale en France

En 2018

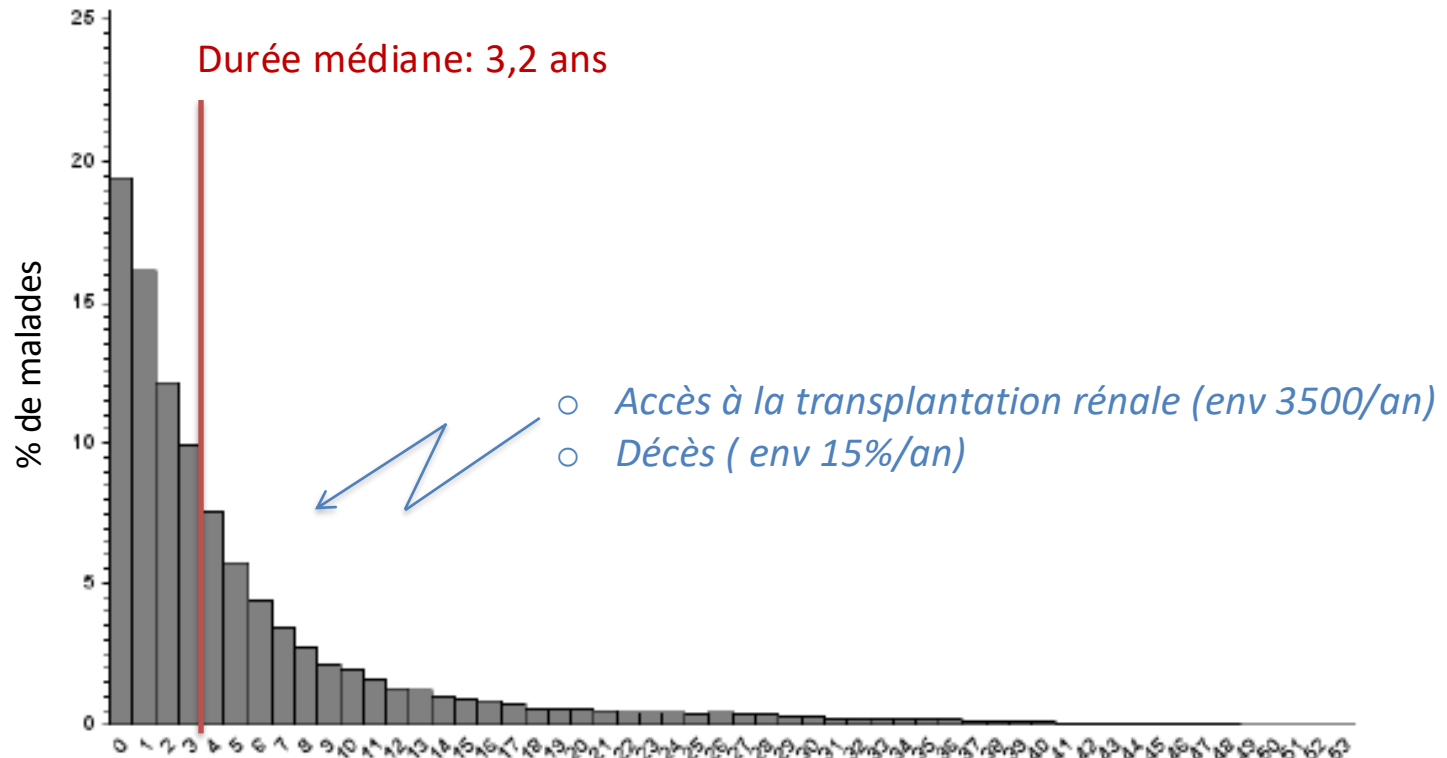


11343 nouveaux patients traités pour
insuffisance rénale terminale
Incidence : 168 pmh



8962 décès
Taux de mortalité: 9,8%
Age médian: 78 ans

Ancienneté en dialyse



Délai écoulé entre le premier traitement en dialyse et le 31/12/2018 (années)

Figure 2-9. Distribution de l'ancienneté du premier traitement de suppléance (années) chez les patients prévalents en dialyse au 31/12/2018

Distribution of prevalent dialysis patients according to the number of years on renal replacement therapy

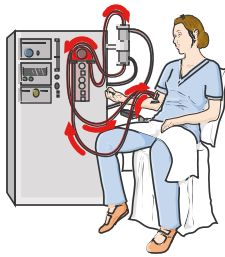
L'INSUFFISANCE RÉNALE TERMINALE EN FRANCE

L'insuffisance rénale chronique terminale (IRCT) correspond à une altération irréversible des différentes fonctions du rein, imposant la mise en œuvre d'une **thérapeutique de suppléance rénale** pour assurer la survie du patient

En France en 2021, **92 535** patients* étaient traités pour insuffisance rénale chronique terminale

Dialyse (>90% Hémodialyse)

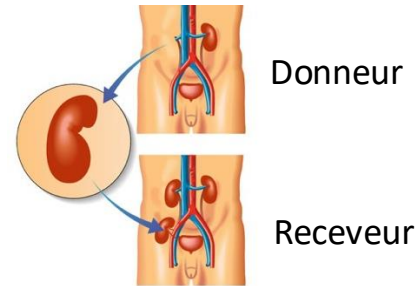
51 325 patients en dialyse
(56%)



8392 décès
i.e. **16.5% / an**

Transplantation rénale

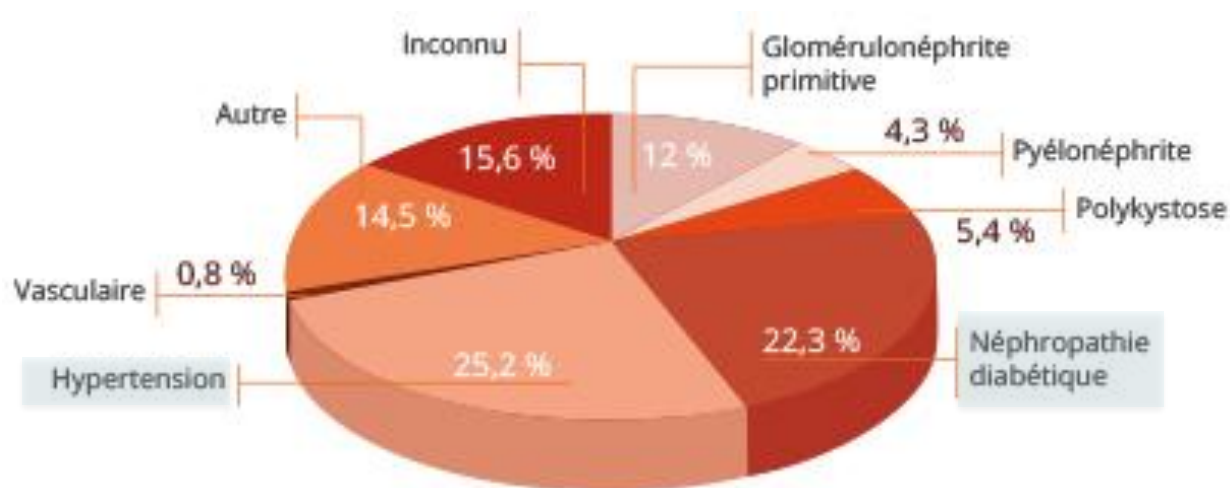
41 210 patients transplantés
(44%)



1613 décès
i.e. **4% / an**

*Rapport R.E.I.N 2021. https://www.agence-biomedecine.fr/IMG/pdf/rapport_rein_2021_2023-06-26.pdf

Quelles sont les principales causes des maladies rénales chroniques en France



Notez que **hypertension artérielle et diabète** représentent près de la moitié des étiologies d'insuffisance rénale (**47,5%**)

Chronic kidney disease

The NEW ENGLAND JOURNAL of MEDICINE

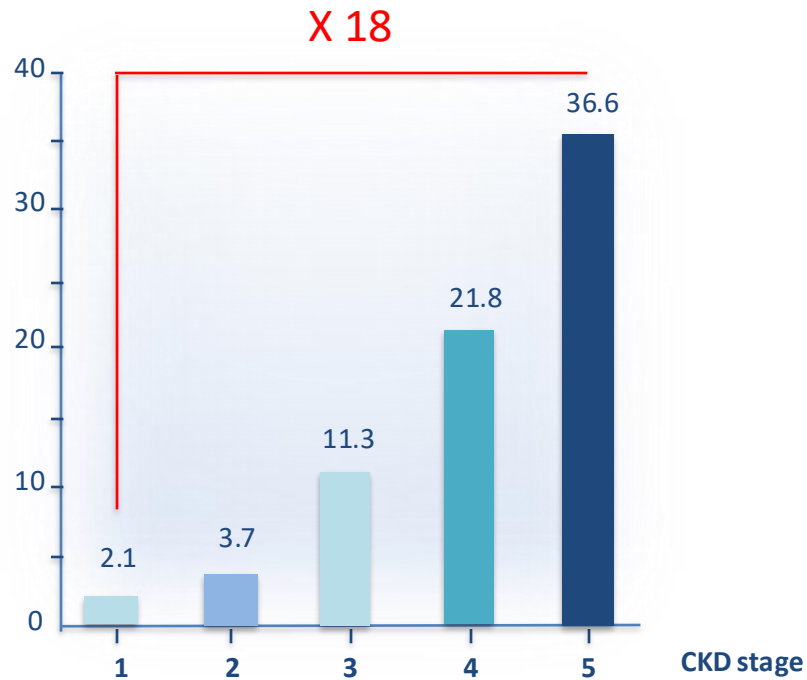
ORIGINAL ARTICLE

Chronic Kidney Disease and the Risks of Death, Cardiovascular Events, and Hospitalization

Alan S. Go, M.D., Glenn M. Chertow, M.D., M.P.H., Dongjie Fan, M.S.P.H.,
Charles E. McCulloch, Ph.D., and Chi-yuan Hsu, M.D.

Chronic Kidney Disease is a major Cardiovascular Risk Factor

Age-Standardized Rate of Cardiovascular Events (per 100 person-yr)



This higher CV risk is however not fully understood...

Chronic kidney disease and mortality

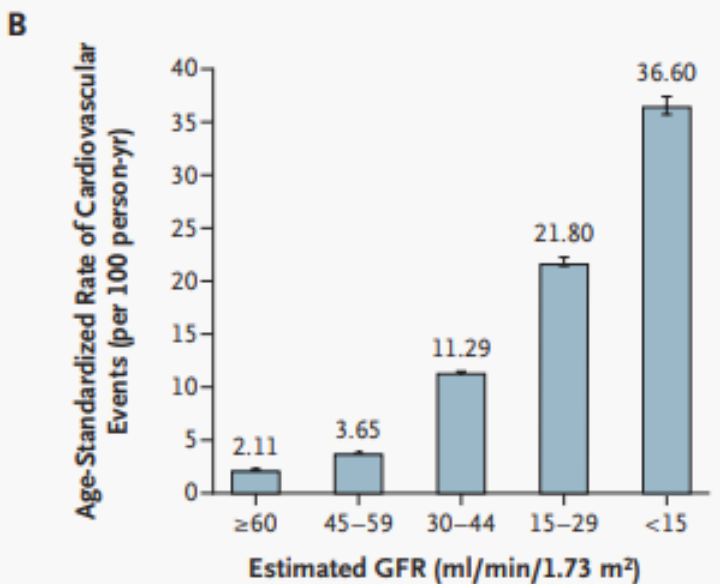
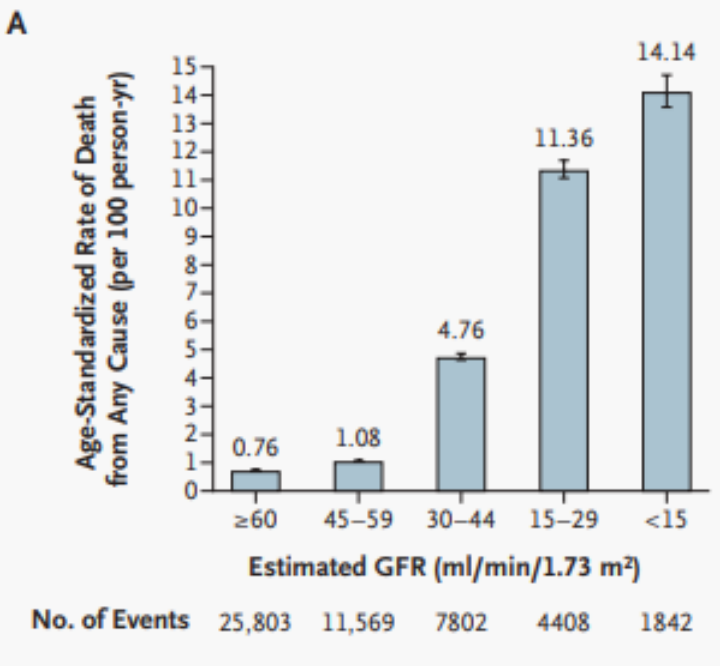


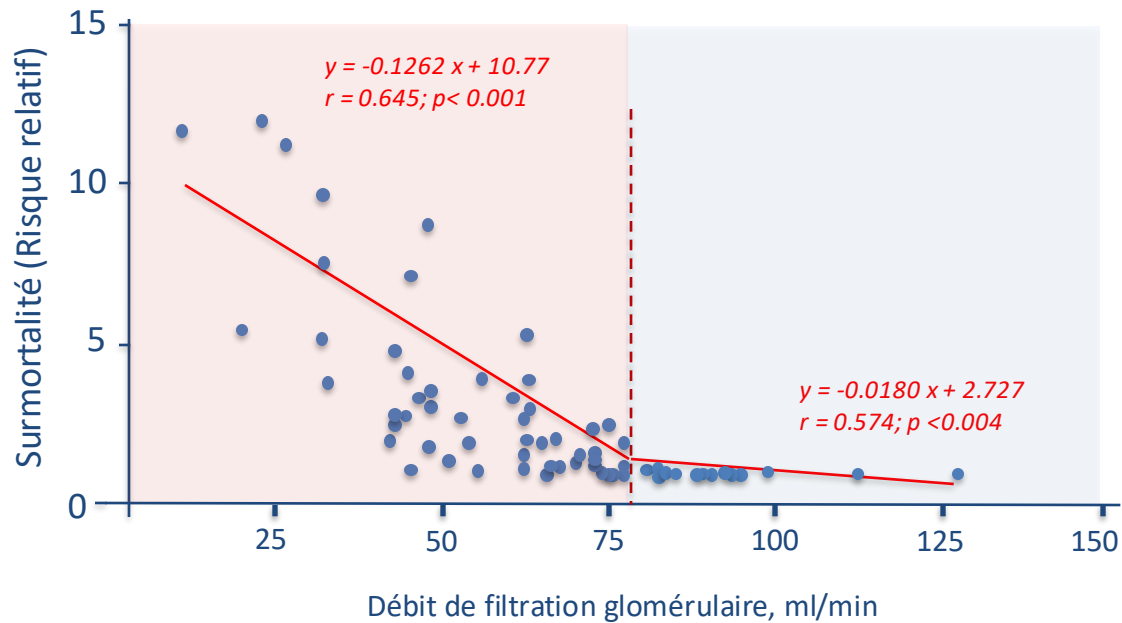
Table 2. Adjusted Hazard Ratio for Death from Any Cause, Cardiovascular Events, and Hospitalization among 1,120,295 Ambulatory Adults, According to the Estimated GFR.*

Estimated GFR	Death from Any Cause	Any Cardiovascular Event	Any Hospitalization
<i>adjusted hazard ratio (95 percent confidence interval)</i>			
≥60 ml/min/1.73 m ² †	1.00	1.00	1.00
45–59 ml/min/1.73 m ²	1.2 (1.1–1.2)	1.4 (1.4–1.5)	1.1 (1.1–1.1)
30–44 ml/min/1.73 m ²	1.8 (1.7–1.9)	2.0 (1.9–2.1)	1.5 (1.5–1.5)
15–29 ml/min/1.73 m ²	3.2 (3.1–3.4)	2.8 (2.6–2.9)	2.1 (2.0–2.2)
<15 ml/min/1.73 m ²	5.9 (5.4–6.5)	3.4 (3.1–3.8)	3.1 (3.0–3.3)

* The analyses were adjusted for age, sex, income, education, use or nonuse of dialysis, and the presence or absence of prior coronary heart disease, prior chronic heart failure, prior ischemic stroke or transient ischemic attack, prior peripheral arterial disease, diabetes mellitus, hypertension, dyslipidemia, cancer, a serum albumin level of 3.5 g per deciliter or less, dementia, cirrhosis or chronic liver disease, chronic lung disease, documented proteinuria, and prior hospitalizations.

† This group served as the reference group.

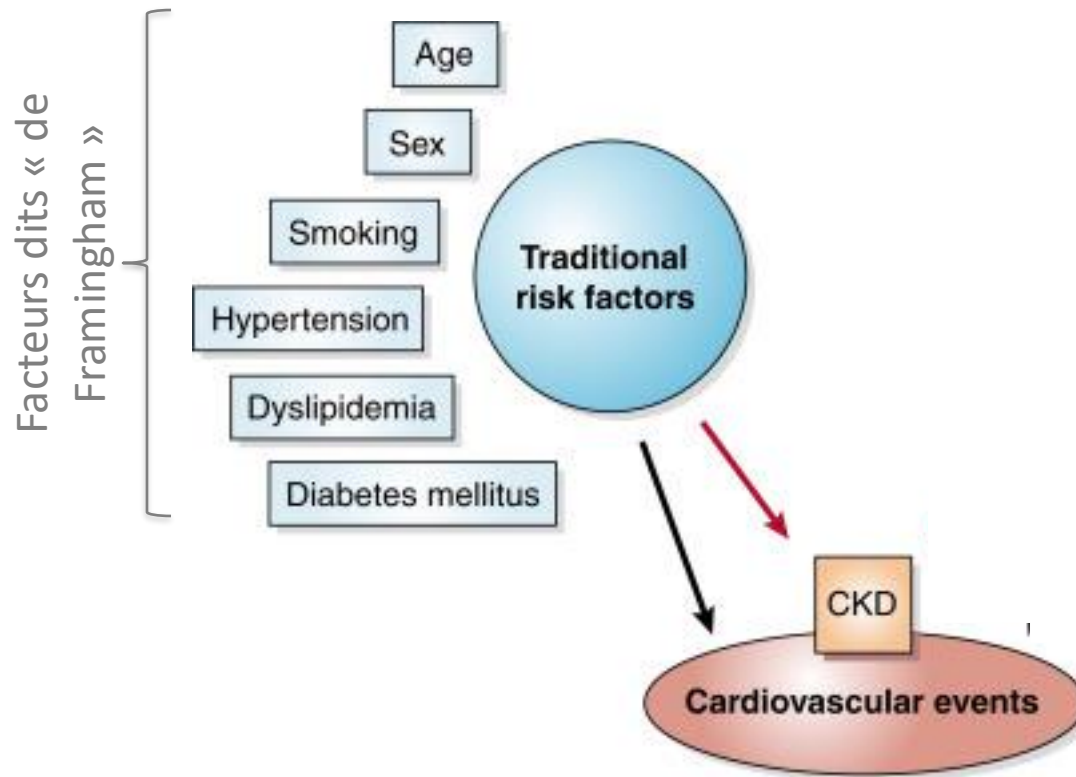
Insuffisance rénale chronique et mortalité



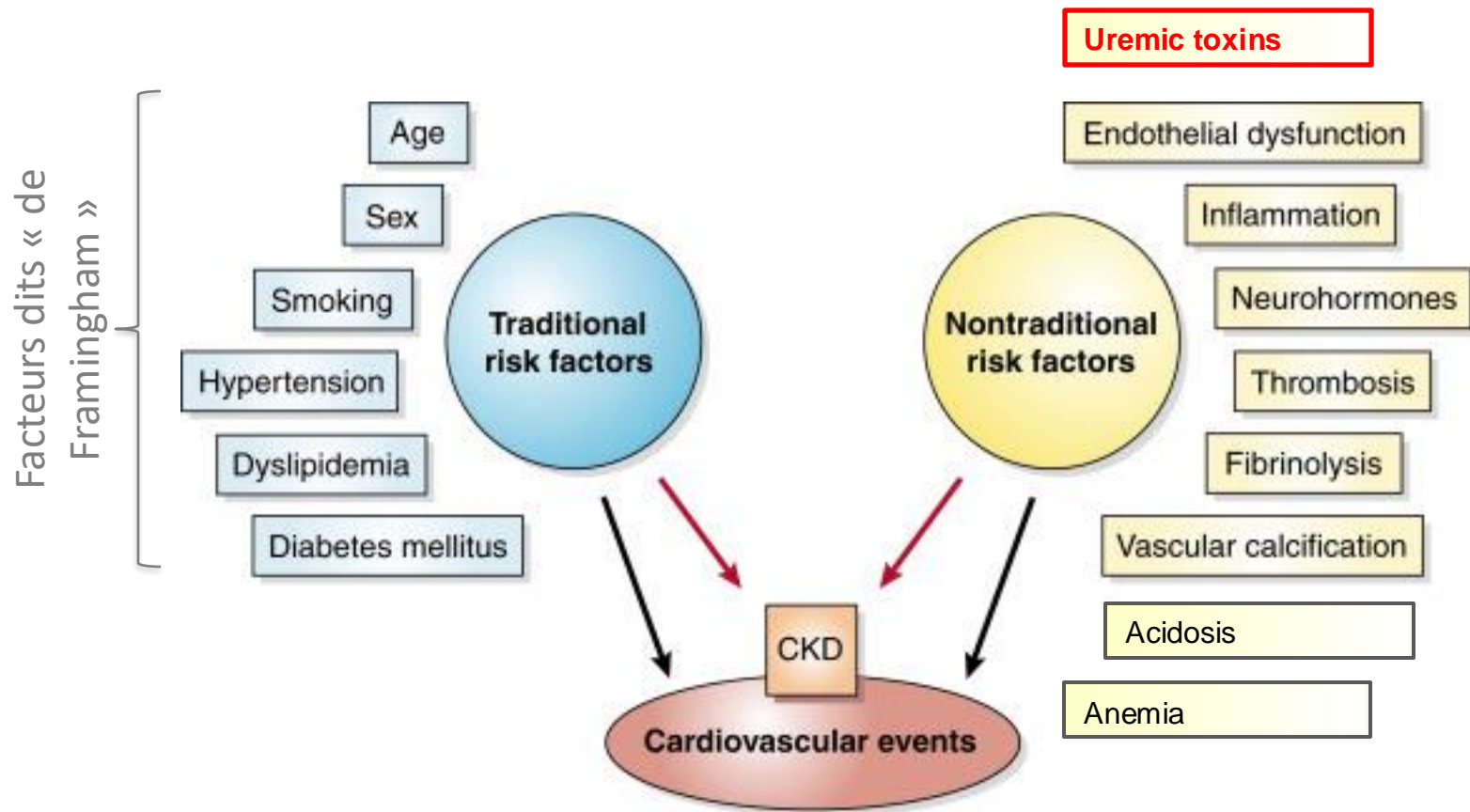
Adapté d'après Vanholder et al, NDT, 20, 1048-1056, 2005

Une surmortalité est observée dès les stades précoces de la MRC
« Décrochage » dès le stade 2...

Facteurs de risques traditionnels et non traditionnels dans l'insuffisance rénale chronique



Facteurs de risques traditionnels et non traditionnels dans l'insuffisance rénale chronique

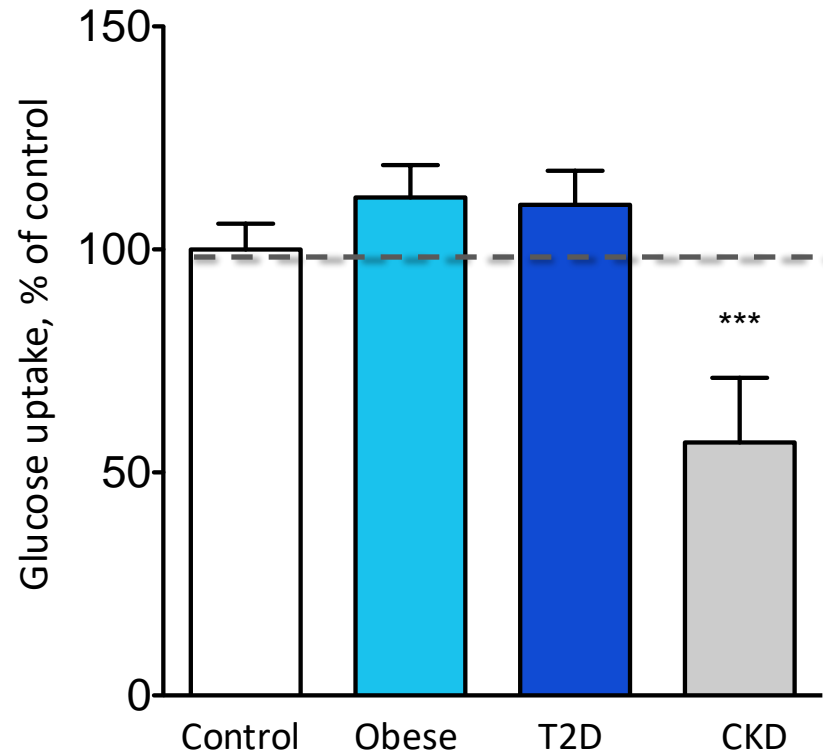


« Qu'y a-t-il dans la soupe urémique »



Uremic plasma induces insulin resistance...

Rat adipocytes incubated with human serum
[¹⁴C]-Glucose uptake after stimulation with insulin (1000μU/ml)



One (or many) specific factor(s) accumulate(s) in plasma from CKD patients...



(An) **uremic factor**(s) accumulate(s) in CKD and impair(s) insulin signaling...

- The uremic factor is now what we call **an uremic toxin...**
- Some of the uremic factors are **poorly dialyzable ...** maybe a large molecule ...

Definition of an uremic toxin (according to EuTox)



A compound is an **Uremic toxin** if and only if:

- Its concentration increases in chronic kidney disease as a result of a decreased renal clearance
- It causes at least one pathophysiological effect

Definition of an uremic toxin (according to EuTox)

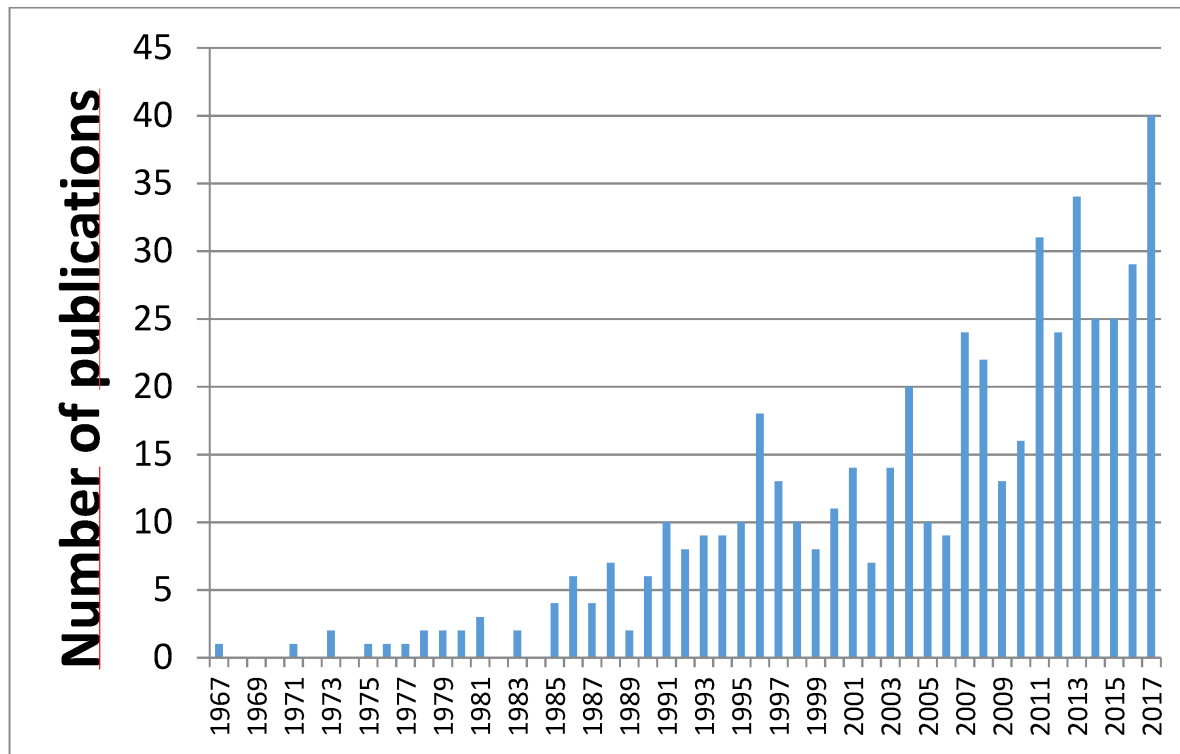


A compound is an **Uremic toxin** if and only if:

- Its concentration increases in chronic kidney disease as a result of a decreased renal clearance (1)
- It causes at least one pathophysiological effect (2)

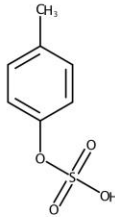
If (1) but not (2) the compound is an **Uremic Retention Solute (URS)**

Number of publications on Uremic Toxins per year of appearance included in the reference list of Vanholder et al, 2018



Classification of uremic toxins (according to EuTox)

POORLY
DIALYSABLE

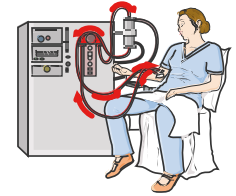


Ex: *p*-cresyl sulfate (MW 188 D)



Ex: Urea (MW 60 D)

DIALYSABLE
(HD, PD)



Protein bound molecules
(<0.5kDa, n=23)

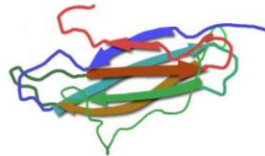
26%

Small water soluble
molecules
(<0.5 kDa, n=40)

46%

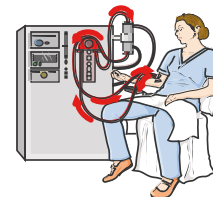
28%

Middle molecules
(0.5-60kDa, n= 25)



Ex: β_2 -microglobulin (11.8 kD)

± DIALYSABLE
(HFD)



Uremic toxins: > 100 compounds...

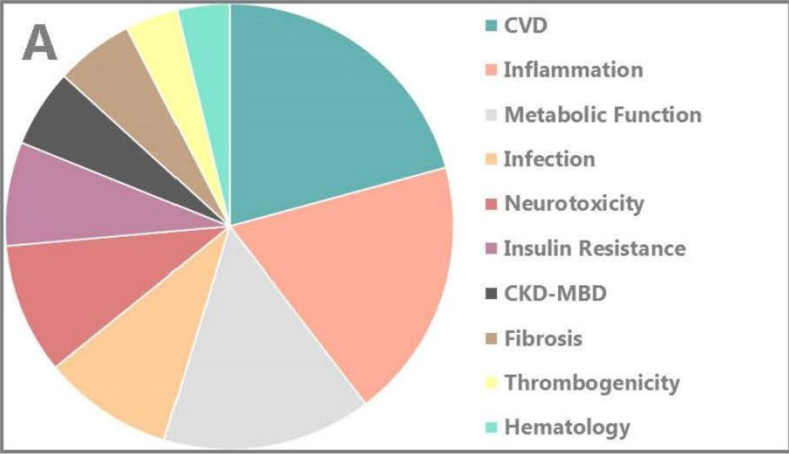
Table 1. Classification of major uremic toxins.

Small Water-Soluble Compounds (<500 Da)	Middle Molecule (≥500 Da)	Protein Bound Compounds (Mostly < 500 Da)
ADMA	ANP	AGEs
Carbamylated compounds	β ₂ -microglobulin	Homocysteine
Creatinine	Endothelin	Indoxyl sulfate
SDMA	FGF23	Indole acetic acid
TMAO	Ghrelin	Kynurenes
Urea	Immunoglobulin light chains	p-cresylsulfate
Uric acid	Interleukin-6	Phenyl acetic acid
	Interleukin-8	
	Interleukin-18	
	Lipids and lipoproteins	
	Neuropeptide Y	
	PTH	
	Retinol binding protein	
	TNF-α	

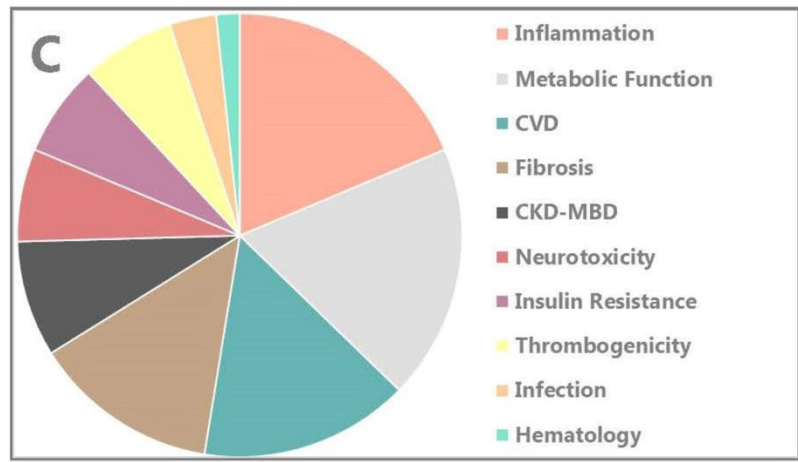
ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; TMAO, trimethylamine-N-oxide; ANP, atrial natriuretic peptide; FGF23, fibroblast growth factor 23; PTH, parathyroid hormone; TNF-α, tumor necrosis factor-α, AGEs, advanced glycation end products.

Number of toxins within a group affecting specific systems

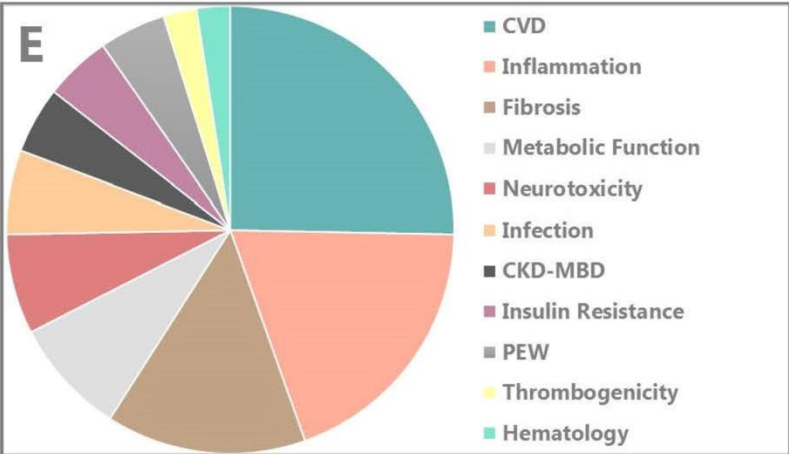
Small water-soluble compounds



Protein-bound compounds

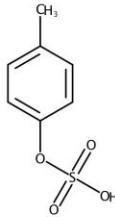


Middle molecules



Classification of uremic toxins (according to EuTox)

POORLY
DIALYSABLE



Ex: p-cresyl sulfate (MW 188 D)

Protein bound molecules
(<0.5kDa, n=23)

26%

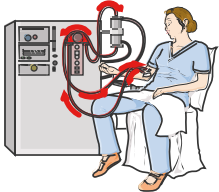


Ex: Urea (MW 60 D)

Small water soluble
molecules
(<0.5 kDa, n=40)

46%

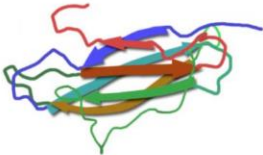
DIALYSABLE
(HD, PD)



Protein Bound Uremic Toxins
(PBUTs)

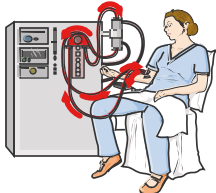
28%

Middle molecules
(0.5-60kDa, n= 25)



Ex: β_2 -microglobulin (11.8 kD)

± DIALYSABLE
(HFD)

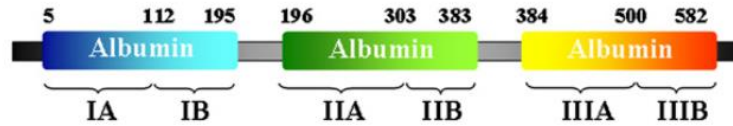


Main plasma binding proteins

Proteins	MW, Da	Concentration	
		g/L	μM
Albumin	67 000	35-50	500-700
α ₁ -glycoprotein acid	42 000	0.4-1.0	9-23
Lipoproteins	2 10 ⁵ -2.4 10 ⁶	variable	
Transcortin	53 000	0.03-0.07	0.6-1.4

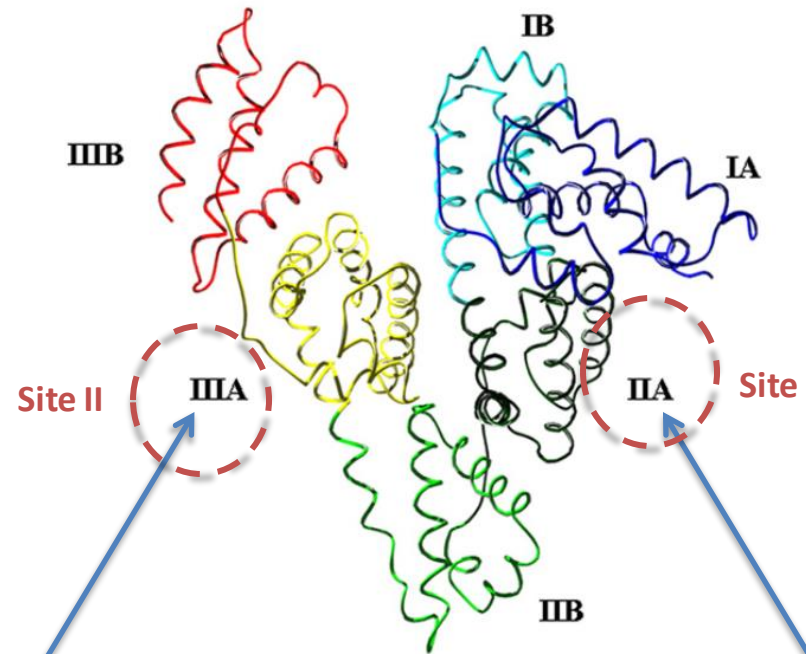
Structure of Human Serum Albumin (HSA)

Crystal structure of HSA



Plasma: 34 à 46 g L⁻¹

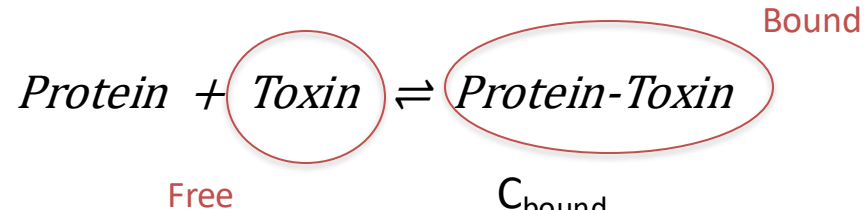
Fixation **réversible** i.e. non covalente (liaisons faibles (H, Van der Waal, hydrophobes, électrostatiques,...))



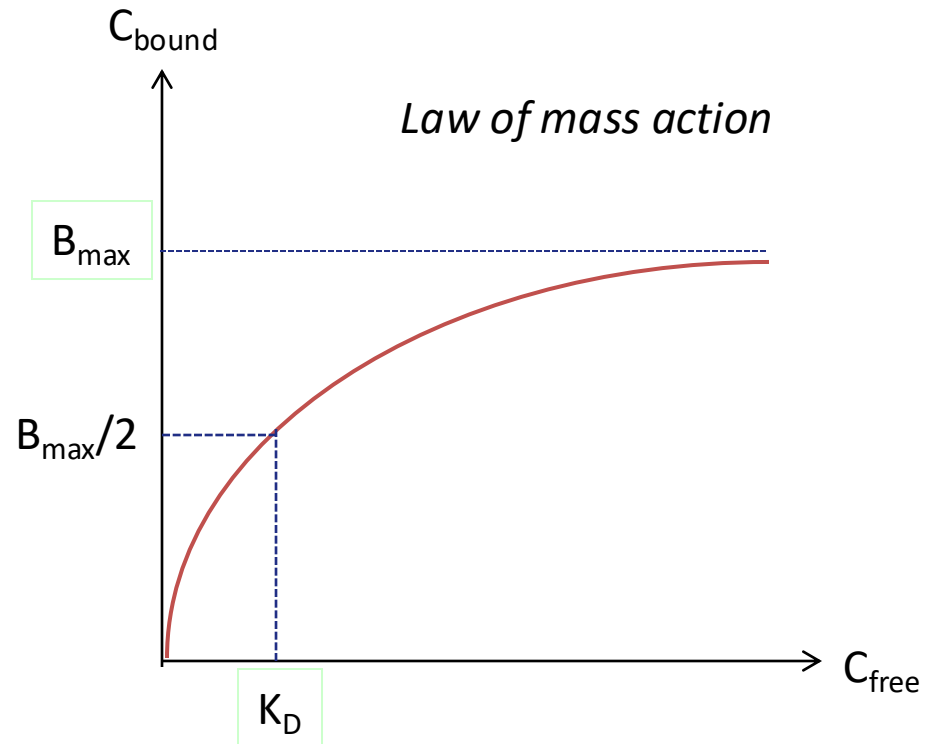
Benzodiazepins
Ibuprofen
Indoxyl-sulfate
P-cresyl sulfate

Warfarine
CMPF

Dynamics of protein binding



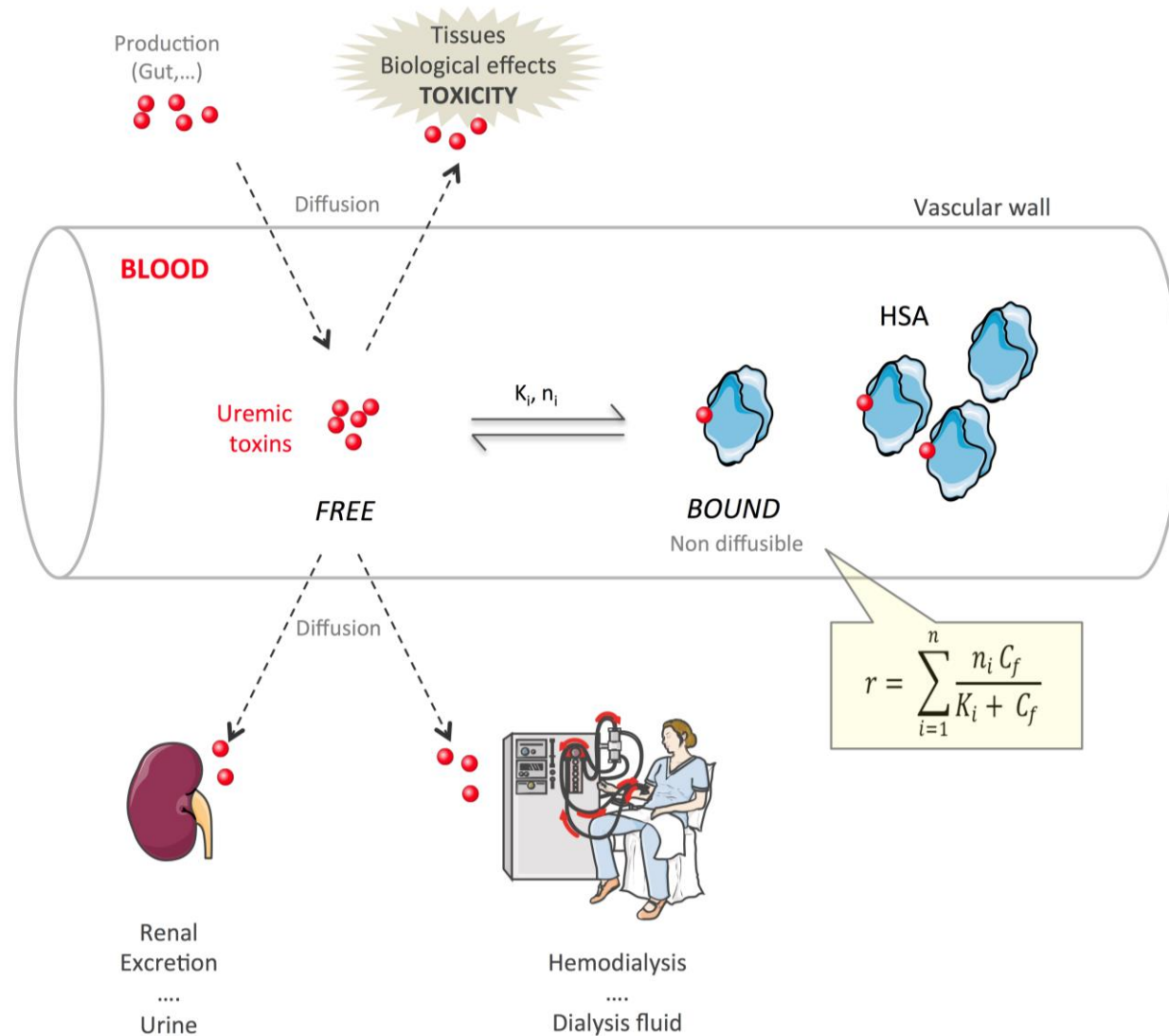
$$C_{\text{bound}} = \frac{B_{\text{max}} \cdot C_{\text{free}}}{K_D + C_{\text{free}}}$$



B_{max} : maximal concentration of binding sites ---- proportionnal to plasma protein concentration

K_D : free drug concentration corresponding to half maximal binding ---- inversely proportional to drug affinity for the protein

Only the **free fraction** is biologically active...



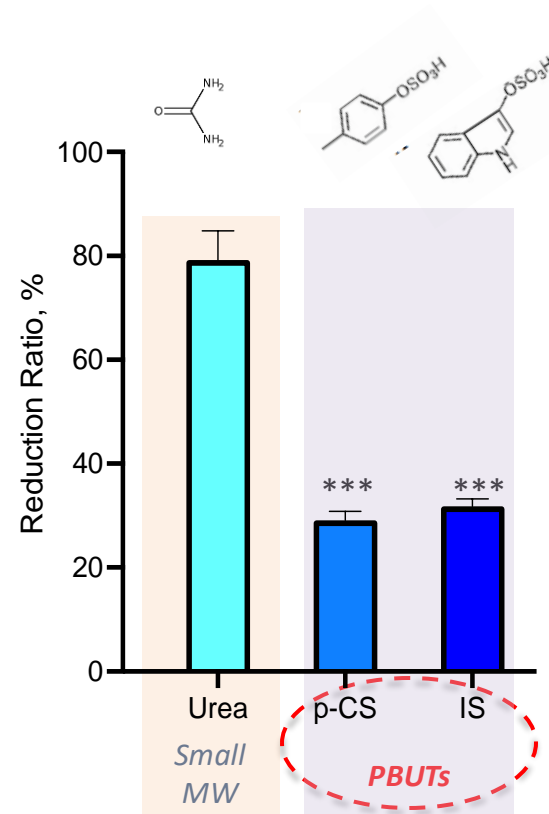
Reduction of uremic toxin concentrations after a typical hemodialysis session (4h)

Protein binding

Urea : no protein binding

p-CS: $95.1 \pm 0.6\%$

IS: $97.7 \pm 0.2\%$



Poorly removed by HD therefore accumulate +++ in CKD patients

Origine des toxines urémiques



Certaines toxines (parmi les plus toxiques...) sont produites dans l'intestin... par notre **microbiote intestinal...**

Données historiques de Einheber & Carter (1965) chez les rats anéphriques

THE ROLE OF THE MICROBIAL FLORA IN UREMIA

I. SURVIVAL TIMES OF GERM-FREE, LIMITED-FLORA, AND CONVENTIONALIZED RATS AFTER BILATERAL NEPHRECTOMY AND FASTING

BY ALBERT EINHEBER, PH.D., AND DARRYL CARTER,* M.D.

(From the Department of Germfree Research, Division of Surgery, Walter Reed Army Institute of Research, Washington, D. C.)

(Received for publication 20 September 1965)

TABLE I

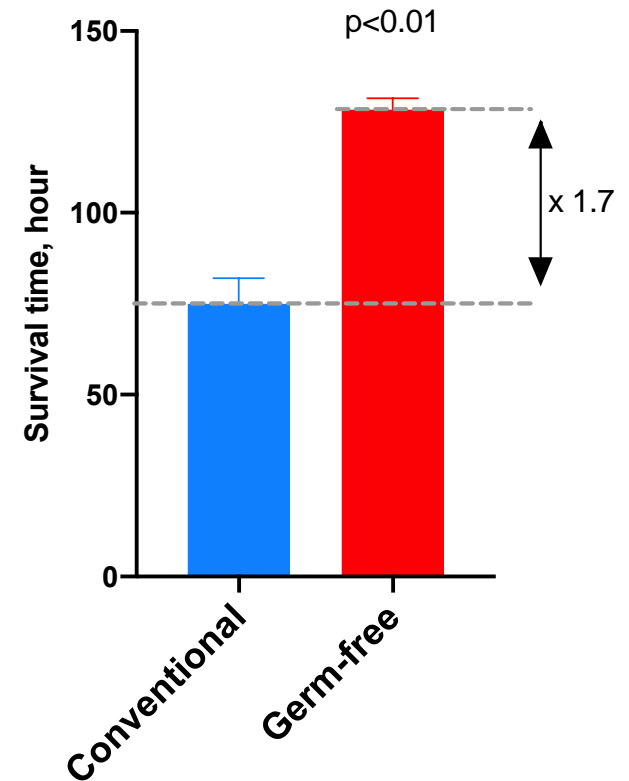
Comparison of Preoperative Body Weights and Postoperative Survival Times of Fasting GF and CONV Rats Dying of Uremia After Bilateral Nephrectomy

Experiment	Sex	Body wt		Survival time		
		GF	CONV	GF	CONV	P value
I	M	204±8.4 (4 rats)	172±1.8 (4 rats)	122±3.7 (113-130)*	89±5.2 (75-100)	<0.01
II	M	207±6.4 (7 rats)	194±8.4 (6 rats)	132±5.8 (108-151)	68±6.2 (48-84)	<0.01
	F	143±3.3 (4 rats)	156±3.0 (3 rats)	131±9.1 (119-147)	68±14.3 (41-90)	<0.01

Mean ± SE of mean.

* Observed range.

Survie après néphrectomie bilatérale



Many protein-bound uremic toxins are issued from gut microbiota metabolism

CLINICAL RESEARCH www.jasn.org

Colonic Contribution to Uremic Solutes

Pavel A. Aronov,* Frank J.-G. Luo,† Natalie S. Plummer,† Zhe Quan,‡ Susan Holmes,§
Thomas H. Hostetter,‡ and Timothy W. Meyer†

*Vincent Coates Foundation Mass Spectrometry Laboratory, and §Department of Statistics, Stanford University, Stanford, California; †Departments of Medicine, Veterans Administration Palo Alto Health Sciences Center and Stanford University, Palo Alto, California; and ‡Albert Einstein College of Medicine, New York, New York

Table 2. Solutes measured by HPLC and urea

Solute	Dialysis Intact Colon (n = 9)	Dialysis Colectomy (n = 6)	Normal Control (n = 7 to 10)
PCS			
plasma pretreatment mg/dl	4.1 ± 1.6 ^{a,b}	0.06 ± 0.09	0.19 ± 0.13
reduction ratio	30 ± 7	–	–
IS			
plasma pretreatment mg/dl	2.8 ± 1.3 ^{a,b}	0.08 ± 0.06	0.06 ± 0.02
reduction ratio	33 ± 7	31 ± 11	–
KYNA			
plasma pretreatment nM	799 ± 404 ^b	634 ± 292 ^b	29 ± 7
reduction ratio	36 ± 7	39 ± 16	–
Hippurate			
plasma pretreatment mg/dl	7.9 ± 4.5 ^b	4.6 ± 5.9 ^b	0.3 ± 0.2
reduction ratio	68 ± 4	72 ± 19	–
DMA			
plasma pretreatment μg/dl	1032 ± 155 ^b	890 ± 103 ^b	218 ± 33
reduction ratio	38 ± 10	43 ± 7	–
MMA			
plasma pretreatment μg/dl	58 ± 10 ^b	54 ± 9 ^b	32 ± 4
reduction ratio	30 ± 10	23 ± 6	–
Urea			
plasma pretreatment mg/dl	50 ± 8 ^b	43 ± 16 ^b	14 ± 3
reduction ratio	74 ± 4	78 ± 7	–

Values are mean ± SD. The plasma PCS concentration was below the limits of detection in one normal subject and in three pretreatment samples and four post-treatment samples from dialysis patients without colons; a reduction ratio for PCS in the dialysis colectomy group was therefore not determined. The plasma hippurate was below the limits of detection in one patient without a colon.

^aP < 0.05 dialysis intact colon group versus dialysis colectomy group.

^bP < 0.05 dialysis intact colon group or dialysis colectomy group versus normal control group.

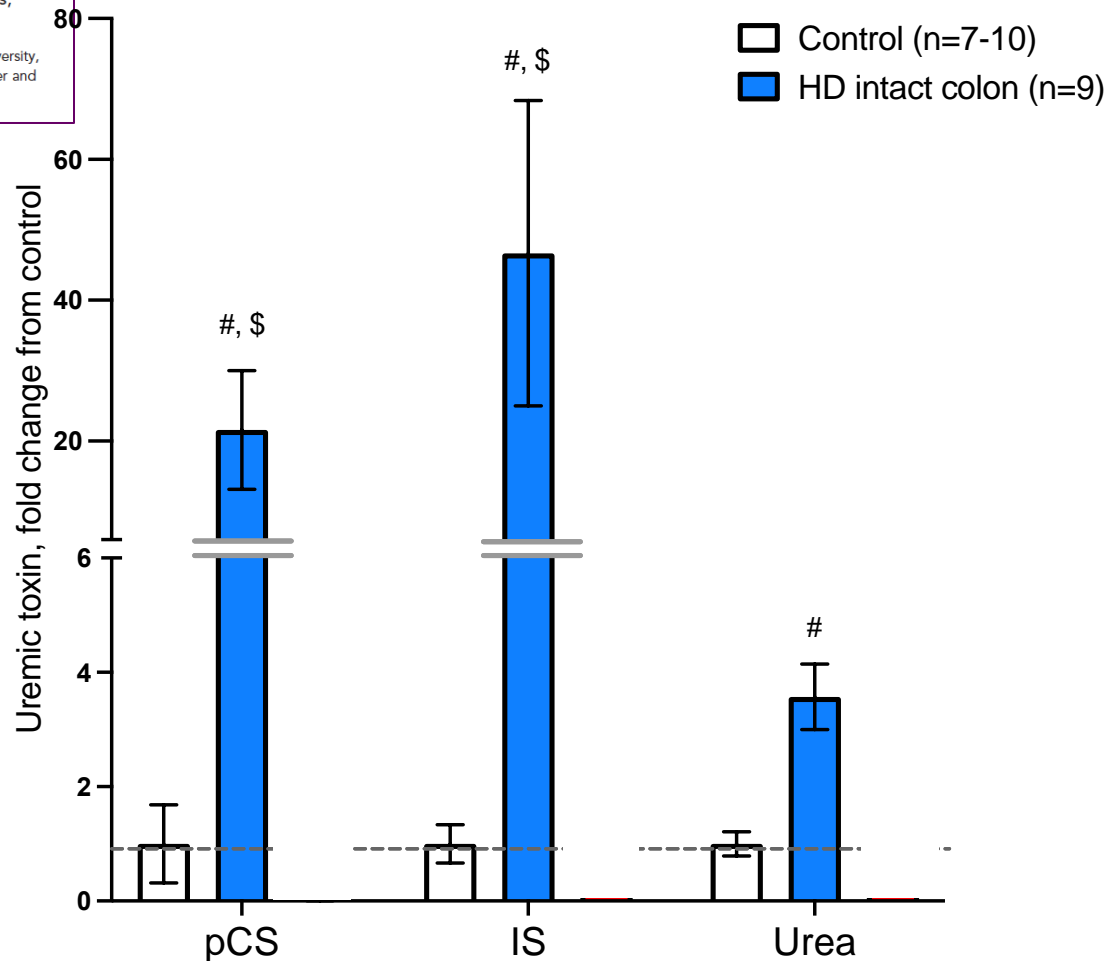
Many uremic toxins are issued from gut microbiota

CLINICAL RESEARCH www.jasn.org

Colonic Contribution to Uremic Solutes

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difference from control

Many uremic toxins are issued from gut microbiota:

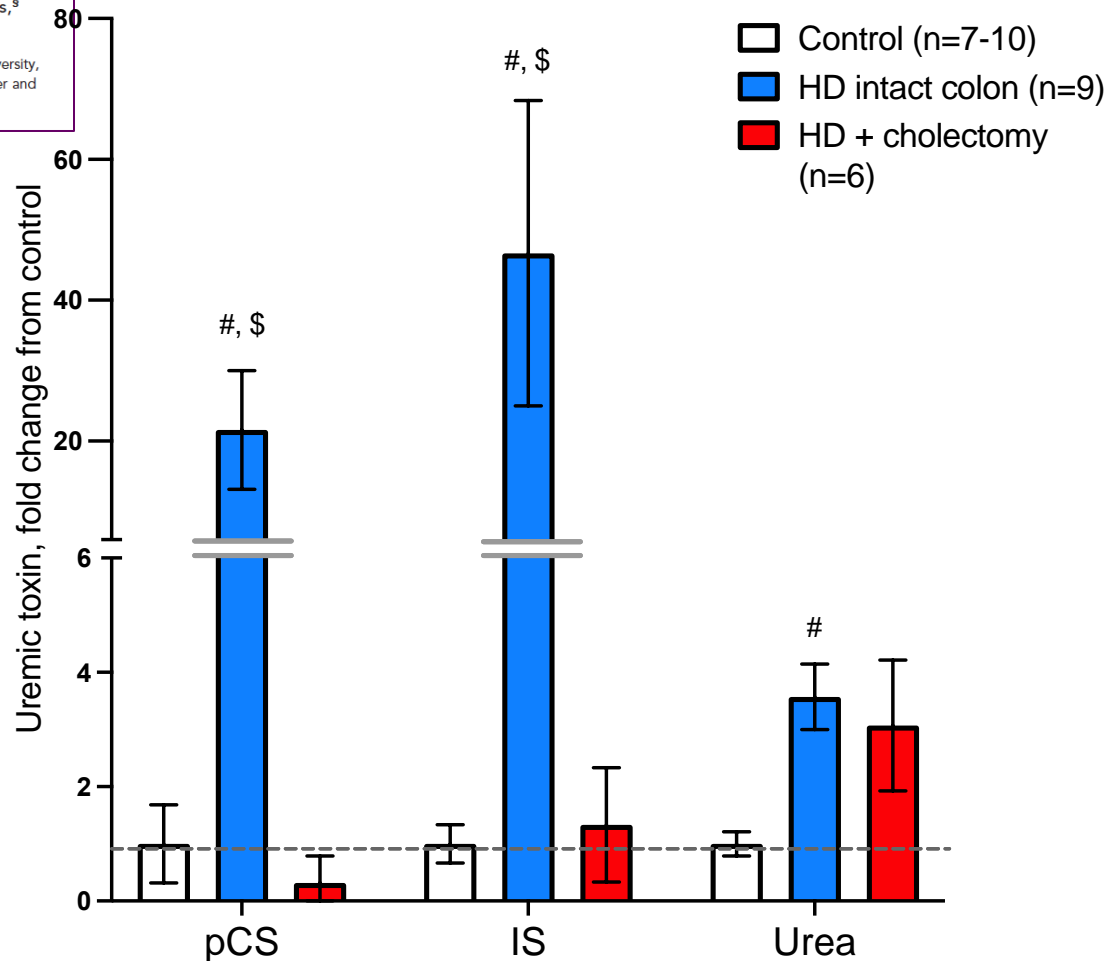
Human data

CLINICAL RESEARCH www.jasn.org

Colonic Contribution to Uremic Solutes

Pavel A. Aronov,* Frank J.-G. Luo,[†] Natalie S. Plummer,[†] Zhe Quan,[‡] Susan Holmes,[§] Thomas H. Hostetter,[†] and Timothy W. Meyer[†]

*Vincent Coates Foundation Mass Spectrometry Laboratory, and [§]Department of Statistics, Stanford University, Stanford, California; [†]Departments of Medicine, Veterans Administration Palo Alto Health Sciences Center and Stanford University, Palo Alto, California; and [‡]Albert Einstein College of Medicine, New York, New York



difference from control

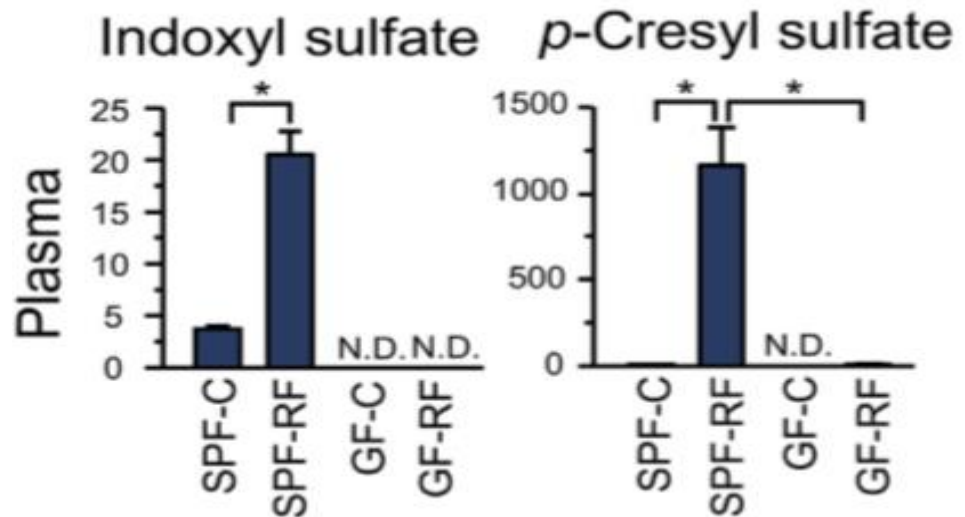
\$ difference from patients with colectomy

Uremic toxins sources

Evaluation of the impact of gut microbiota on uremic solute accumulation by a CE-TOFMS-based metabolomics approach

OPEN

Eikan Mishima^{1,2,8}, Shinji Fukuda^{3,4,8}, Chikahisa Mukawa⁵, Akinori Yuri⁵, Yoshitomi Kanemitsu⁵, Yotaro Matsumoto⁵, Yasutoshi Akiyama¹, Noriko N. Fukuda³, Hiroki Tsukamoto⁵, Kei Asaji⁵, Hisato Shima¹, Koichi Kikuchi¹, Chitose Suzuki¹, Takehiro Suzuki¹, Yoshihisa Tomioka⁵, Tomoyoshi Soga³, Sadayoshi Ito¹ and Takaaki Abe^{6,7}

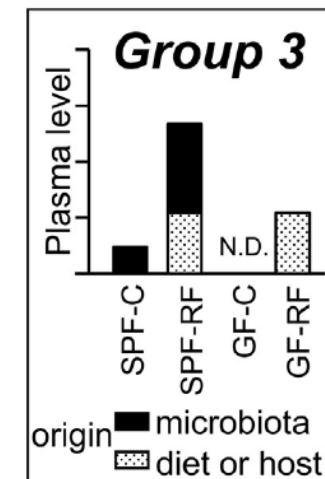
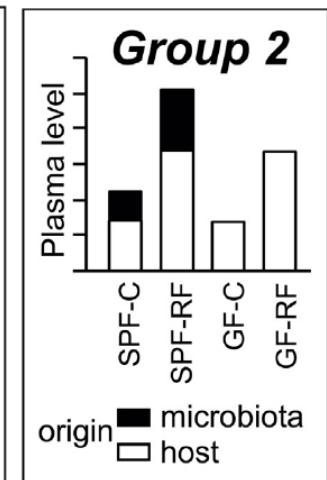
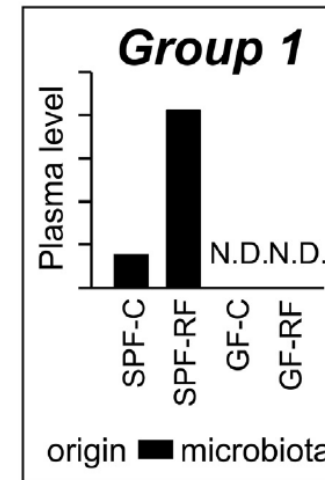


Evaluation of the impact of gut microbiota on uremic solute accumulation (using Germ free mice with renal failure)

Table 2 | Classification of the microbiota-derived uremic solutes. The microbiota-derived uremic solutes (M-USs) are classified into Groups 1, 2, and 3 depending on their presence in the plasma of germ-free control (GF-C) and GF renal failure (GF-RF) mice

	Group 1		Group 2		Group 3	
	GF-C	GF-RF	GF-C	GF-RF	GF-C	GF-RF
Plasma	–	–	+	+	–	+
Feces	–	–	+	+	+ or –	+ or –
Urine	–	–	+	+	+	+
Solute	Indoxyl sulfate		Dimethylglycine		TMAO	
	<i>p</i> -Cresyl sulfate		γ -Guanidinobutyrate		Phenaceturate	
	Phenyl sulfate		Glutarate			
	Cholate		2-Hydroxypentanoate			
	Hippurate					

+, detected in the indicated sample; –, not detected or nearly undetectable in the indicated sample; TMAO, trimethylamine-*N*-oxide.

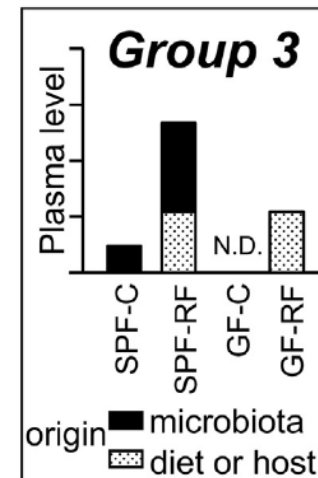
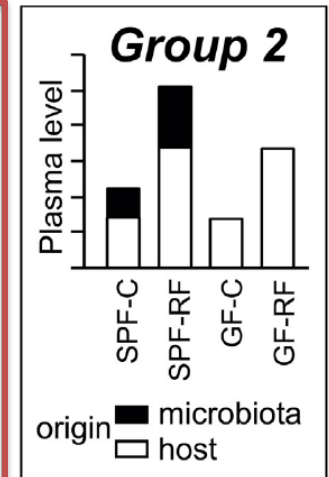
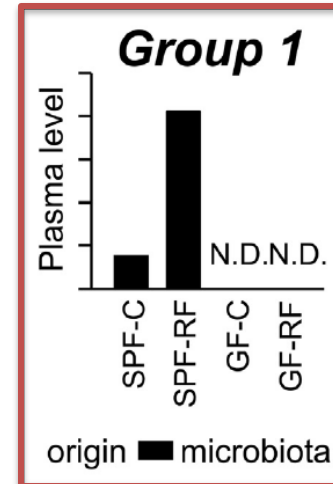


Evaluation of the impact of gut microbiota on uremic solute accumulation (using Germ free mice with renal failure)

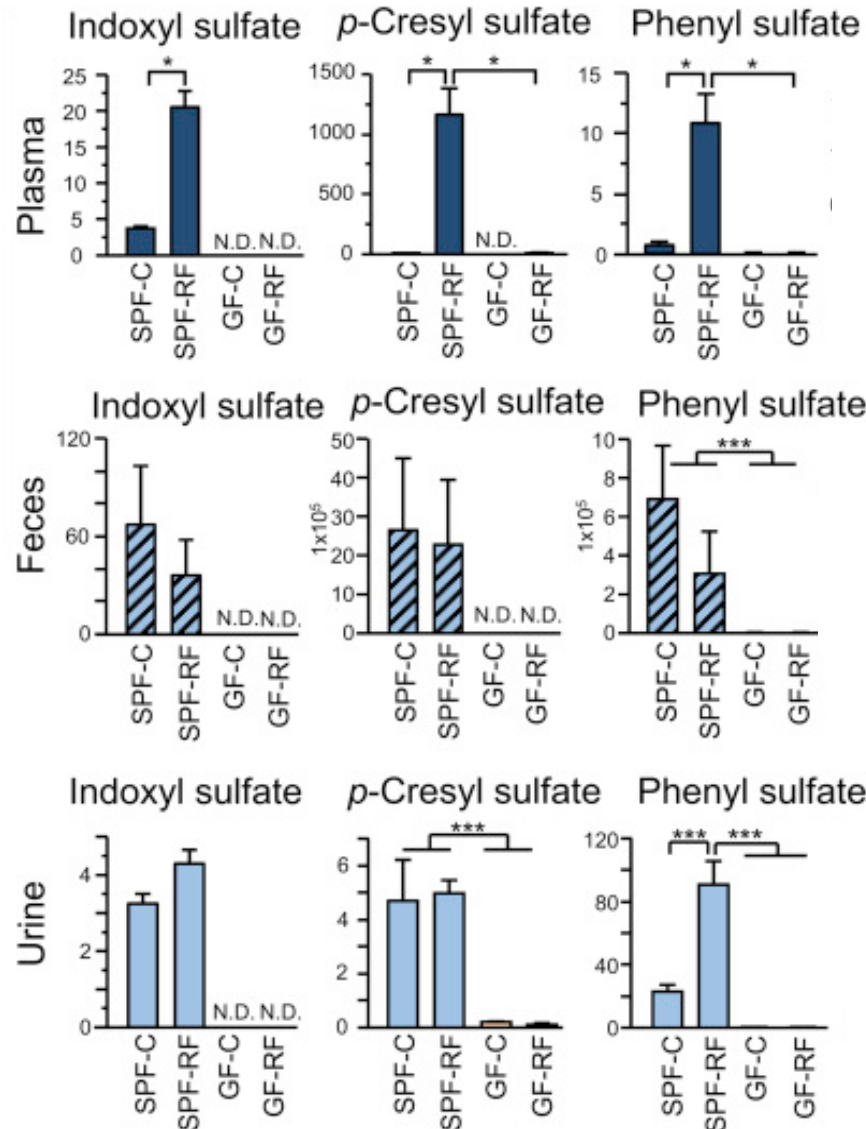
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	Group 1		Group 2		Group 3	
	GF-C	GF-RF	GF-C	GF-RF	GF-C	GF-RF
Plasma	-	-	+	+	-	+
Feces	-	-	+	+	+ or -	+ or -
Urine	-	-	+	+	+	+
Solute	Indoxyl sulfate <i>p</i> -Cresyl sulfate Phenyl sulfate Cholate Hippurate		Dimethylglycine γ -Guanidinobutyrate Glutarate 2-Hydroxypentanoate		TMAO Phenaceturate	

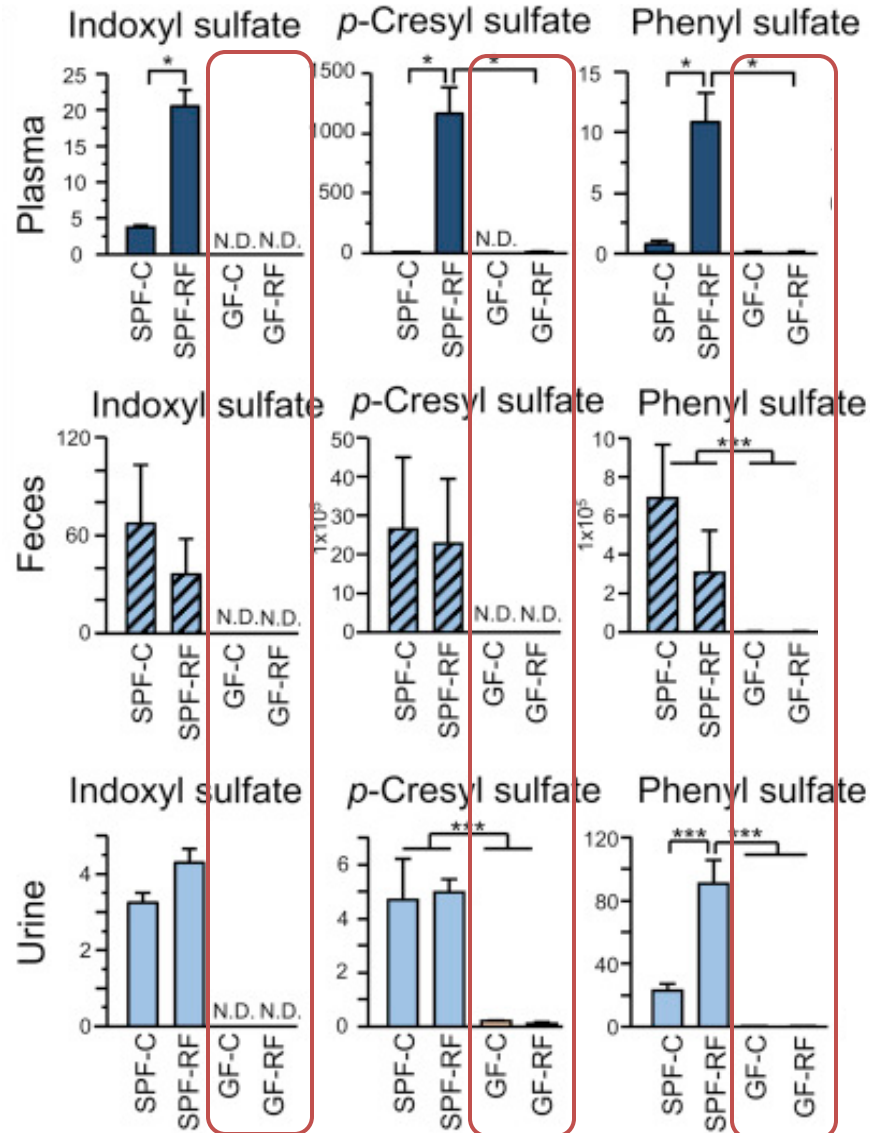
+, detected in the indicated sample; -, not detected or nearly undetectable in the indicated sample; TMAO, trimethylamine-*N*-oxide.



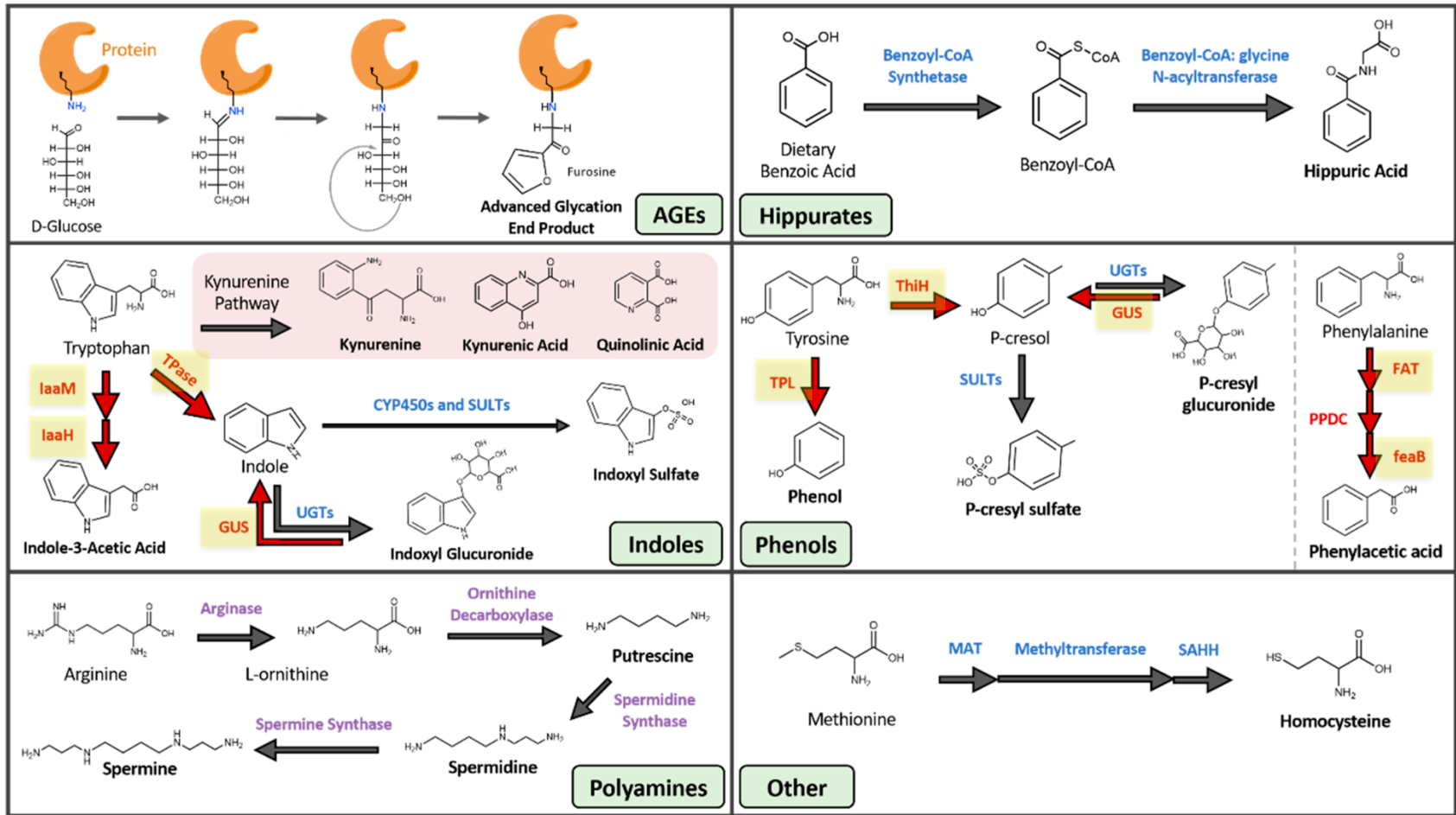
Many protein-bound uremic toxins are produced only by gut microbiota



Many protein-bound uremic toxins are produced only by gut microbiota



Panorama of gut derived uremic toxins



Gut-Derived Protein-Bound Uremic Toxins. **Blue text: human enzymes, red text: microbial enzymes, purple text: both human and microbial enzymes.** AGE, advanced glycation end product; CYP450, cytochrome P450; FAT, phenylalanine dehydrogenase or transaminase; feaB, phenylacetaldehyde dehydrogenase; GUS, beta-glucuronidase; IaaH, indole-3-acetamide hydrolase; IaaM, tryptophan 2-monoxygenase; MAT, methionine adenosyltransferase; PPDC, phenylpyruvate decarboxylase; SAHH, S-adenosylhomocysteine hydrolase; SULT, sulfotransferase; ThiH, tyrosine lyase; TPase, tryptophanase; TPL, tyrosine phenol-lyase; UGT, UDP-glucuronosyltransferase.

Altération du microbiote intestinal au cours de l'IRC

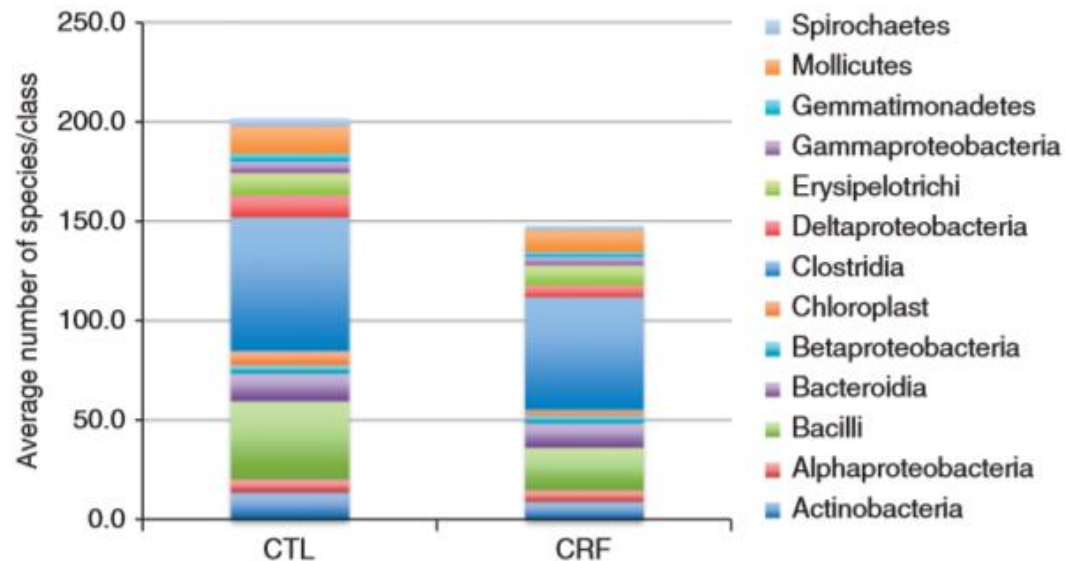
Chronic kidney disease alters intestinal microbial flora

Nosratola D. Vaziri¹, Jakk Wong², Madeleine Pahl¹, Yvette M. Piceno², Jun Yuan¹, Todd Z. DeSantis³, Zhenmin Ni¹, Tien-Hung Nguyen² and Gary L. Andersen²

¹Division of Nephrology and Hypertension, UC Irvine Medical Center, Irvine, California, USA; ²Center for Environmental Biotechnology, Lawrence Berkeley National Laboratory, Berkeley, California, USA and ³Second Genome, San Bruno, California, USA

Diminution
de la
richesse

Modification
de la
biodiversité



The NEW ENGLAND
JOURNAL *of* MEDICINE

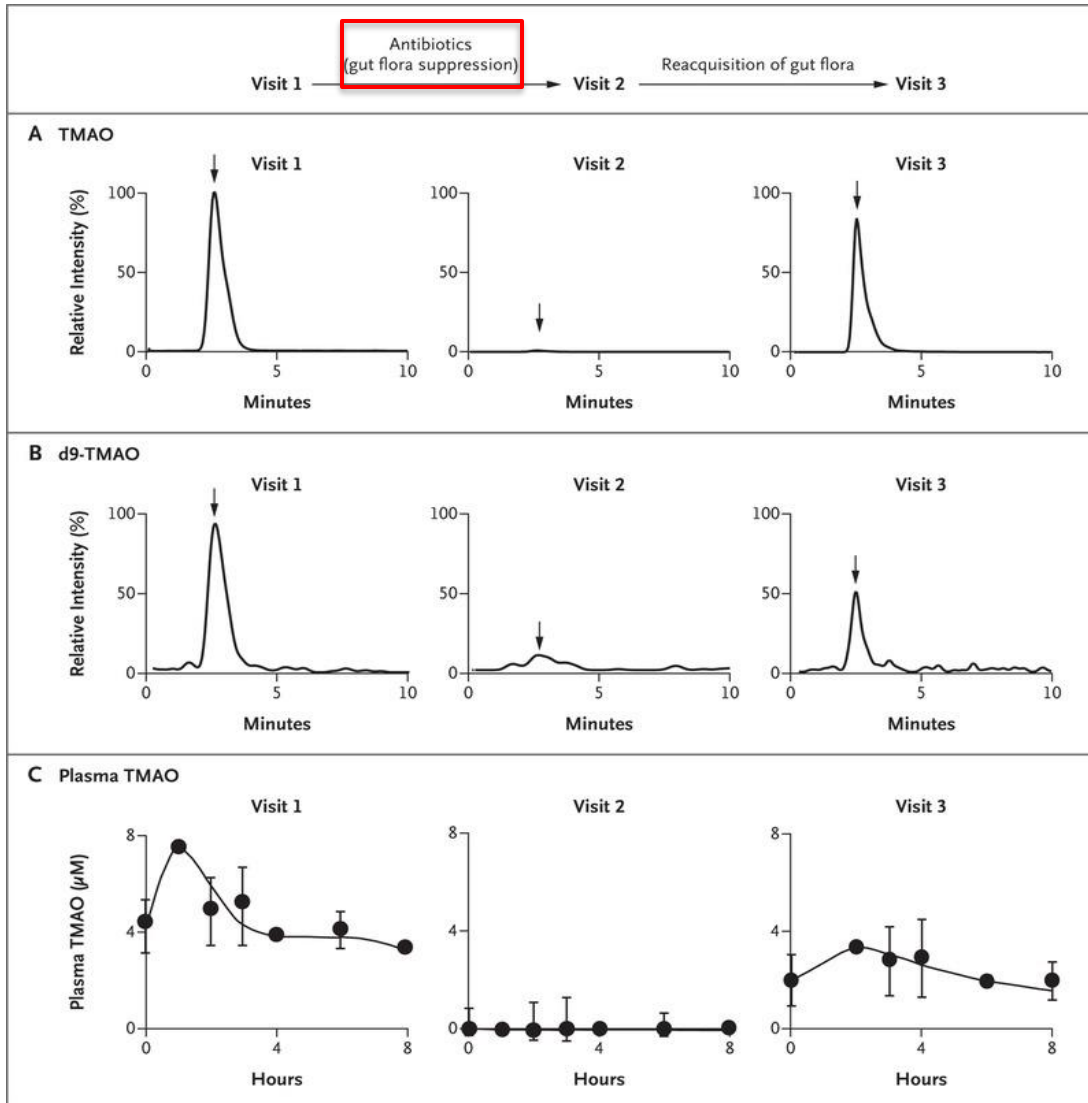
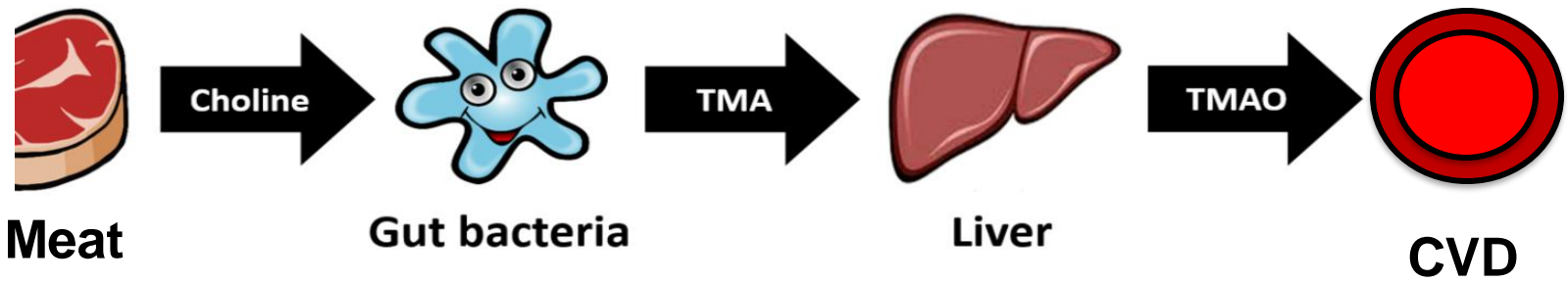
ESTABLISHED IN 1812

APRIL 25, 2013

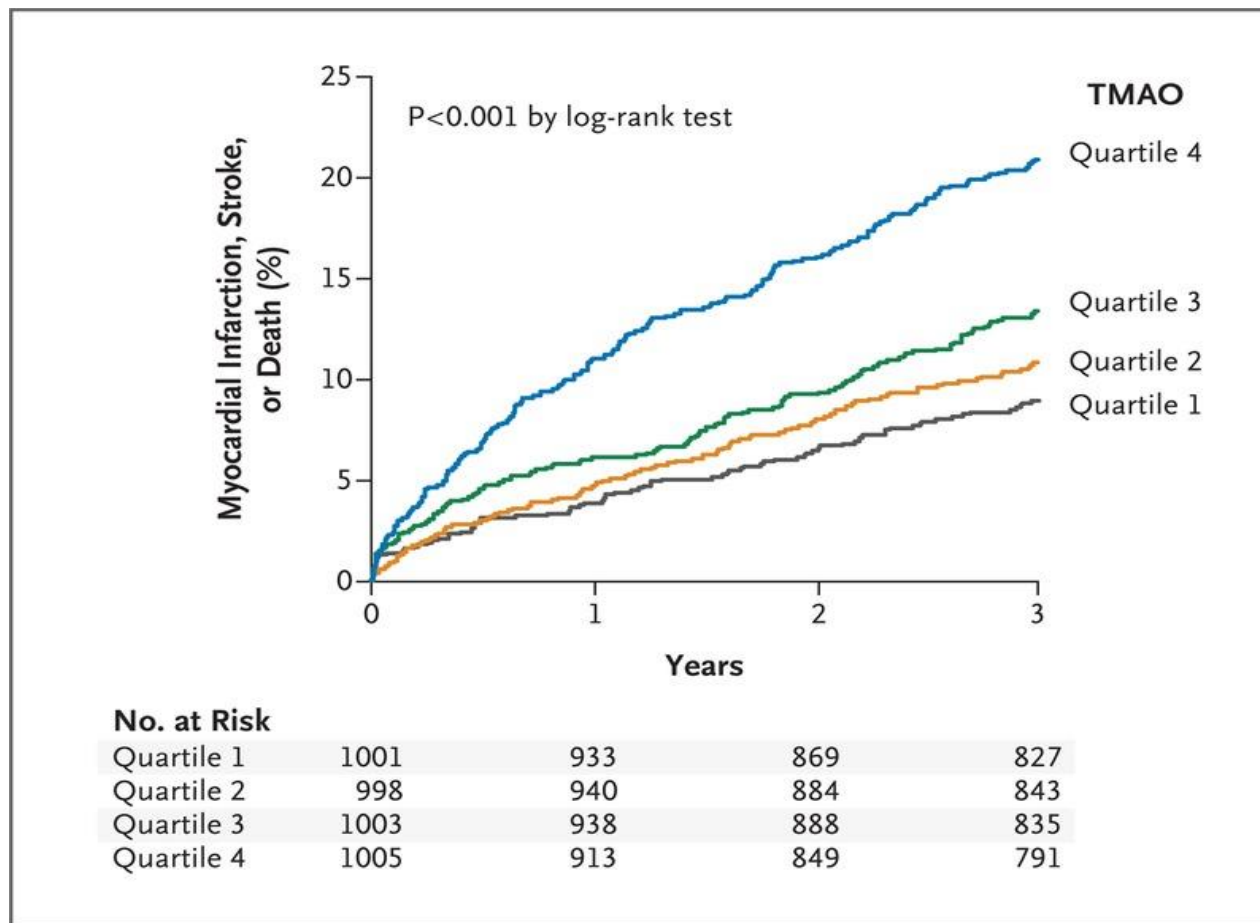
VOL. 368 NO. 17

Intestinal Microbial Metabolism of Phosphatidylcholine
and Cardiovascular Risk

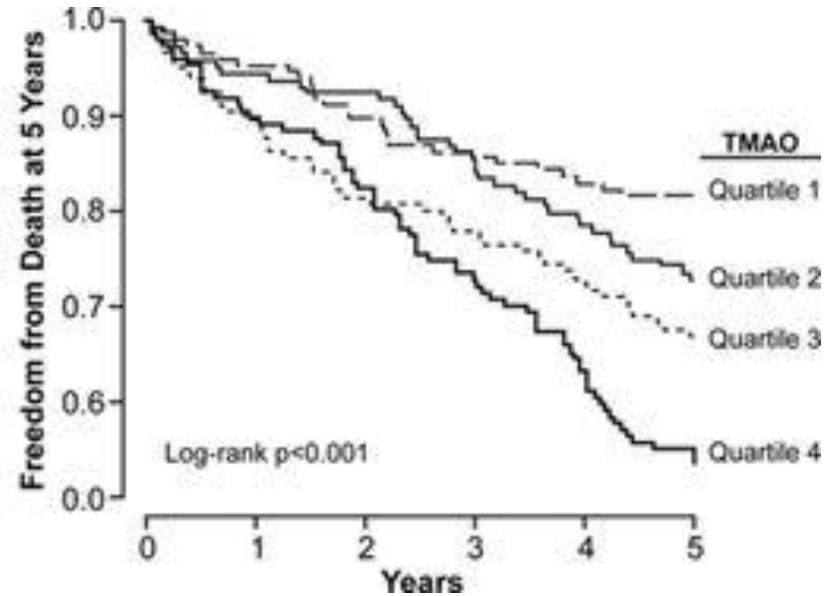
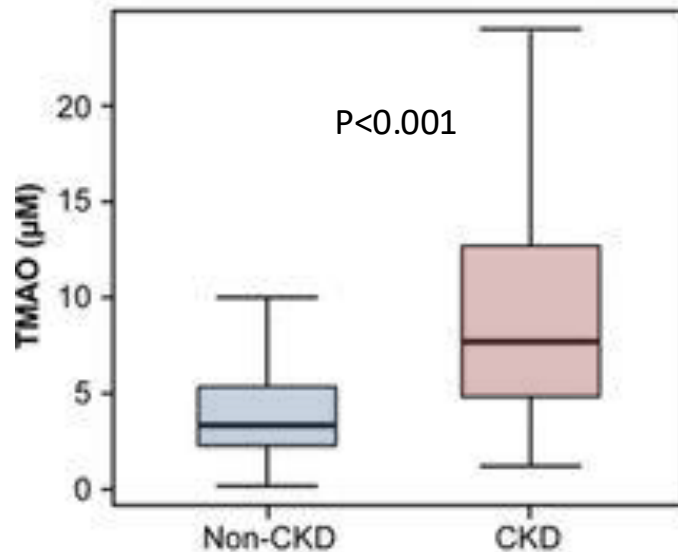
W.H. Wilson Tang, M.D., Zeneng Wang, Ph.D., Bruce S. Levison, Ph.D., Robert A. Koeth, B.S., Earl B. Britt, M.D.,
Xiaoming Fu, M.S., Yuping Wu, Ph.D., and Stanley L. Hazen, M.D., Ph.D.



Kaplan–Meier Estimates of Major Adverse Cardiovascular Events, According to the Quartile of TMAO Level



TMAO et maladie rénale chronique



Quantile 4: HR **1.93** (1.13–3.29)

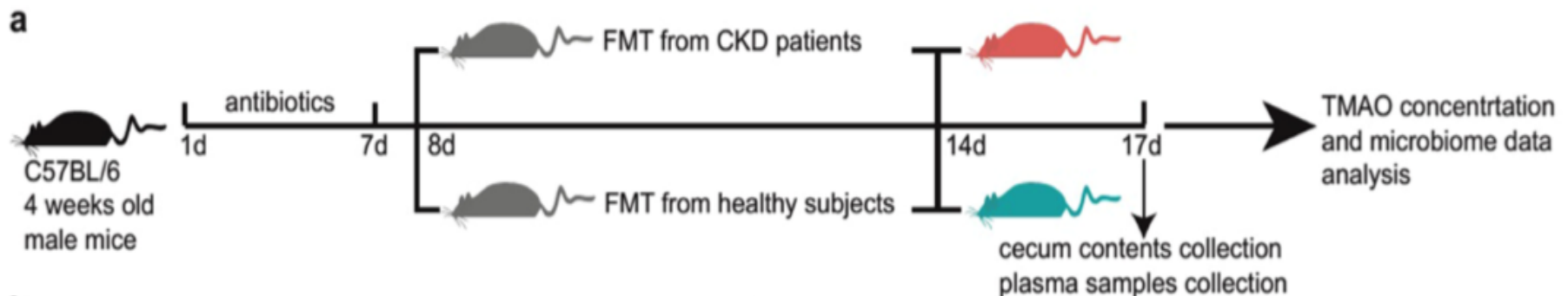


W.H. Wilson Tang. Circulation Research. Gut Microbiota-Dependent Trimethylamine N-Oxide (TMAO) Pathway Contributes to Both Development of Renal Insufficiency and Mortality Risk in Chronic Kidney Disease, Volume: 116, Issue: 3, Pages: 448-455, DOI: (10.1161/CIRCRESAHA.116.305360)

Fecal microbiota transplantation

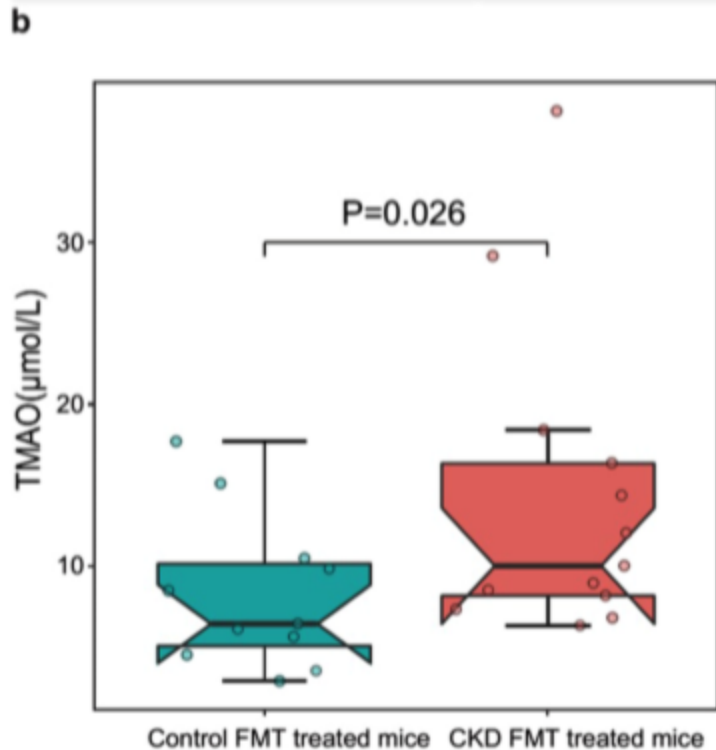
Impaired renal function and dysbiosis of gut microbiota contribute to increased trimethylamine-N-oxide in chronic kidney disease patients

Kai-Yu Xu¹, Geng-Hong Xia¹, Jun-Qi Lu^{2,3}, Mu-Xuan Chen³, Xin Zhen⁴, Shan Wang³, Chao You¹, Jing Nie⁴, Hong-Wei Zhou³ & Jia Yin¹

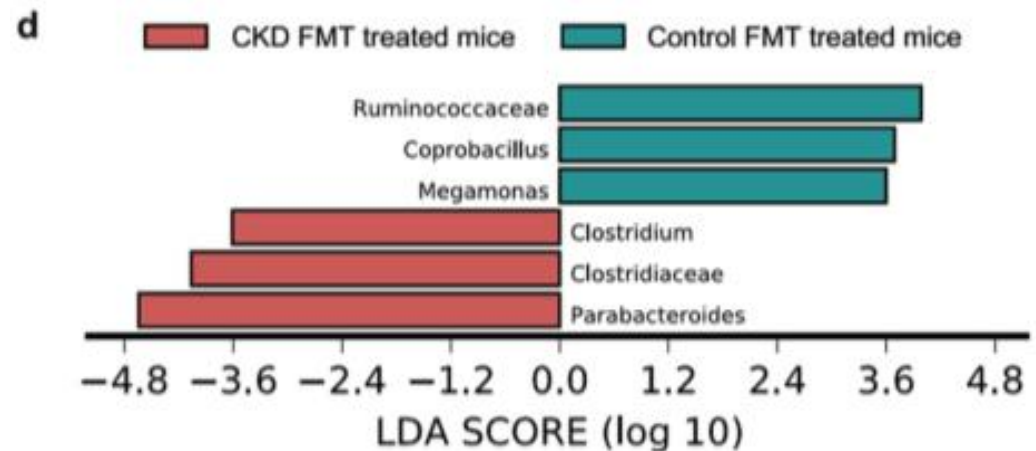


Fecal microbiota transplantation

Transplantation of the CKD patient microbiota induced an increased TMAO level and dysbiosis of the gut microbiota in antibiotic-treated mice. To investigate whether changes in the gut microbiome contributed to the increased TMAO level in the CKD patients, we subjected antibiotic-treated mice to the FMT technique (Fig. 4a)²¹. The mouse group that received the gut microbiota from the CKD patients showed a significantly higher TMAO concentration compared to the group that received faecal microbes from the healthy controls (Mann-Whitney U test, $P = 0.026$) (Fig. 4b).



« Sélection » de souches produisant des toxines urémiques



Modification de la perméabilité intestinale

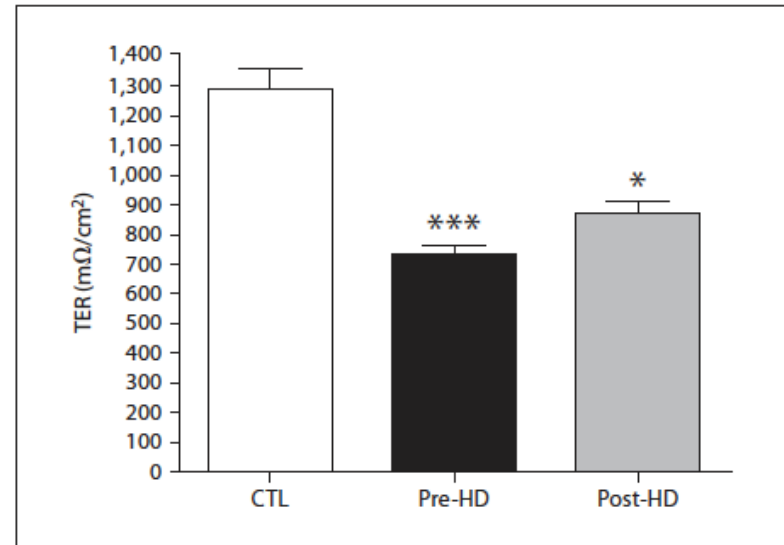
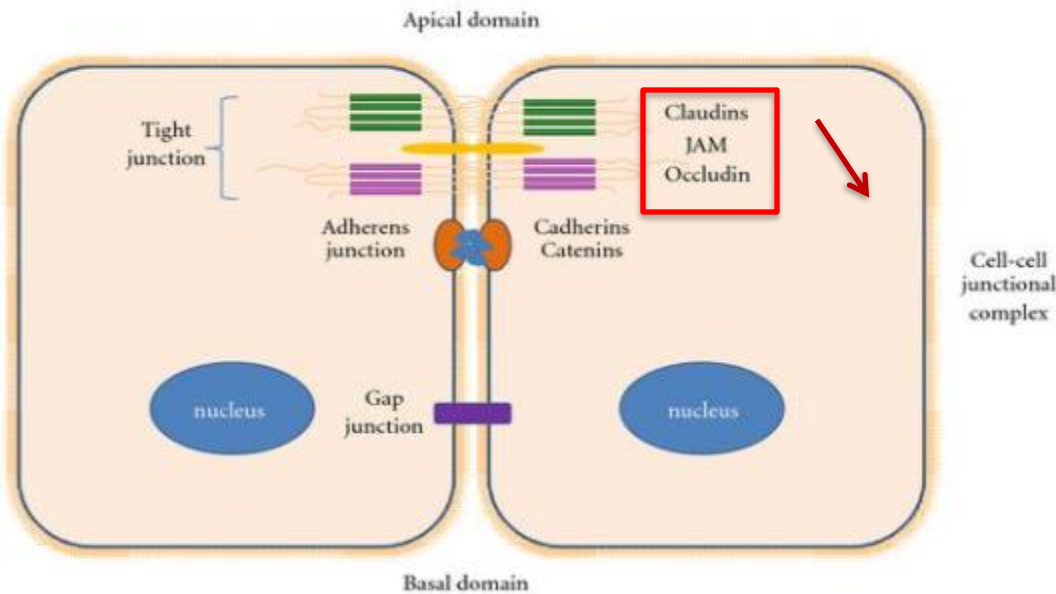
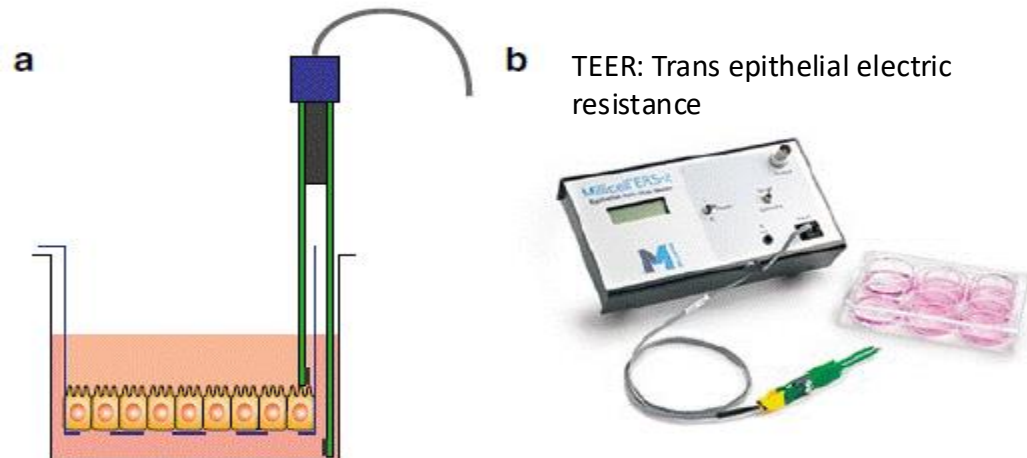


Fig. 1. Bar graph depicting TER in intestinal epithelial T84 cell monolayers exposed to media containing plasma obtained from healthy control (CTL) individuals and those containing prehemodialysis (Pre-HD) and posthemodialysis (Post-HD) plasma samples from ESRD patients. CTL vs. pre-HD, $p = 0.0003$; pre-HD vs. post-HD, $p = 0.027$.

Vaziri et al, Am J of Nephrol, 2012 36(5):438-443



Vaziri ND, Yuan J, Norris K. Role of Urea in Intestinal Barrier Dysfunction and Disruption of Epithelial Tight Junction in Chronic Kidney Disease. American Journal of Nephrology, 2013.

Modification de la perméabilité intestinale

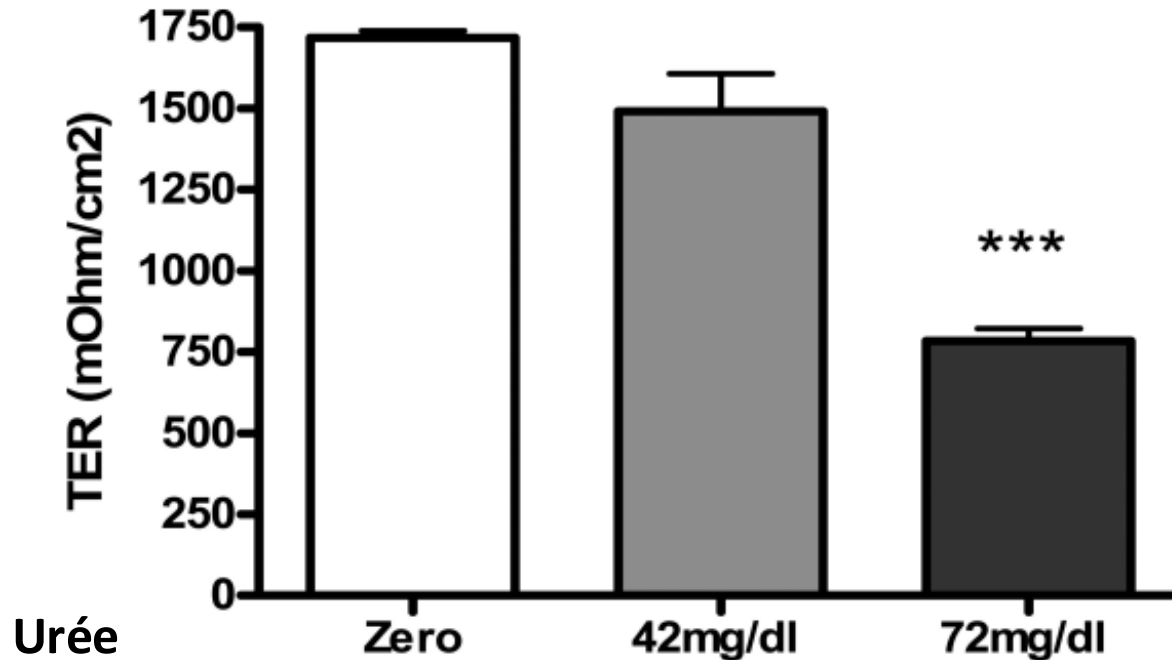
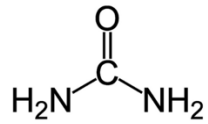


Figure 1.
Bar graphs depicting the trans-epithelial electrical resistance (TER) in intestinal epithelial T84 cell monolayers incubated for 24 hr in regular media and those incubated in media containing 42 or 72 mg/dl urea.

Vaziri ND, Yuan J, Norris K. Role of Urea in Intestinal Barrier Dysfunction and Disruption of Epithelial Tight Junction in Chronic Kidney Disease. *American Journal of Nephrology*, 2013.

Modification de la perméabilité intestinale



Urée plasmatique



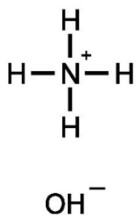
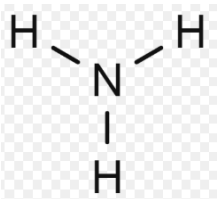
Passage dans la lumière intestinale



Ammoniac



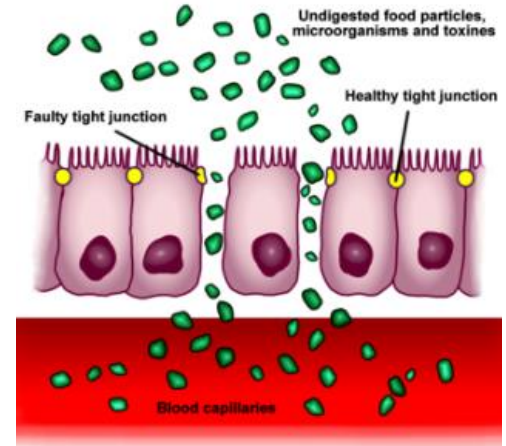
Ammonium hydroxyde



Uréeses microbiennes



Baisse du pH



Augmentation perméabilité intestinale

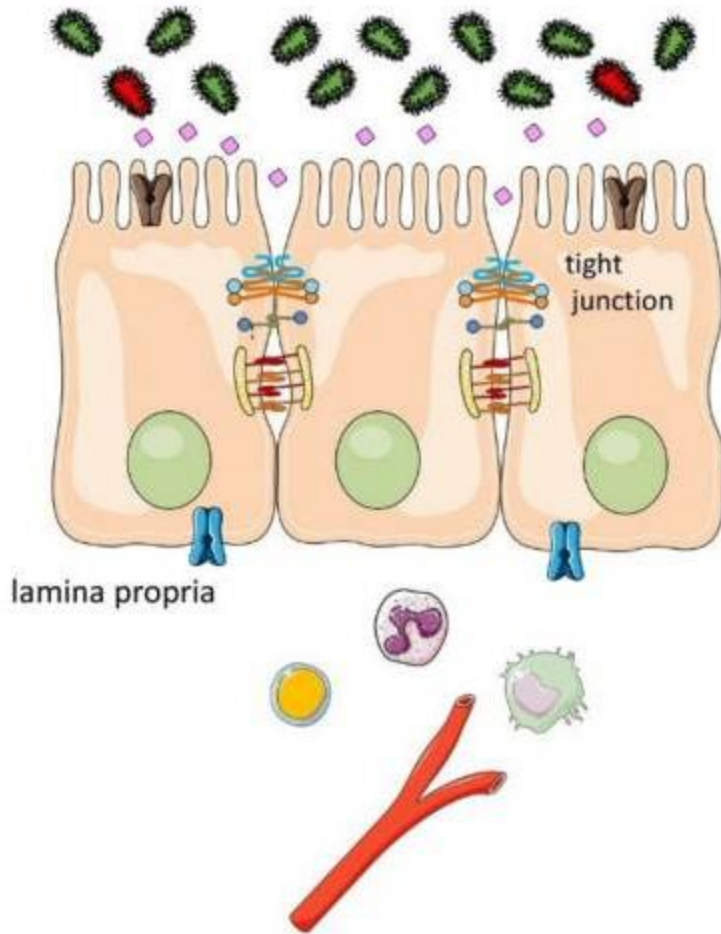


Inflammation intestinale

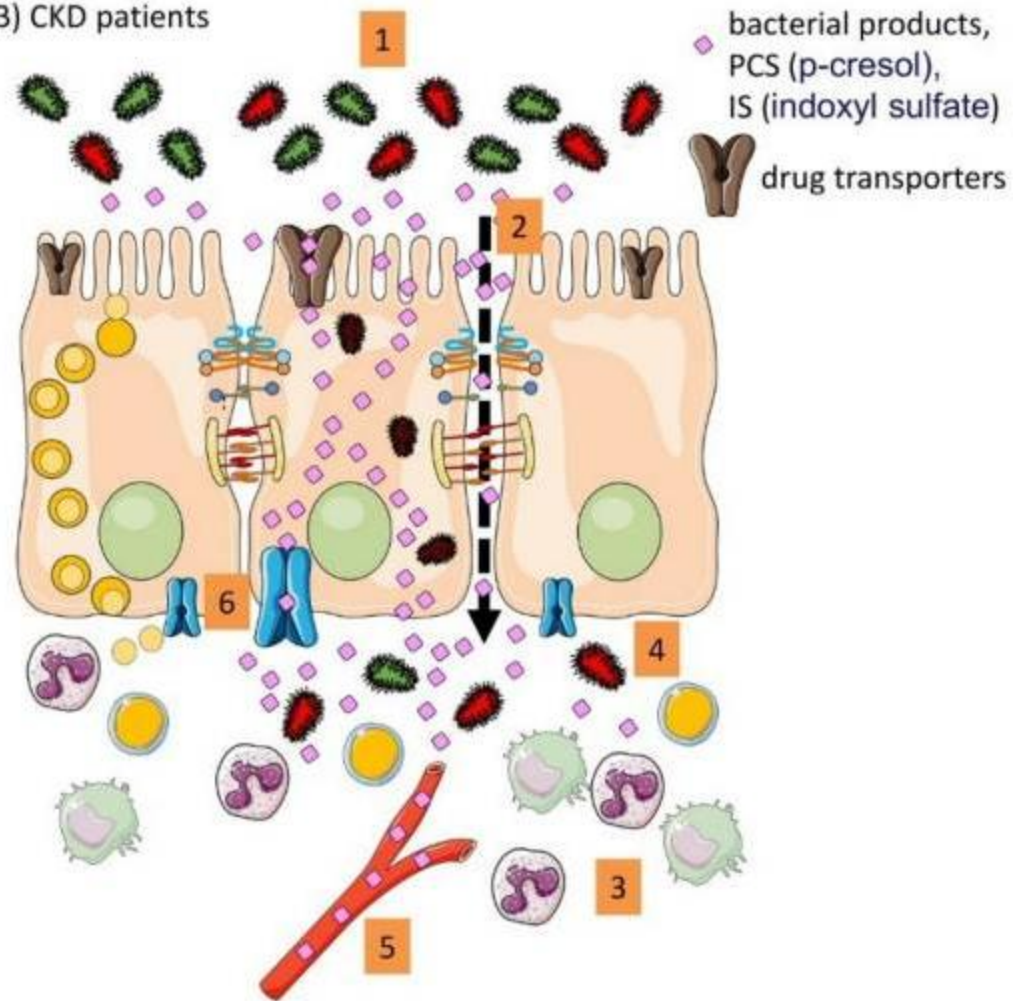


Modification de la perméabilité intestinale (« leaky gut »)

A) Healthy



B) CKD patients



Low grade inflammation

IRC et inflammation chronique de bas grade

Table 2. Plasma levels of inflammatory markers and acute phase proteins by eGFR category

Biomarker	eGFR (ml/min per 1.73 m ²) (n=3939)					P Value ^a
	>60 (n=412)	50–59 (n=754)	40–49 (n=1042)	30–39 (n=967)	<30 (n=764)	
Acute phase protein						
hs-CRP (mg/L)	1.9 (0.9, 4.2)	2.5 (1.0, 6.8)	2.7 (1.1, 6.0)	2.8 (1.2, 7.1)	2.8 (1.1, 7.1)	<0.001
Fibrinogen (g/L)	3.5 (3.0, 4.1)	3.9 (3.3, 4.5)	4.0 (3.4, 4.7)	4.2 (3.5, 5.0)	4.5 (3.8, 5.4)	<0.001
Albumin (g/dl)	4.1 (3.8, 4.3)	4.0 (3.8, 4.3)	4.0 (3.7, 4.2)	3.9 (3.6, 4.2)	3.9 (3.6, 4.2)	<0.001
Cytokines						
IL-1 β (pg/ml)	0 (0, 0.7)	0 (0, 0.9)	0.2 (0, 1.3)	0.3 (0, 1.4)	0.4 (0, 2.0)	<0.001
IL-1RA (pg/ml)	605.3 (312.6, 1262.8)	636.6 (350.3, 1340.3)	698.7 (389.6, 1529.8)	805.9 (448.8, 1642.6)	850.5 (426.6, 1771.6)	<0.001
IL-6 (pg/ml)	1.2 (0.7, 2.1)	1.6 (1.0, 2.6)	1.8 (1.2, 2.9)	2.2 (1.3, 3.5)	2.4 (1.5, 3.9)	<0.001
TNF- α (pg/ml)	1.4 (1.0, 2.1)	1.7 (1.2, 2.6)	2.1 (1.5, 2.9)	2.5 (1.8, 3.5)	3.0 (2.2, 4.1)	<0.001
TGF- β (pg/ml)	11.3 (6.3, 19.4)	10.1 (5.8, 16.6)	11.4 (6.8, 18.2)	11.2 (6.9, 18.1)	10.6 (6.4, 17.5)	0.01

Values are presented as the median (interquartile range). A value of 0 indicates that it was below the detection limit. P values <0.001 were significant after Bonferroni correction for multiple comparisons. eGFR, estimated GFR; hs-CRP, high-sensitivity C-reactive protein; IL-1RA, IL-1 receptor antagonist.

^aKruskal-Wallis test between categories of eGFR

IRC et inflammation chronique de bas grade

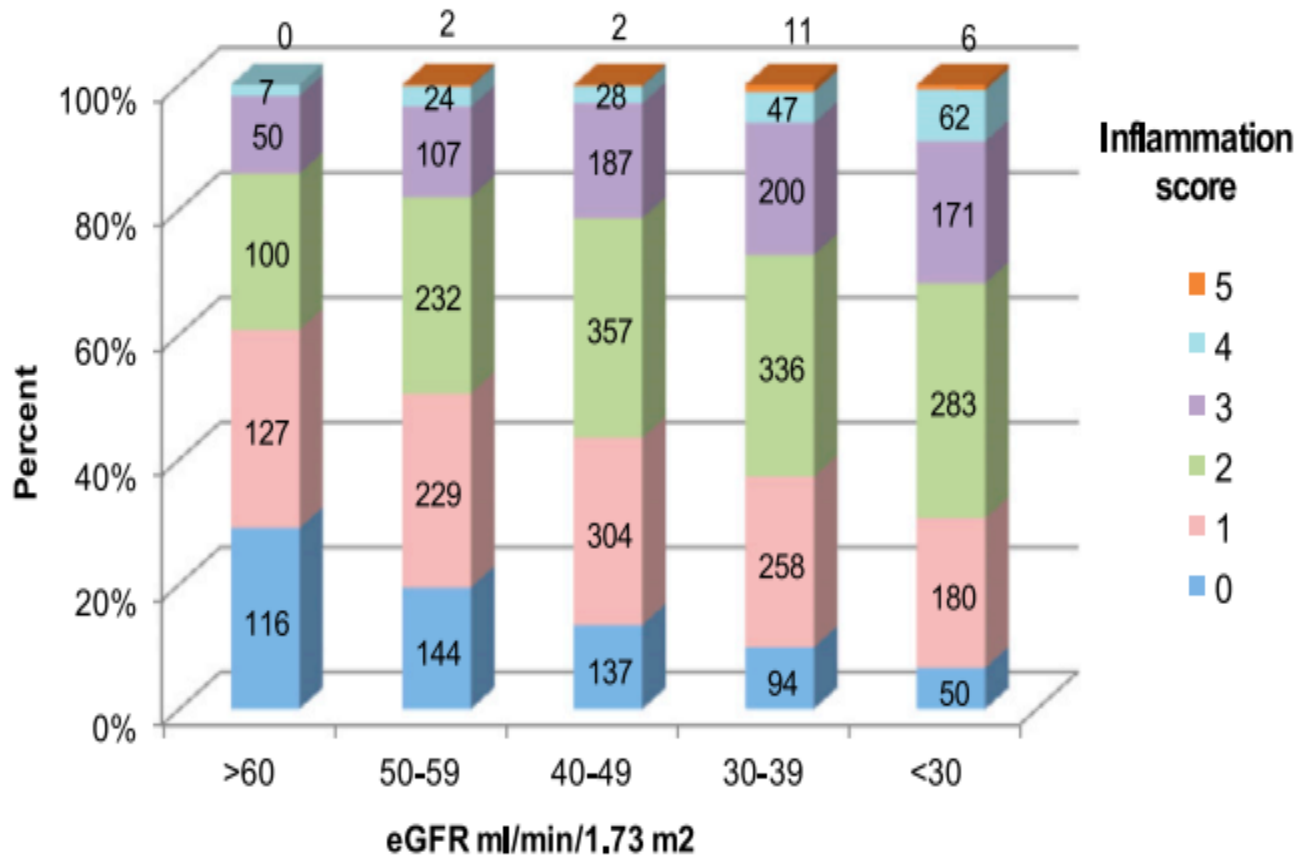
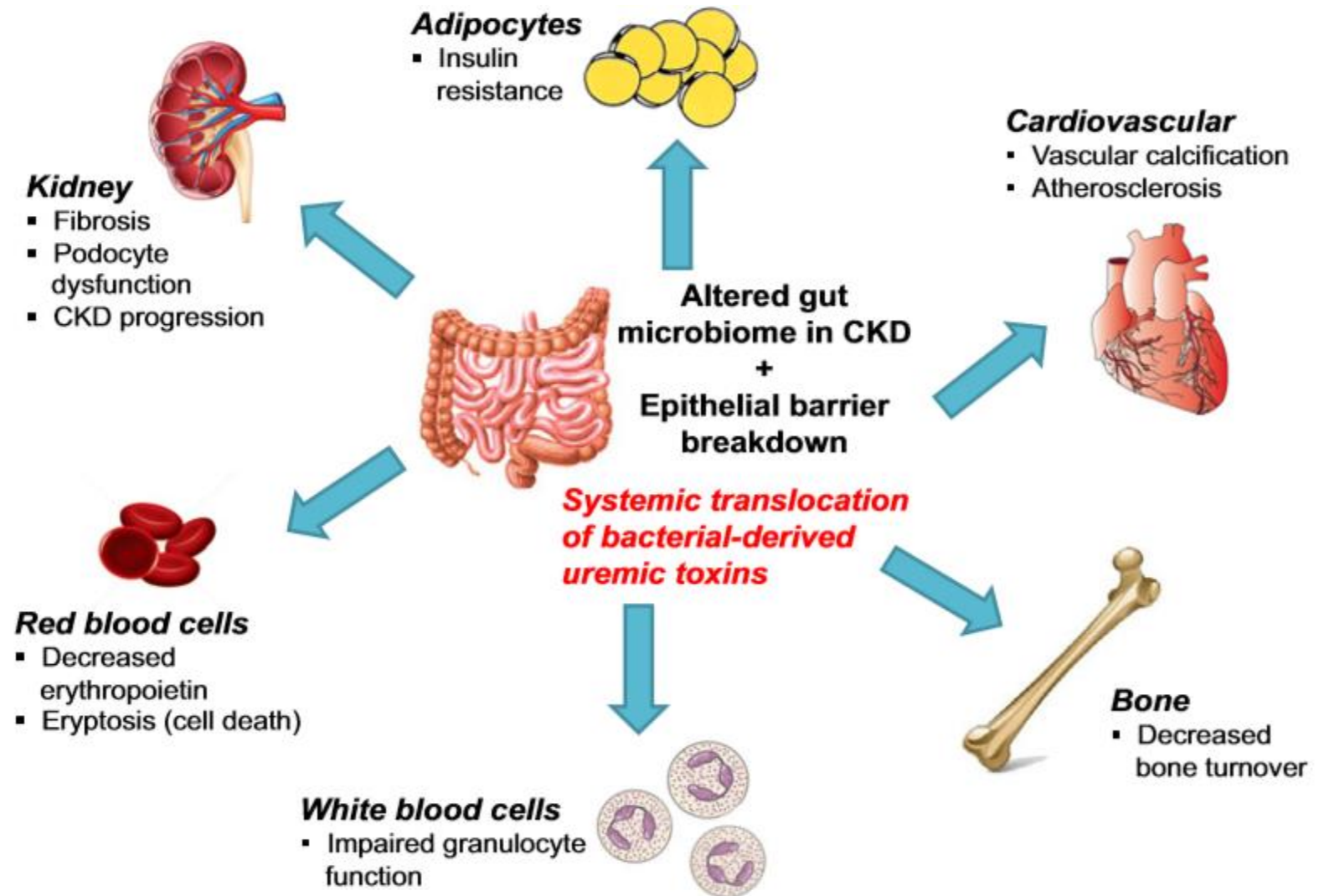


Figure 1. | Distribution of inflammation score by categories of eGFR (n=3850). P value for inflammation score among categories of eGFR <0.001 by Kruskal–Wallis test. eGFR, estimated GFR.

Altération du microbiote intestinal au cours de l'IRC



Lau WL & al. Altered microbiome in chronic kidney disease: systemic effects of gut-derived uremic toxins. Clinical Science. 2018.

Colonic Transit Time in Long-Term Dialysis Patients

Ming-Ju Wu, MD, Chi-Sen Chang, MD, Chi-Hung Cheng, MD, Cheng-Hsu Chen, MD, Wen-Chin Lee, MD, Yu-Huang Hsu, MD, Kuo-Hsiung Shu, MD, and Ming-Jer Tang, MD, PhD

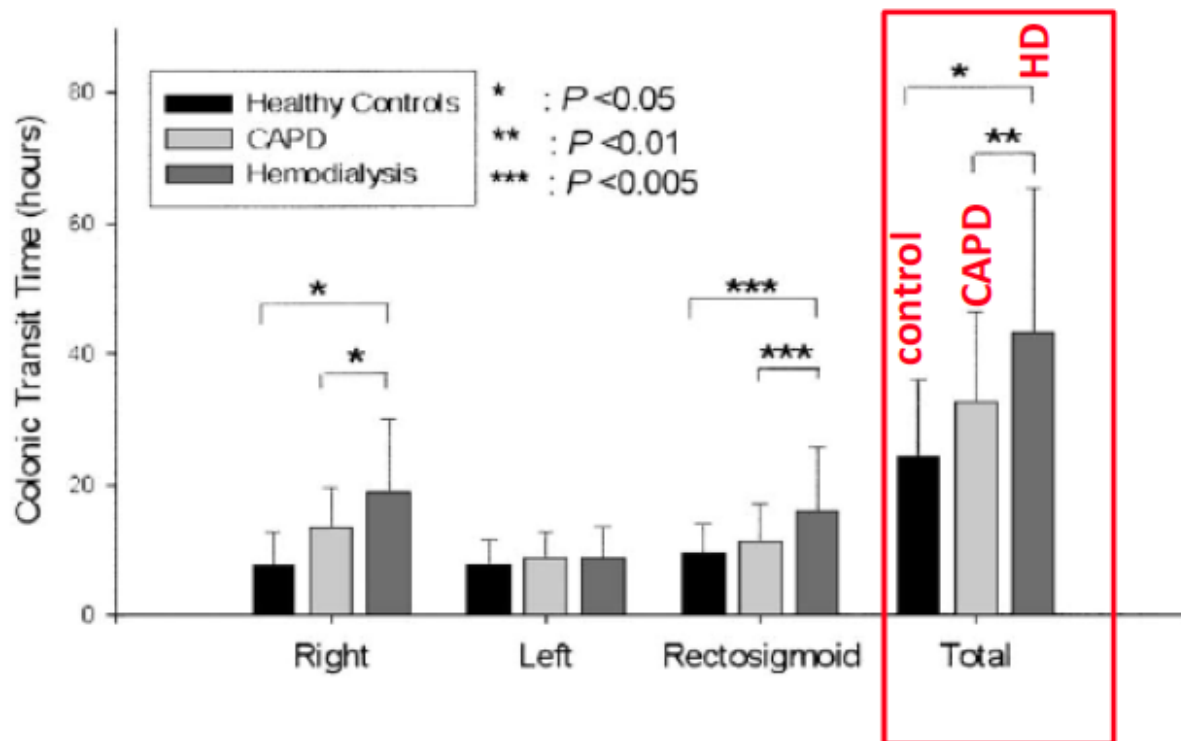


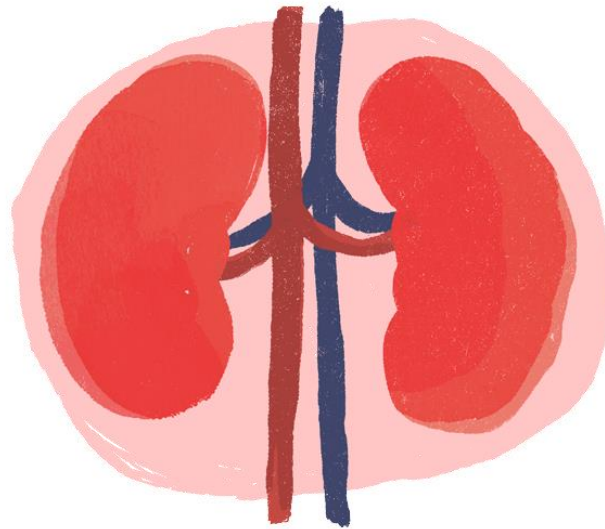
Fig 2. Total and segmental colonic transit times in dialysis patients and controls.

Gut, the forgotten organ in uremia ???

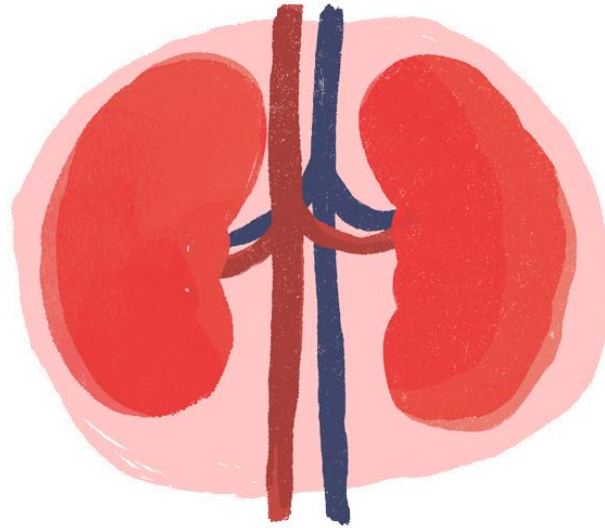


- Many gut derived uremic toxins
- Dysbiosis= alteration of gut microbiota in CKD
- Increased paracellular intestinal permeability (“leaky gut”)
- Source of inflammation +++

Toxines urémiques et complication de la MRC



Effets métaboliques
Effets sur le système cardio-vasculaire



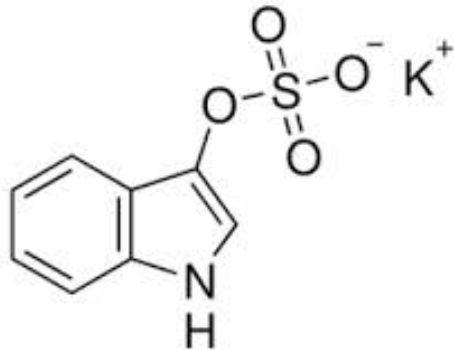
Exemple de toxicité:

Toxicité cardiovasculaire du p-crésyl sulfate (p-CS) et de l'indoxyl-sulfate (IS)

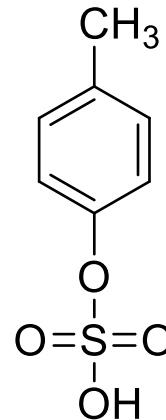
2 major uremic toxins: Indoxyl sulfate & p-cresyl-sulfate

Uremic toxins (or uremia retention solutes) are compounds that accumulate in CKD patients due to a defect in their renal clearance and exert biological deleterious effect(s)

Indoxyl sulfate

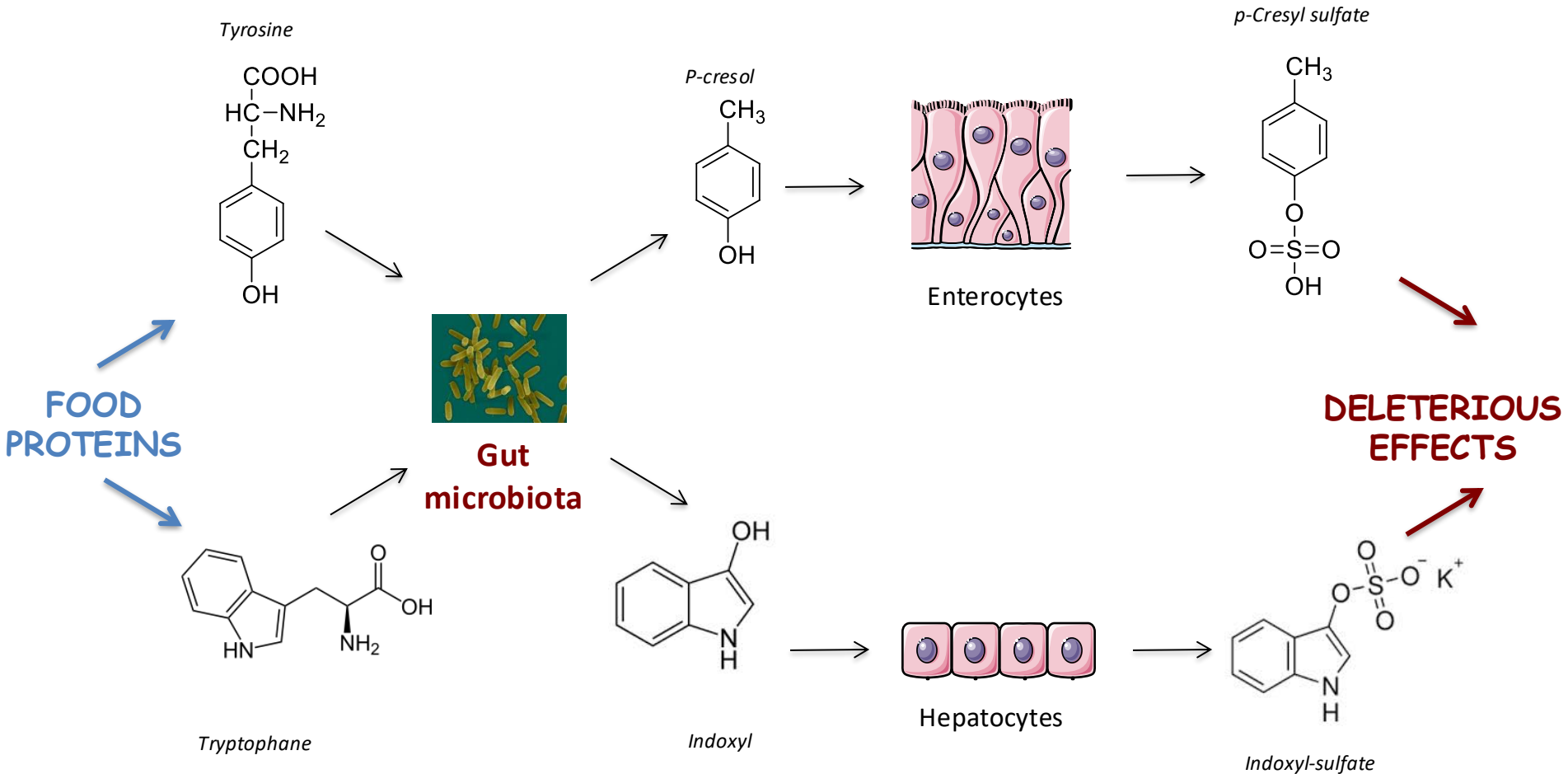


p-Cresyl sulfate



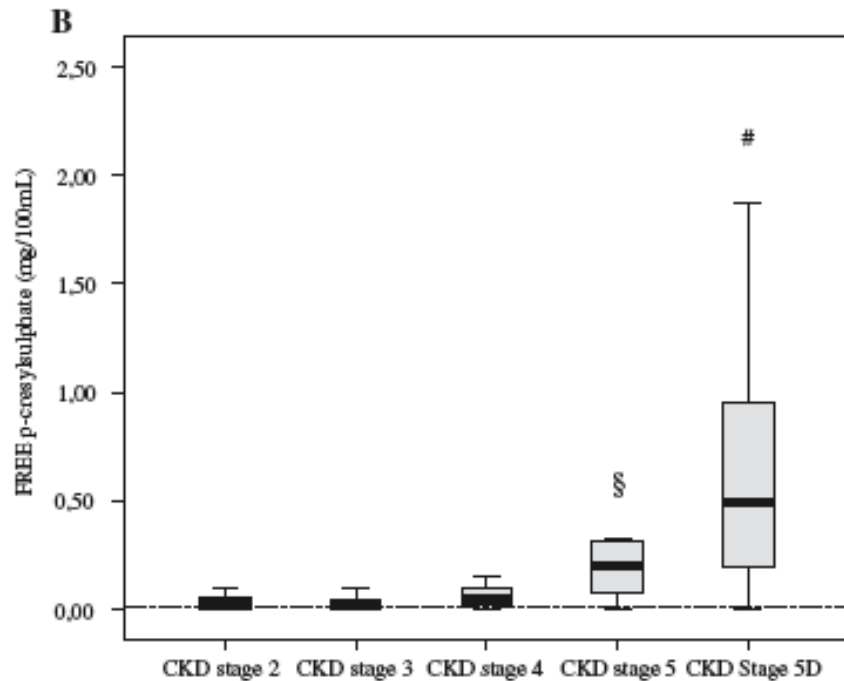
- Plasma concentration is highly correlated with mortality in CKD patients
- Mostly bound to plasma proteins (>90%)
- **POORLY REMOVED BY HEMODIALYSIS**

Indoxyl /p-cresyl sulfate are (only) produced in the gut by the microbiota

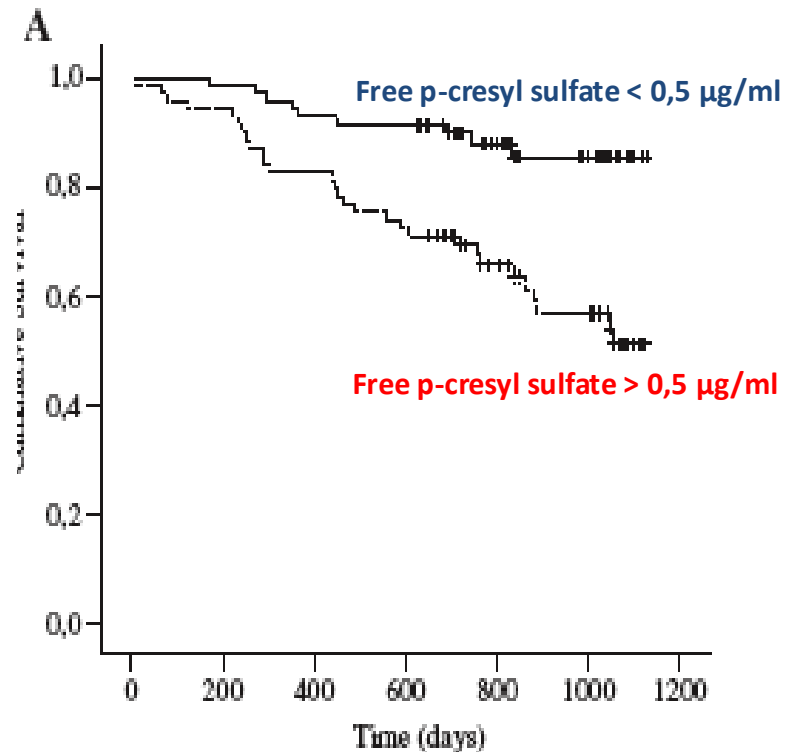


p-cresyl-sulfate and mortality in CKD patients

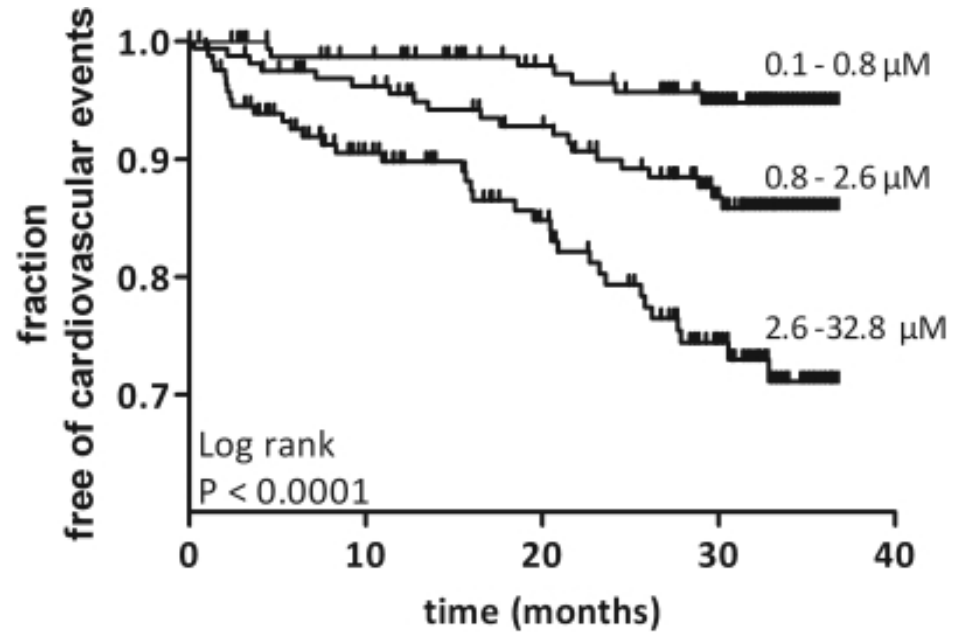
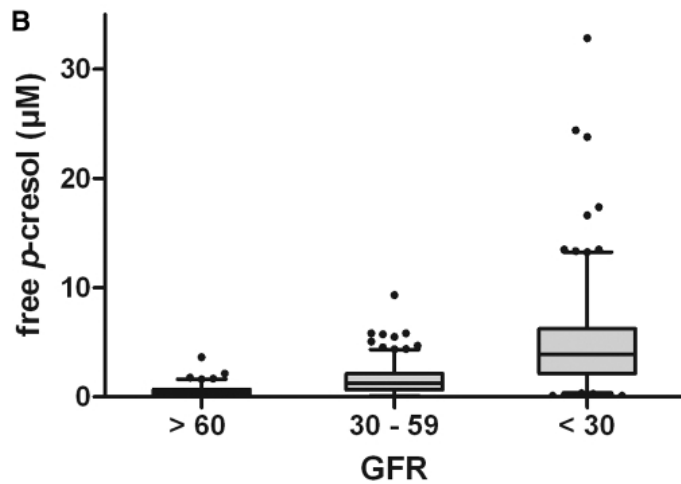
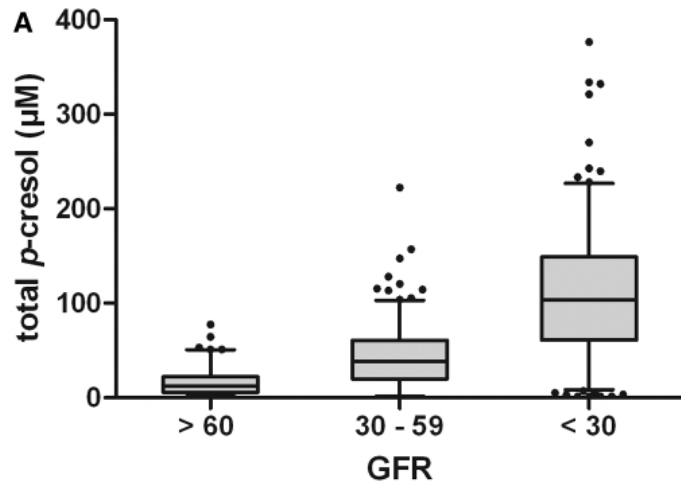
P-cresyl sulfate accumulates in ESRD



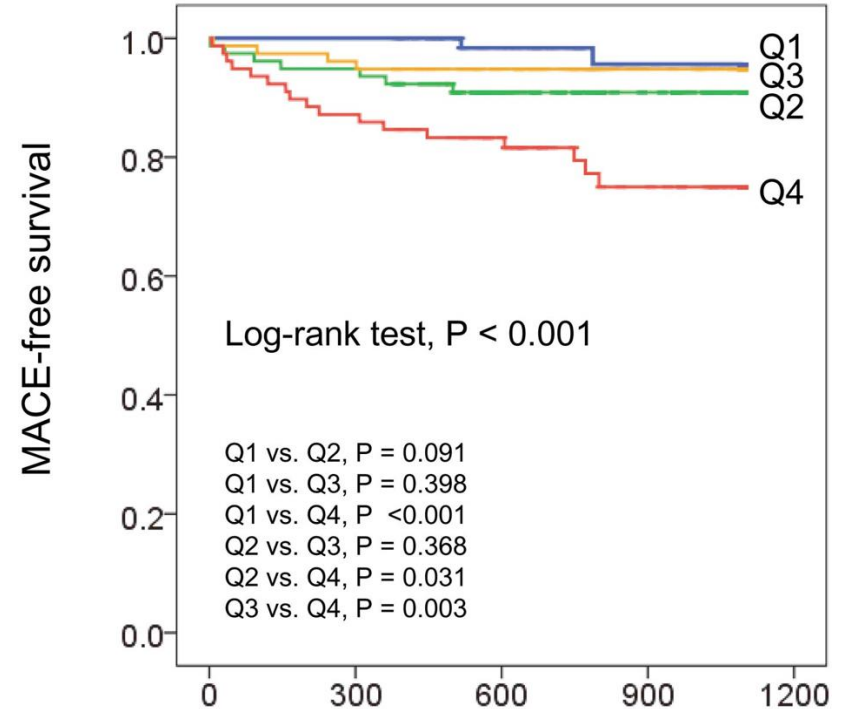
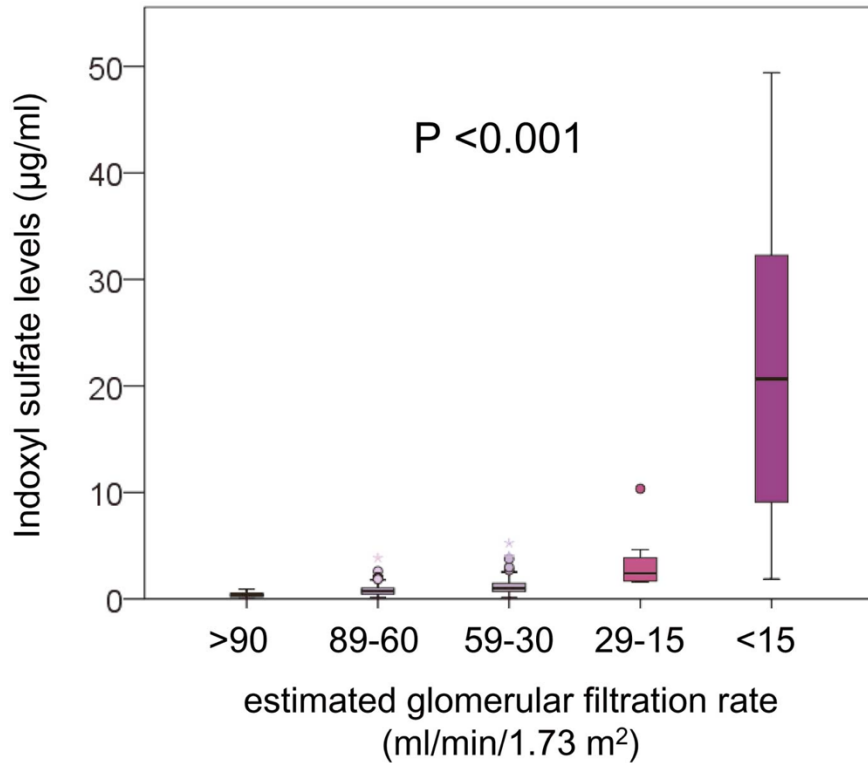
Free p-cresyl sulfate is a predictor of mortality in CKD patients



p-Cresol level predicts cardiovascular risk in CKD patients



Indoxyl-sulfate and mortality in CKD patients

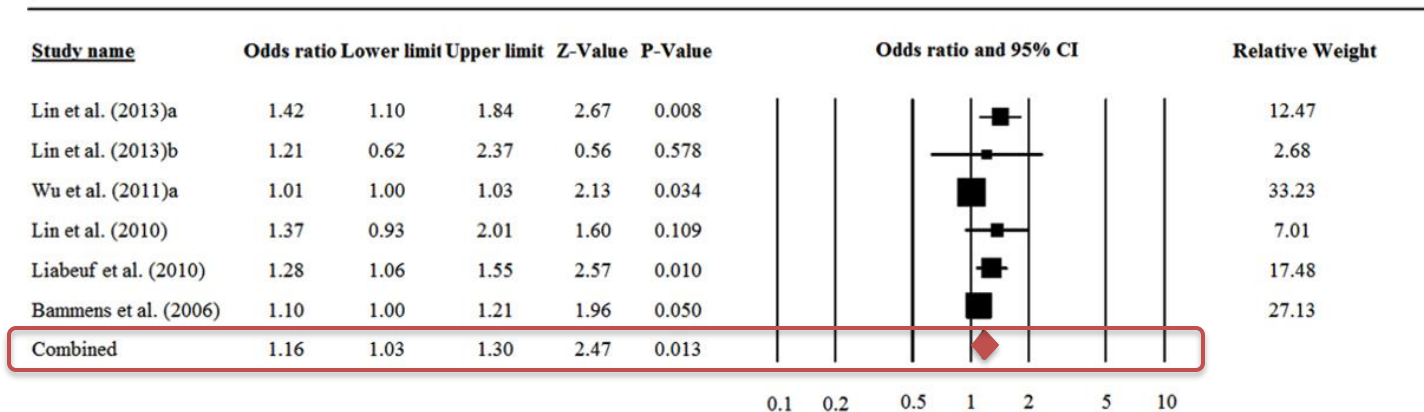


No. at risk

Q1	78	78	54	31	22
Q2	78	74	51	31	15
Q3	77	74	56	25	15
Q4	78	68	51	31	17

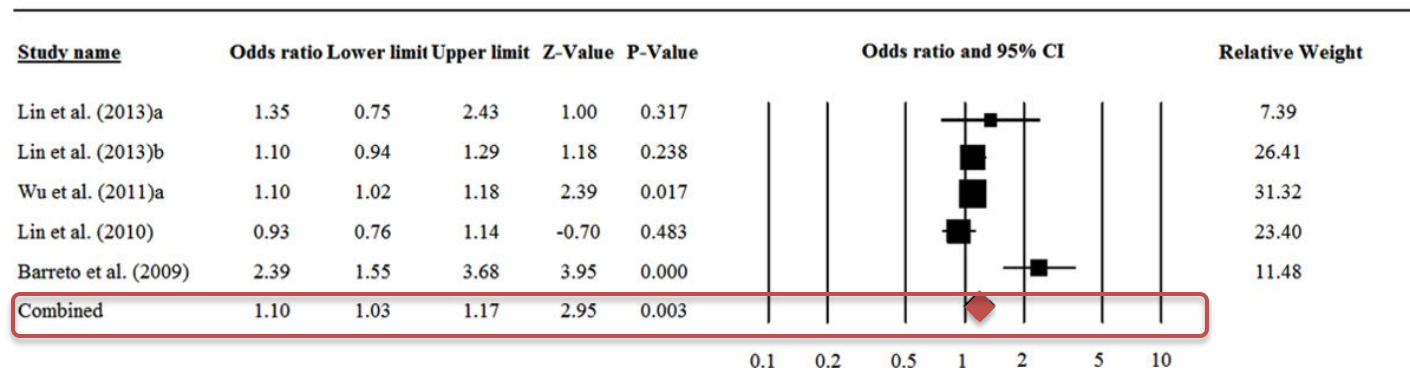
Meta-analysis: Associations between free IS or pCS and all-cause mortality

(A) Free PCS



Heterogeneity test: $Q = 17.55$, $df = 5$, $P = 0.004$, $I^2 = 71.51\%$

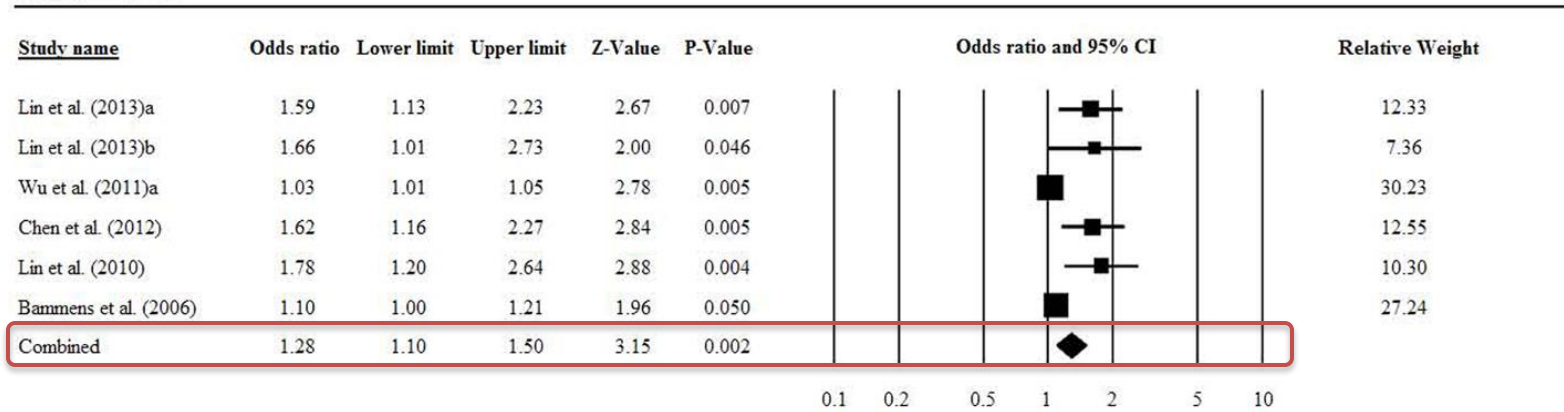
(B) Free IS



Heterogeneity test: $Q = 15.49$, $df = 4$, $P = 0.004$, $I^2 = 74.18\%$

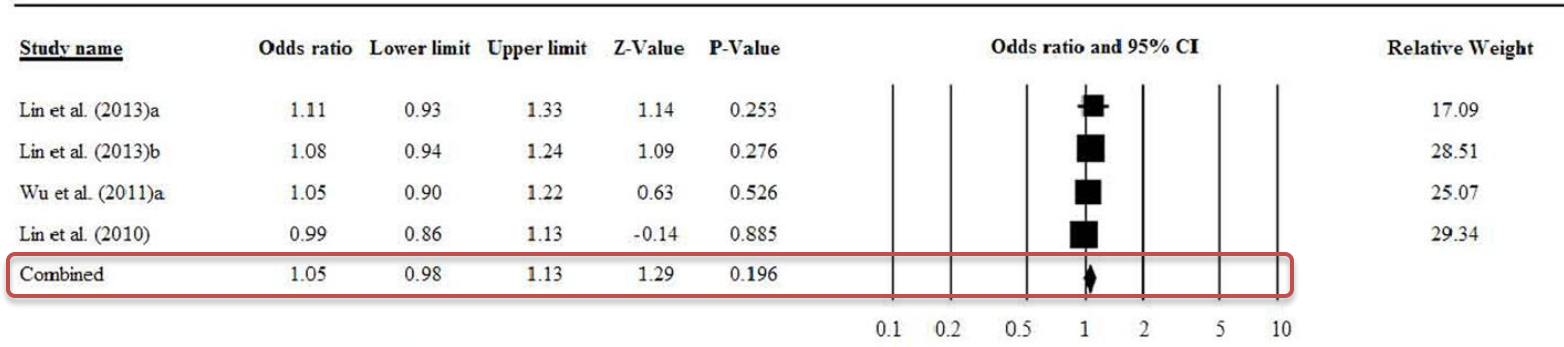
Meta-analysis: Associations between free IS or pCS and cardiovascular events

(A) Free PCS



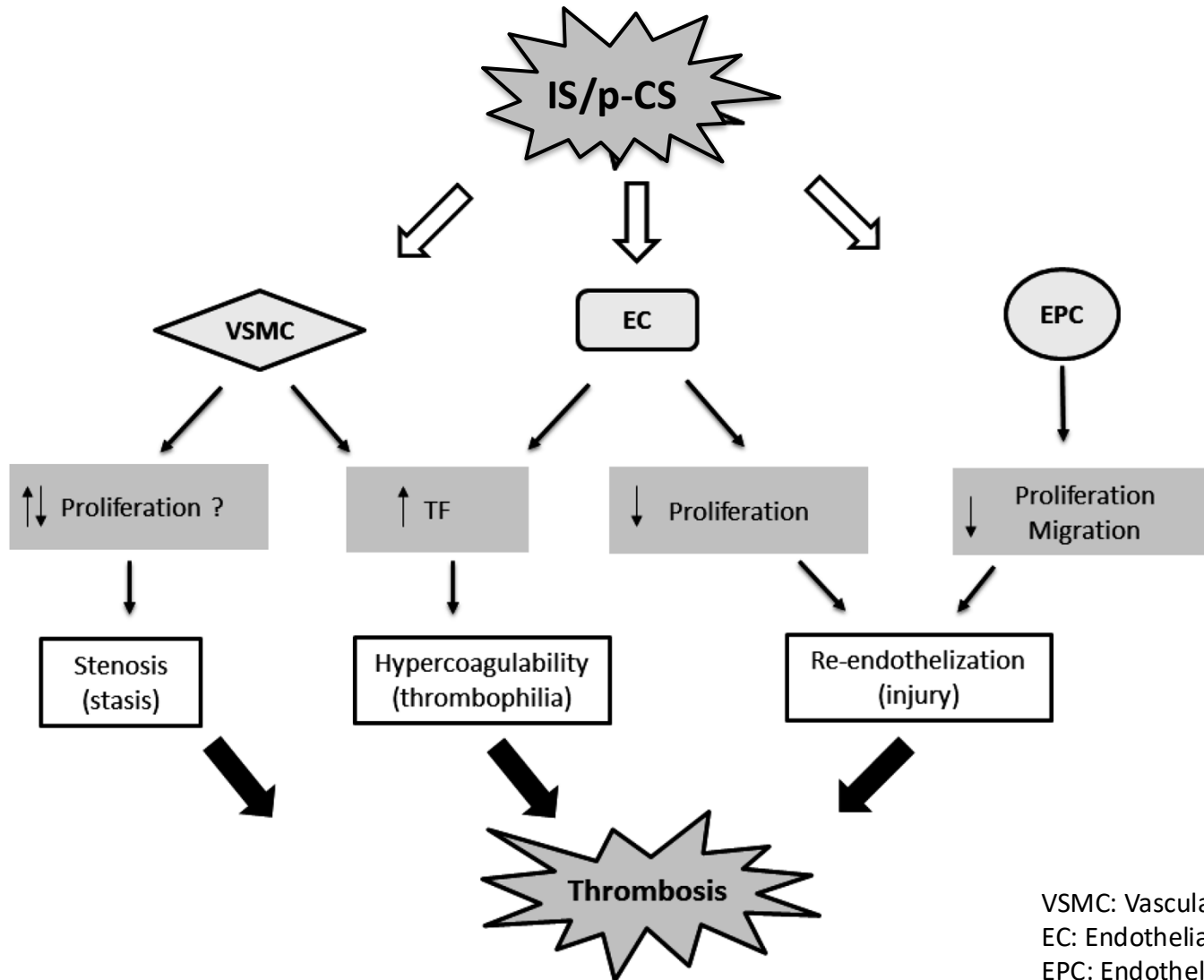
Heterogeneity test: $Q = 25.92$, $df = 5$, $P < 0.001$, I-square = 80.7%

(B) Free IS



Heterogeneity test: $Q = 1.24$, $df = 3$, $P = 0.743$, I-square = 0.0%

IS/pCS and cardiovascular dysfunction



pCS impairs reendothelialization after lesion...

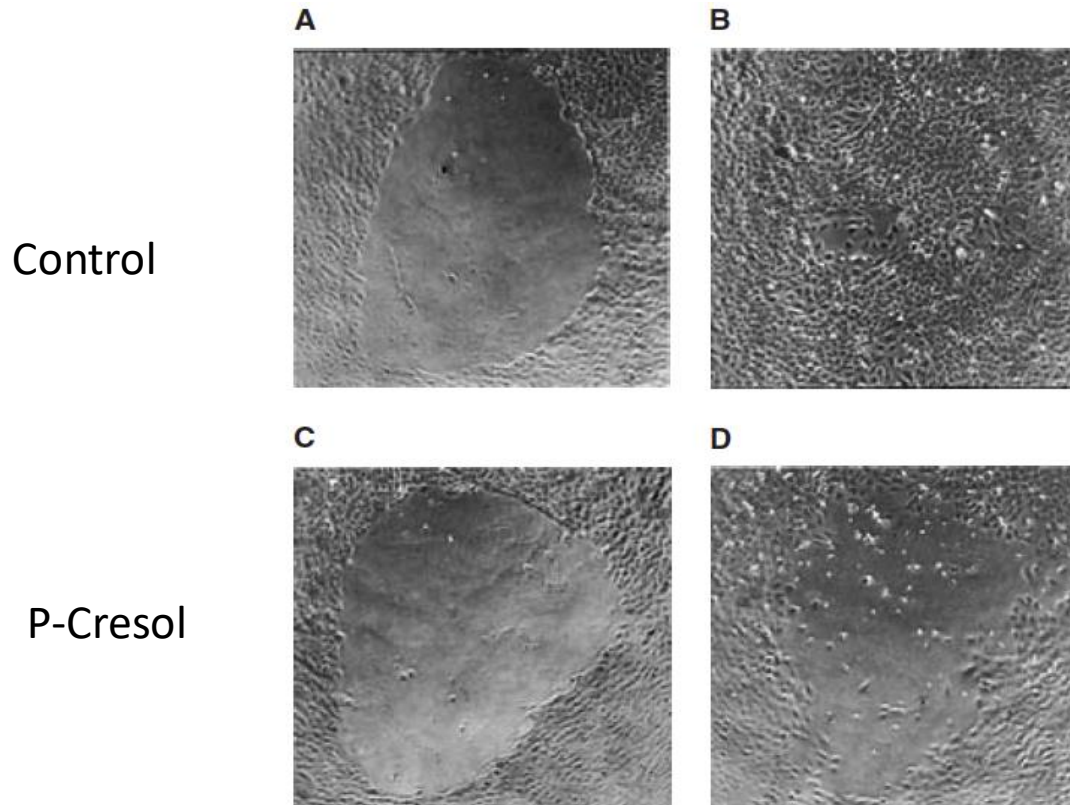


Fig. 6. Pictures of endothelial wound repair in presence of p-cresol. Endothelial monolayers were wounded, incubated with control medium or with p-cresol, and endothelial wound repair was analyzed under videomicroscopy. Pictures of wounds before addition of control medium (A) or p-cresol (C). Pictures of wounds after a 24-hour incubation with control medium (B) or with p-cresol at 50 µg/mL (D). In presence of control medium, the original wound was almost completely repaired after 24 hours of incubation (A and B). In presence of p-cresol, wound repair was inhibited (C and D).

pCS impairs reendothelialization after lesion...

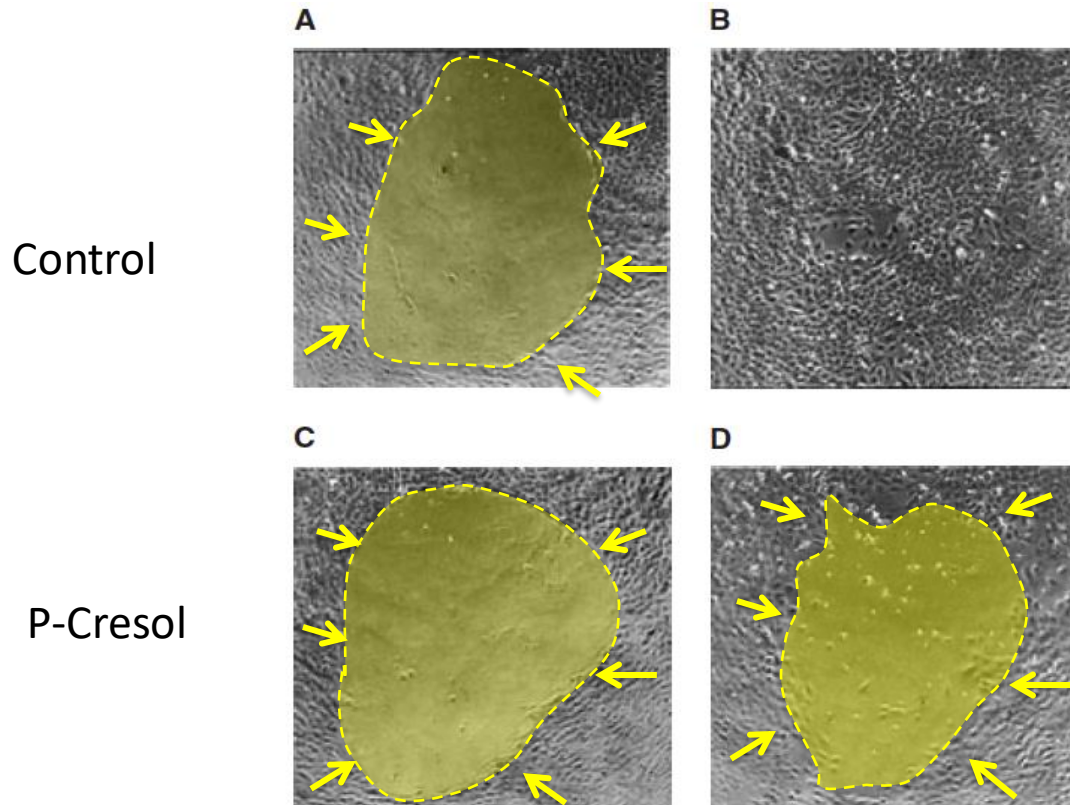
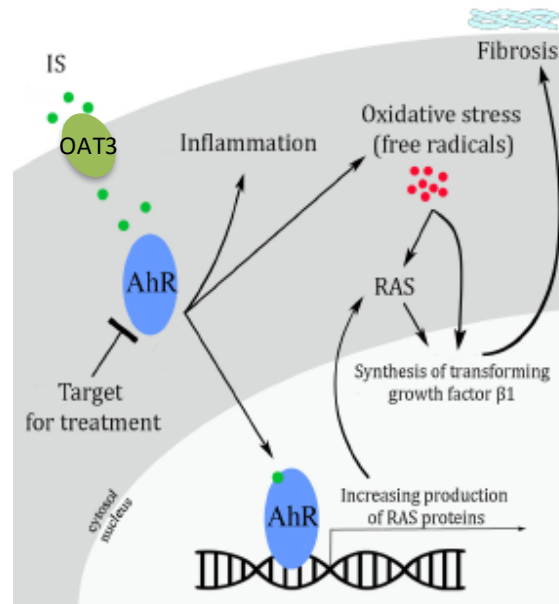


Fig. 6. Pictures of endothelial wound repair in presence of p-cresol. Endothelial monolayers were wounded, incubated with control medium or with p-cresol, and endothelial wound repair was analyzed under videomicroscopy. Pictures of wounds before addition of control medium (A) or p-cresol (C). Pictures of wounds after a 24-hour incubation with control medium (B) or with p-cresol at 50 µg/mL (D). In presence of control medium, the original wound was almost completely repaired after 24 hours of incubation (A and B). In presence of p-cresol, wound repair was inhibited (C and D).

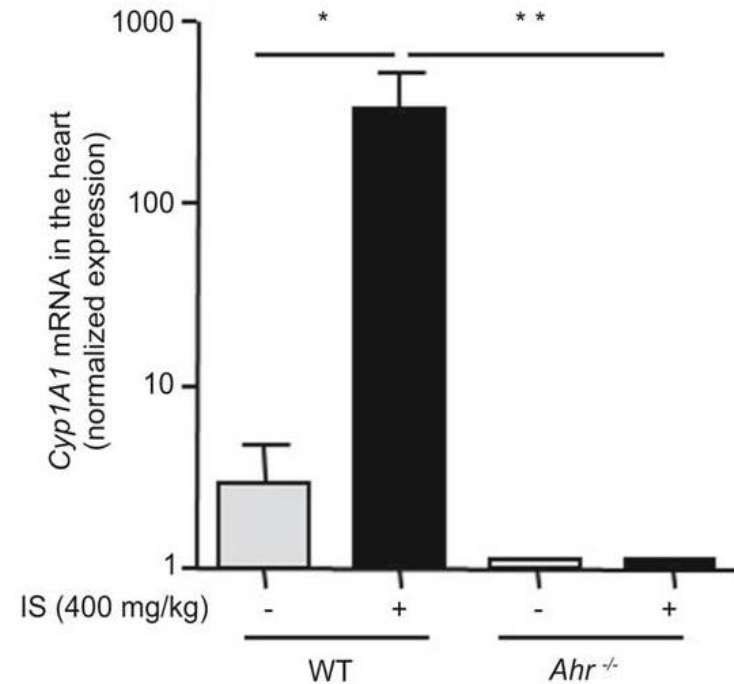
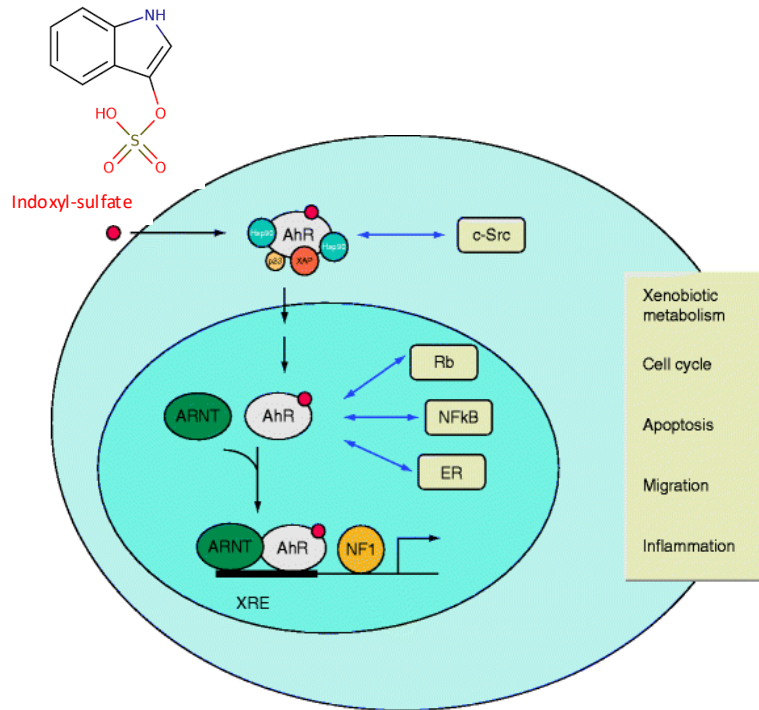
Les effets biologiques de l'indoxy-sulfate (IS) sont médiés par sa liaison avec le récepteur AhR

AhR= Aryl hydrocarbon Receptor = récepteur aux dioxines



The mechanism by which IS (indoxyl sulfate) causes cell damage through the activation of the AhR (aryl hydrocarbon receptor) and RAS (renin-angiotensin system). In cultured cell and animal models of chronic kidney disease (CKD), Sun et al.[3] found that IS causes the activation of the RAS, which in turn leads to an inflammatory reaction, increasing levels of free radicals, and the synthesis of proteins causing fibrosis. Watanabe et al.[4] using a human umbilical cord blood vessel model, showed that IS induces inflammation and cellular oxidative stress via AhR activation. The result of these studies together present the hypothesis that upon IS binding, the AhR induces inflammation, oxidative stress, and an increase in the synthesis of RAS proteins, which ultimately enhance fibrosis. Modified from Pernomian and da Silva.[12]

Le récepteur nucléaire AhR « activé » fonctionne comme un facteur de transcription

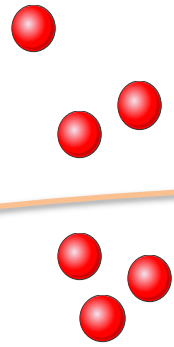
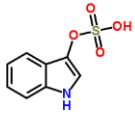


Dou and al. KI 2018

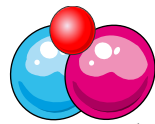
Liaison à des séquences **XRE** (Xenobiotic Response Element) qui contrôle l'expression de gènes de détoxification eg. CYP1A1

A bioassay to monitor AhR activation and detect AhR ligands

Pr Stéphane BURTEY's team, La Timone, Marseille



AhR ligand (indole)



AhR activation

Transactivation

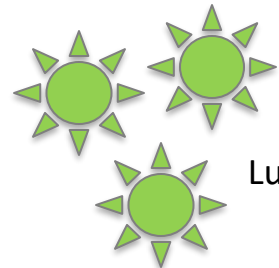
AhR/ARNT



XRE

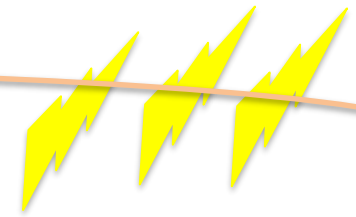
Luciferase gene

Reporter gene

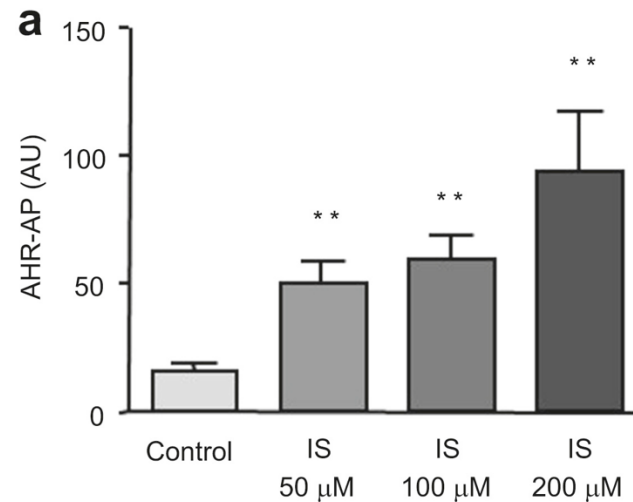


Luciferase

Bioluminescence

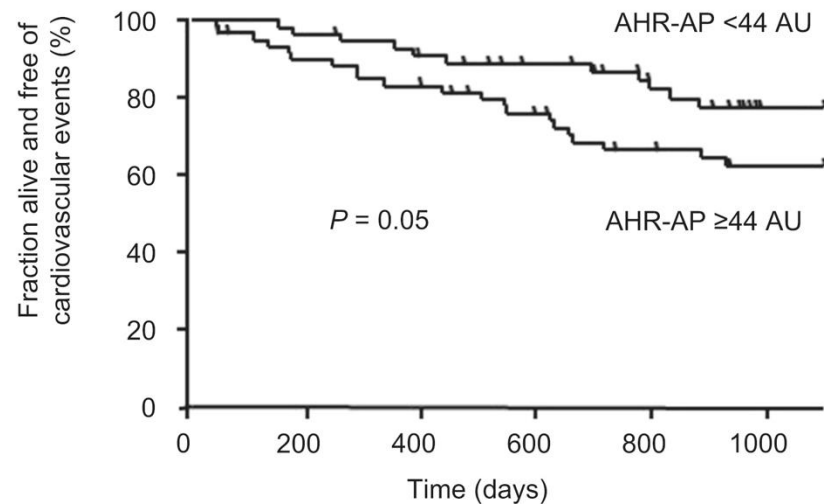
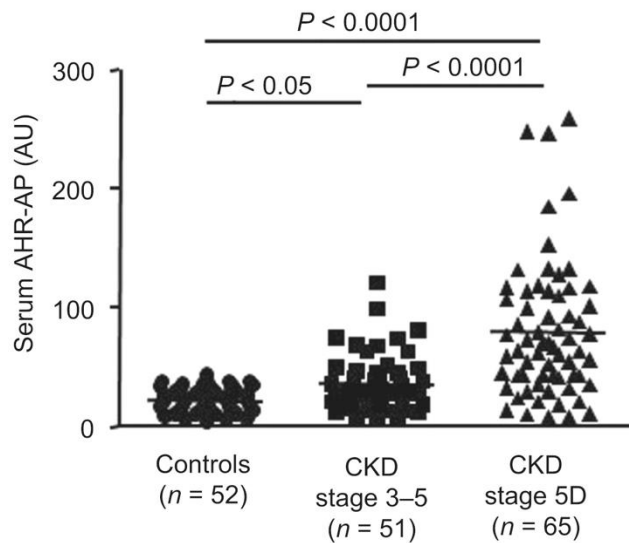


The aryl hydrocarbon receptor (AhR) agonist indoxyl sulfate (IS) induces AHR activation in vitro.



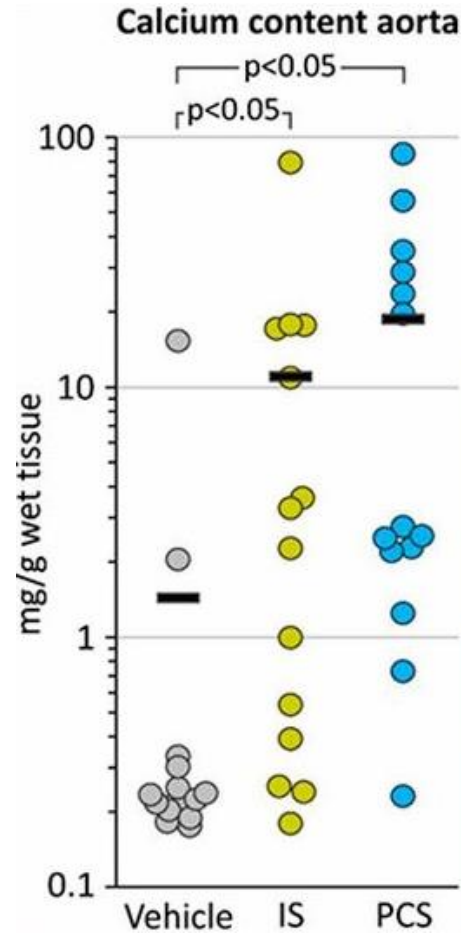
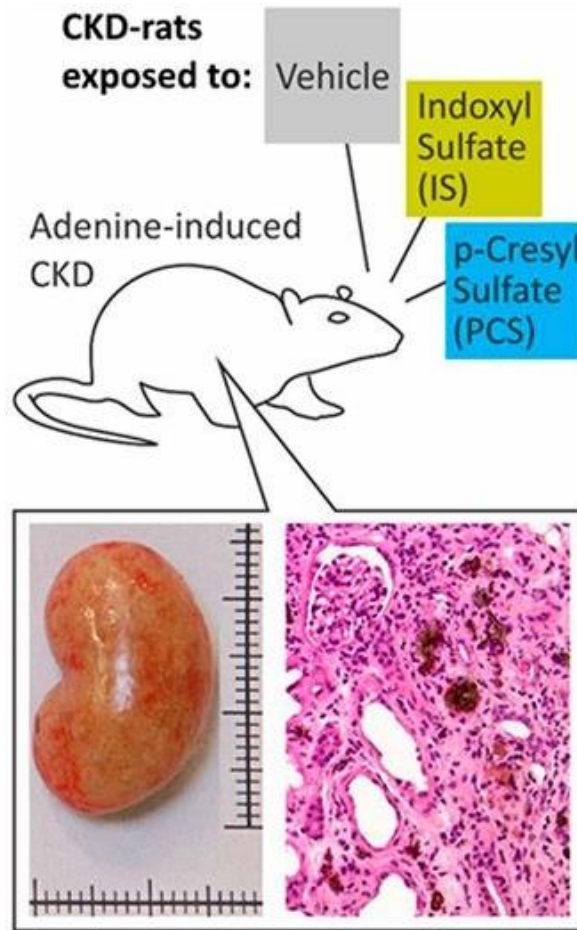
a) Dose effect of AHR activation in HG2L7.5c1 cells induced by IS was studied after 4 hours of incubation in complete medium. Values are expressed as mean \pm SEM of 4 independent experiments. ***P < 0.01 versus potassium chloride control. (b) Kinetic of AHR activation in HG2L7.5c1 cells induced by IS at the uremic concentration of 200 μ M. Values are expressed as mean \pm SEM of 3 independent experiments. (c) Effect of IS (200 μ M) supplementation of control serum on AHR activating potential (AP). Data, expressed in AU, represent the values obtained with 6 different sera. *P < 0.05 versus control serum without IS.

The AhR activation is associated with cardiovascular events and mortality in CKD patients

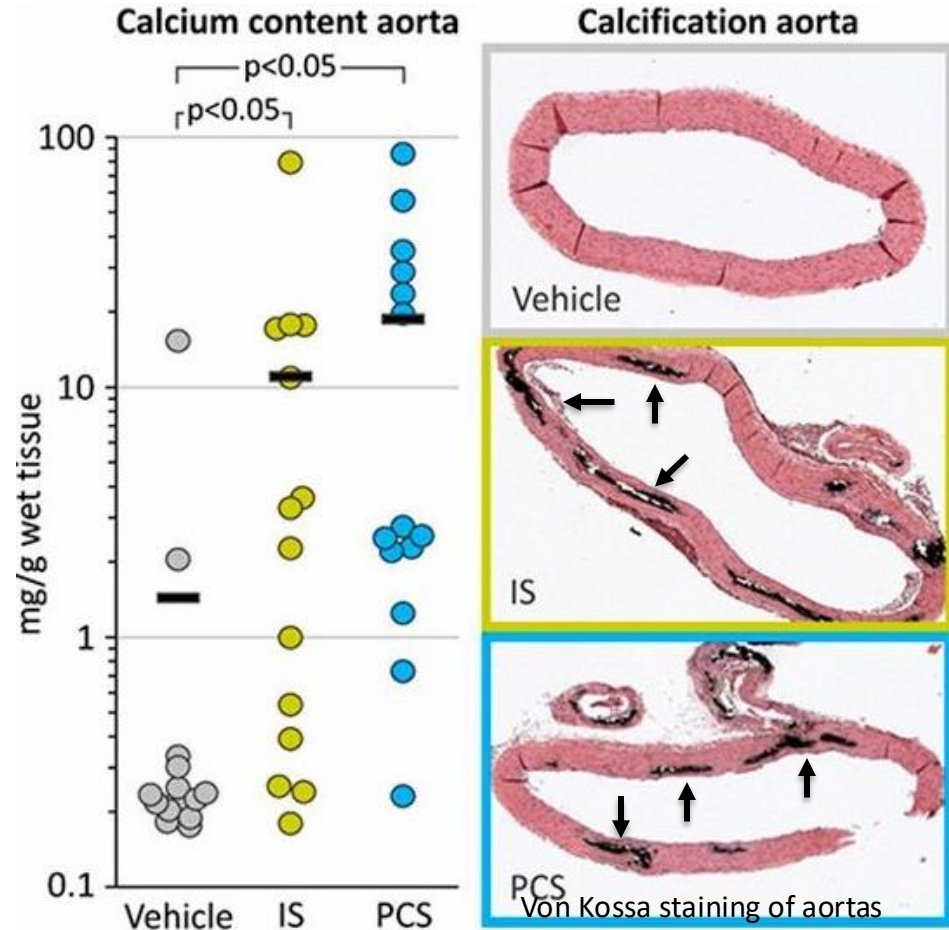
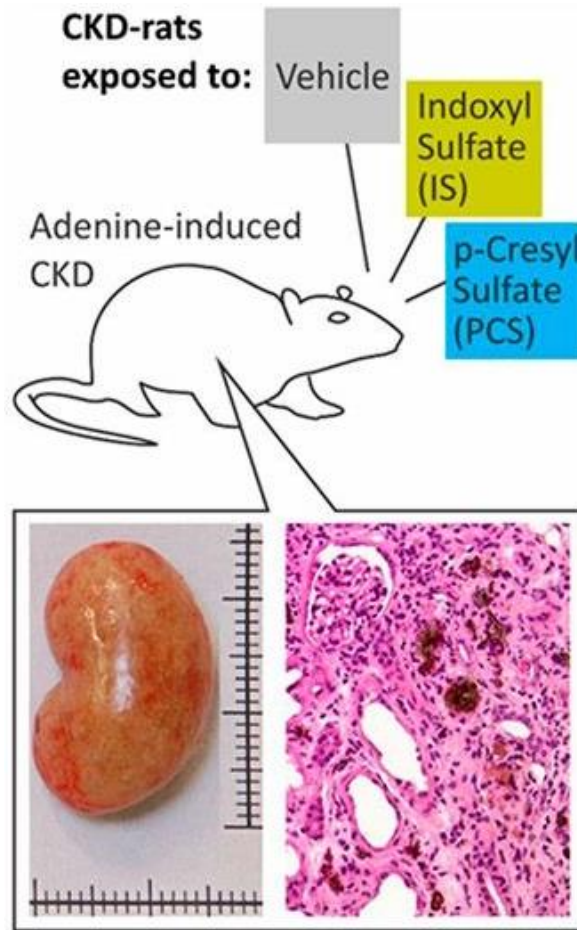


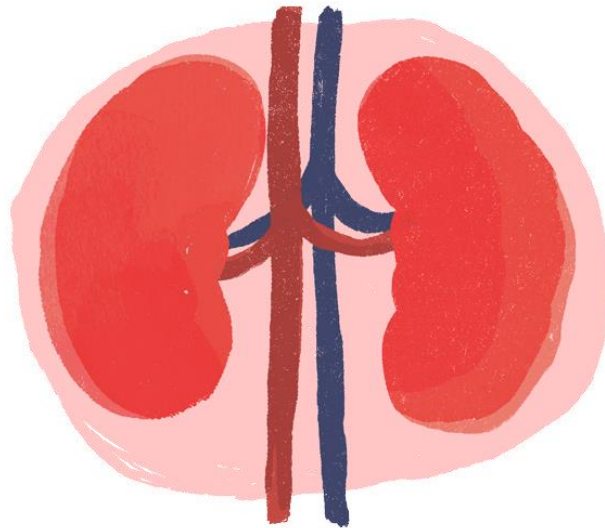
Kaplan-Meier estimates of (a) cumulative survival and (b) major cardiovascular events of all patients according to aryl hydrocarbon receptor-activating potential (AHR-AP) values above and below the median of 44 arbitrary units (AU). (a) $P = 0.19$ in a Gehan-Breslow-Wilcoxon comparison of the curves. (b) $P = 0.05$ in a Gehan-Breslow-Wilcoxon comparison of the curves.

IS and pCS promote vascular calcifications in CKD rats



IS and pCS promote vascular calcifications in CKD rats





Exemple de toxicité:

Toxicité métabolique du p-crésyl sulfate (p-CS) chez la souris

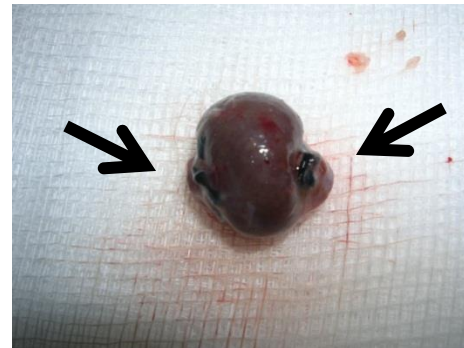
Néphrectomie subtotale des 5/6

Modèle chirurgical d'insuffisance rénale chez le rongeur

Souris **contrôle**



Souris **néphrectomisée 5/6**



Néphrectomie unilatérale et ablation des 2/3 du rein restant
= **reduction néphronique (-80%)**

Les souris Nx5/6 comme les patients IRC développent une insulino-résistance



Souris C57Bl6, 4 semaines d'IRC

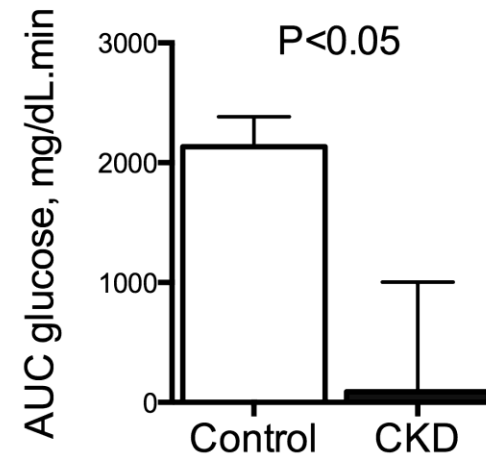
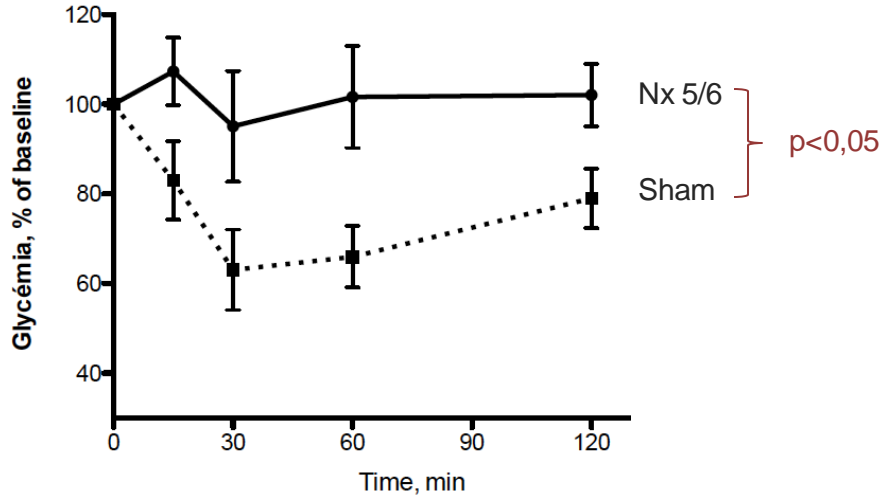
Paramètres plasmatiques	Sham		Nx 5/6		Variation
Urée, mmol/L	12	± 3	34	± 8	+191%***
Glucose mg/dL	84	± 3	98	± 5	+37%**
Insuline, pg/mL	11	± 6	60	± 11	+455%**
HOMA-IR	0.3	± 0.1	1.8	± 0.4	+500%**
Triglycérides, mg/dL	70	± 5	118	± 12	+26%*
Cholestérol total, mg/dL	65	± 5	123	± 11	+55%*

Hyperglycémie + hyperinsulinémie + dyslipidémie...

L'IRC est associée à une insulino-résistance



Test de tolérance à l'insuline

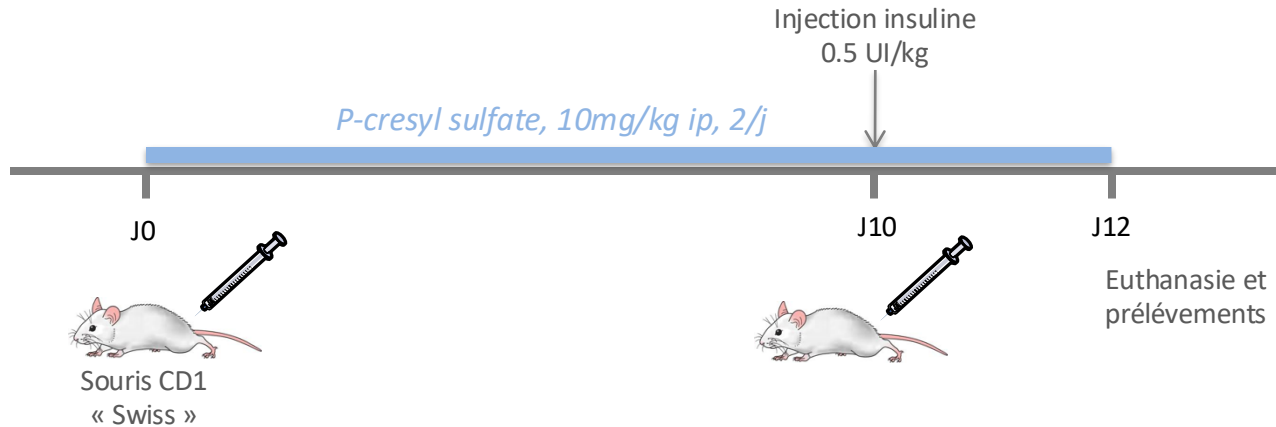


n=8-9, moy. ± SEM, ANOVA-2

P-Cresyl Sulfate Promotes Insulin Resistance Associated with CKD, Koppe & al, JASN, 2013

Profil métabolique des souris supplémentées en p-cresyl sulfate

P-cresyl sulfate, 10mg/kg ip, 2 fois par jour, 10 jours



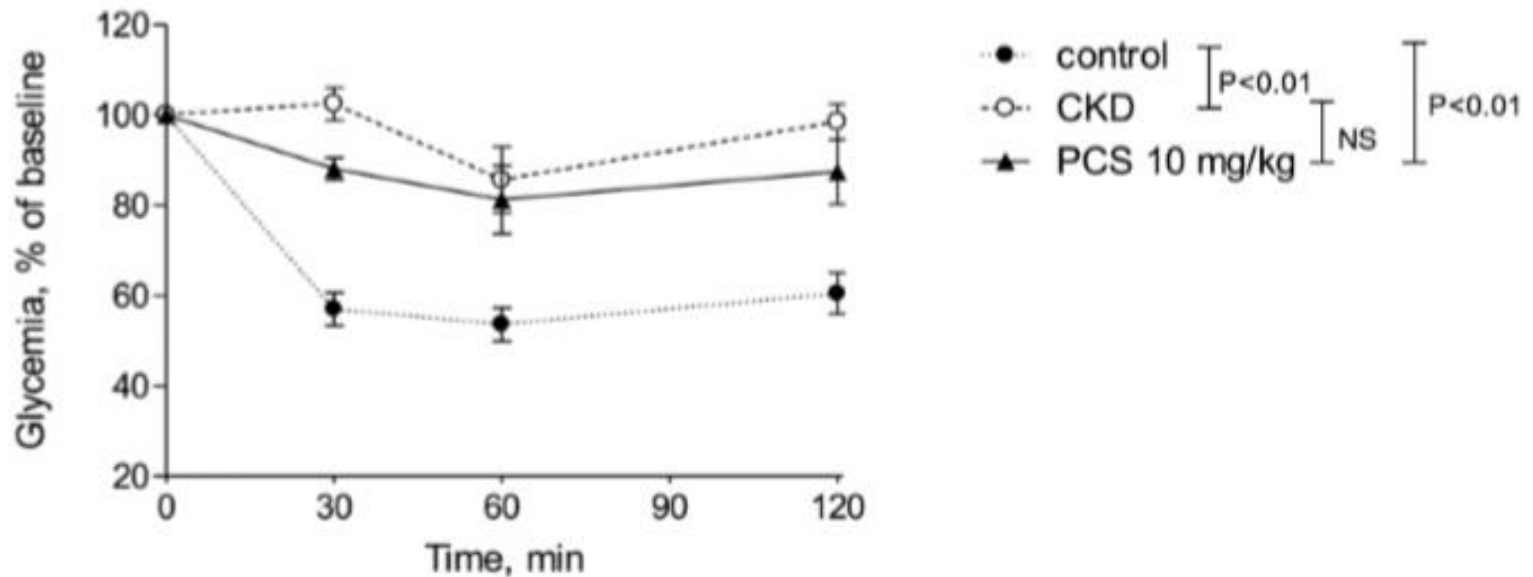
Paramètre plasmatiques	Contrôle		P-cresyl-S		Variation	IRC		Variation
Glucose mg/dL	74	± 6	110	± 4	+37%**	98	± 5	+31%*
Triacylglycerols, mg/dL	51	± 4	65	± 7	+26%*	67	± 3	+30%*
Total cholesterol, mg/dL	98	± 14	152	± 17	+55*	170	± 9	+74%**

Hyperglycémie et dyslipidémie très similaires à celles des modèles animaux IRC (Néphrectomie des 5/6)...

P-crésyl sulfate and insulin resistance

p-Cresyl Sulfate Promotes Insulin Resistance Associated with CKD

Laetitia Koppe,^{*†} Nicolas J. Pillon,[†] Roxane E. Vella,[†] Marine L. Croze,[†] Caroline C. Pelletier,^{*†} Stéphane Chambert,[‡] Ziad Massy,[§] Griet Glorieux,^{||} Raymond Vanholder,^{||} Yann Dugenet,[§] Hédi A. Soula,[†] Denis Fouque,^{*†} and Christophe O. Soulage[†]



How to prevent uremic toxin accumulation in CKD ?

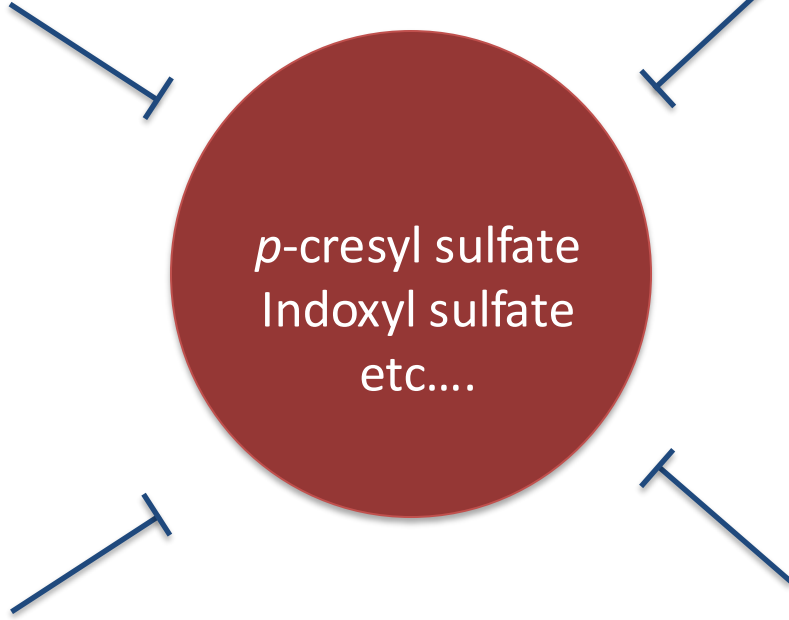
Some toxins such as P-cresyl-S/ indoxyl-S are tightly bound to plasma protein and thus

Poorly dialyzable

Prebiotics/Probiotics

Adsorbants

(Kremezin[®], AST-120)



p-cresyl sulfate
Indoxyl sulfate
etc....

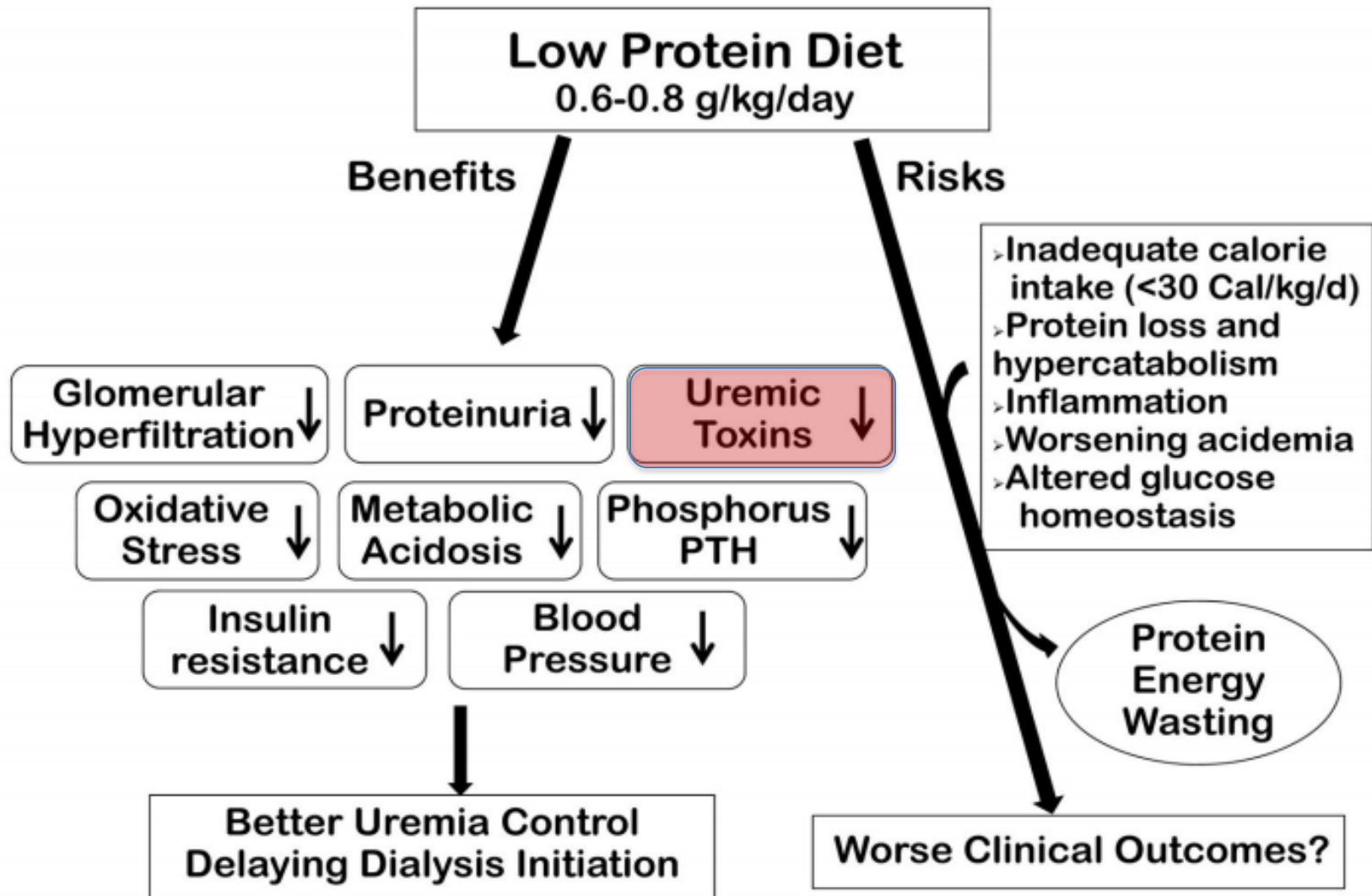
Low protein diets

Bacterial fermentation
inhibitors (acarbose)

Stratégies thérapeutiques

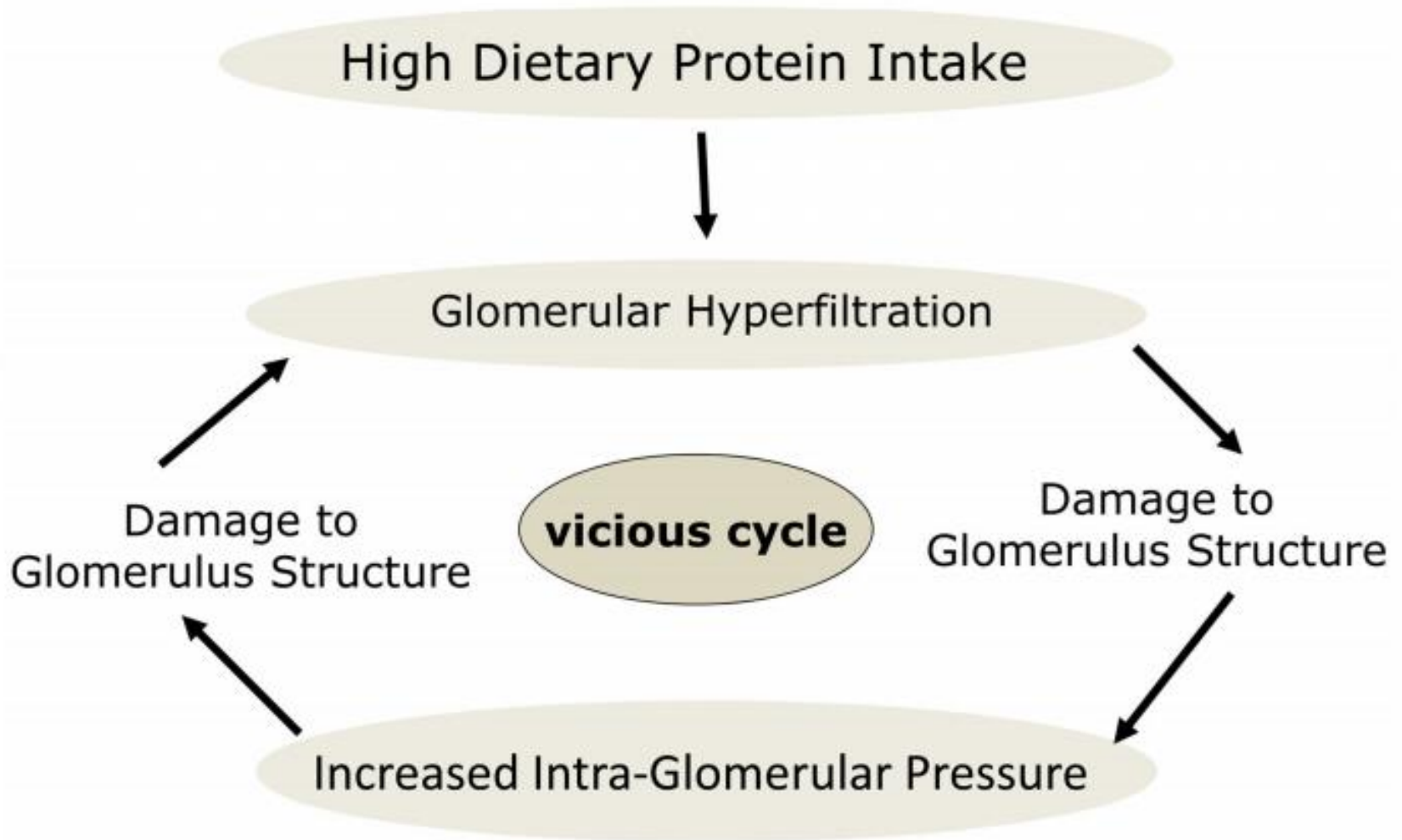
- **Low protein diet**
- **AST-120**
- **Pre and probiotics**
- **Deprivation in aromatic aminoacids**

Low and very low protein diet



Ko GJ, Dietary protein intake and chronic kidney disease: Current Opinion in Clinical Nutrition and Metabolic Care. 2017

Low protein diet (LPD)

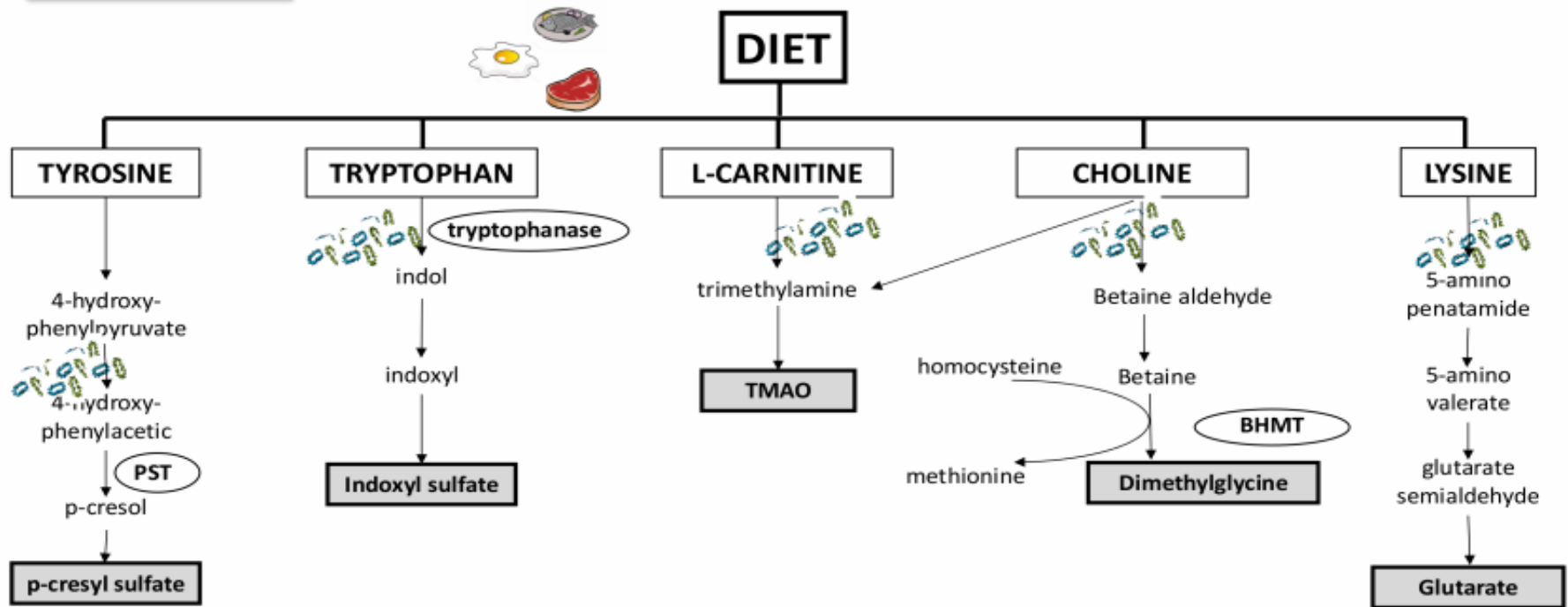


Ko GJ, Dietary protein intake and chronic kidney disease: Current Opinion in Clinical Nutrition and Metabolic Care. 2017

Uremic toxins sources

1. Gut microbiota
2. Endogenous metabolism

3. Food → *Nombreux substrats pour fermentation bactérienne*



Les protéines/acides aminés sont les précurseurs de nombreuses toxines urémiques

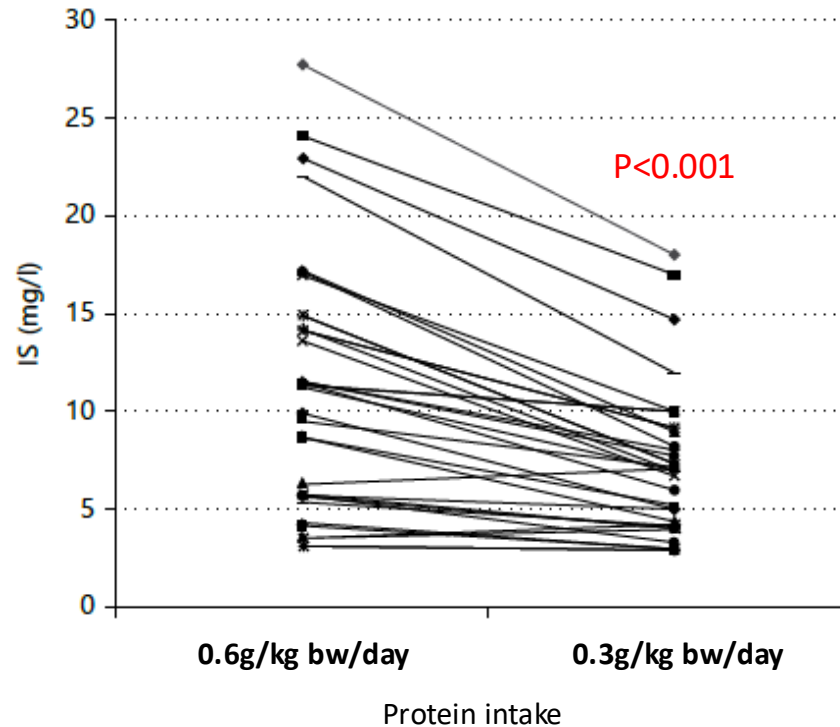
L'efficacité des LPD/VLPD pourrait aussi passer par une réduction de la production de toxines urémiques

Very Low Protein Diet Reduces Indoxyl Sulfate Levels in Chronic Kidney Disease

Stefania Marzocco^a Fabrizio Dal Piaz^a Lucia Di Micco^b Serena Torraca^b
Maria Luisa Sirico^b Domenico Tartaglia^c Giuseppina Autore^a Biagio Di Iorio^b

^aDepartment of Pharmacy, University of Salerno, Salerno, ^bDepartment of Medicine, Nephrology, and
^cPharmaceutical Unit, 'A. Landolfi' Hospital, Solofra, Italy

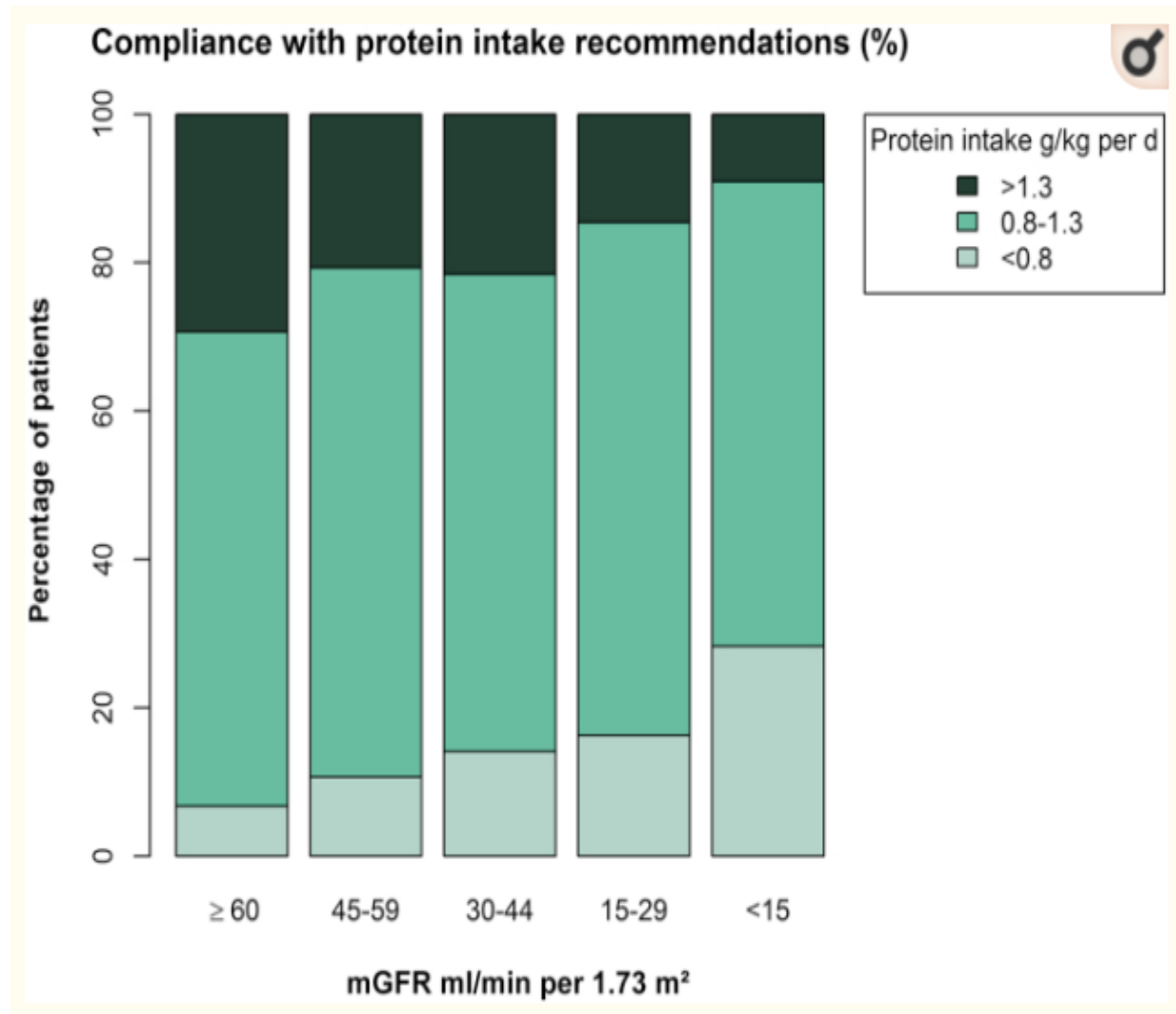
CKD patients, n=24
Cross-over study
Duration: 1 week



La diminution de l'apport protéique diminue la production de toxines urémiques...

Drawbacks of Low protein diets

- 0,6 à 0,8 g/kg/J
- Contraignant:
Problème de compliance
- Risque de dénutrition
- Possible perte de masse musculaire



Association of a Low-Protein Diet With Slower Progression of CKD, Marie Metzger & al, Kidney International report, 2018



Vegetarian diet as a surrogate of LPD/VLPD....

Vegetarian diet: lower protein intake

Crucial role of **Protein/Fibers** ratio

Better compliance ???

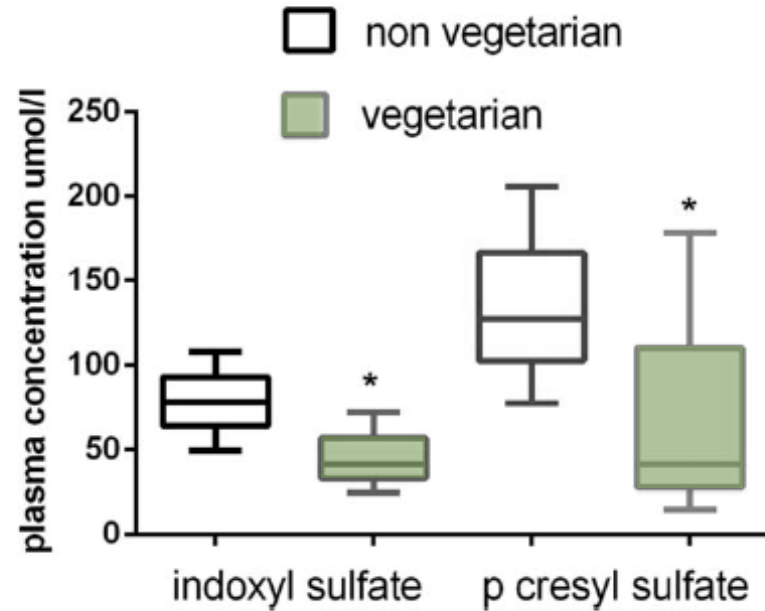
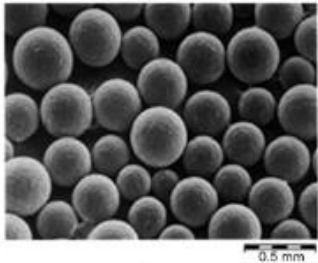


Figure 1 Plasma concentrations of indoxyl sulfate and p cresyl sulfate in non-vegetarian and vegetarian patients. Results expressed as median (interquartile range). *P < 0.05 vs. non-vegetarian.

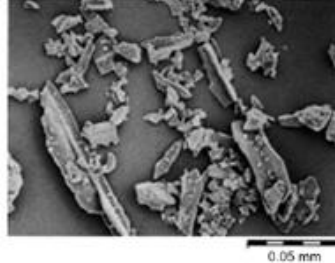
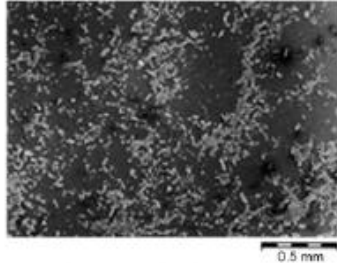
	Standard diet	Vegetarian diet
Urea, mg/dL	18.4 ± 5.2	13.8 ± 3.8 *

Adsorbant: AST-120 (Kremezin®)

AST-120

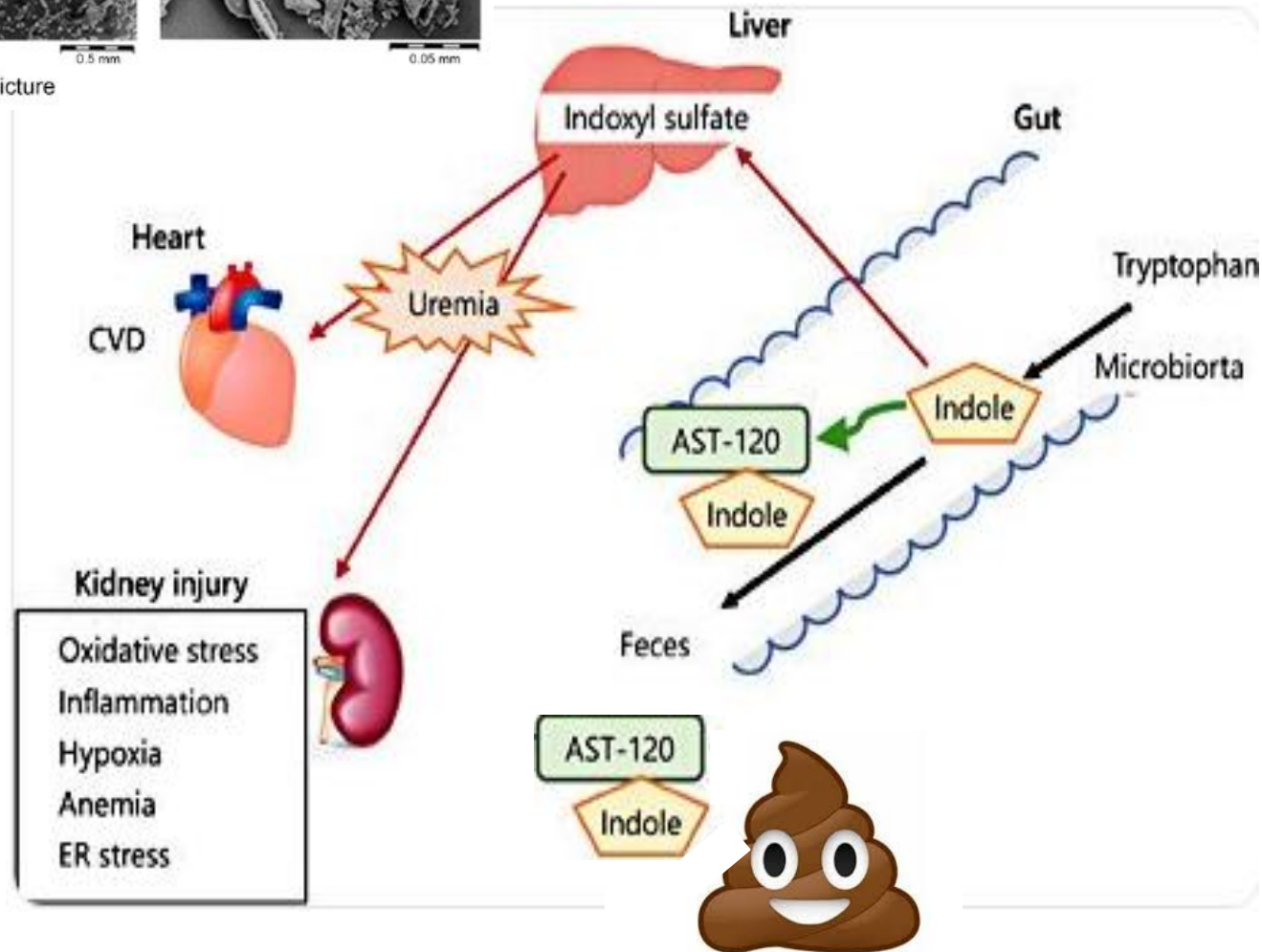


Activated Charcoal



High-resolution transmission electron microscopic picture

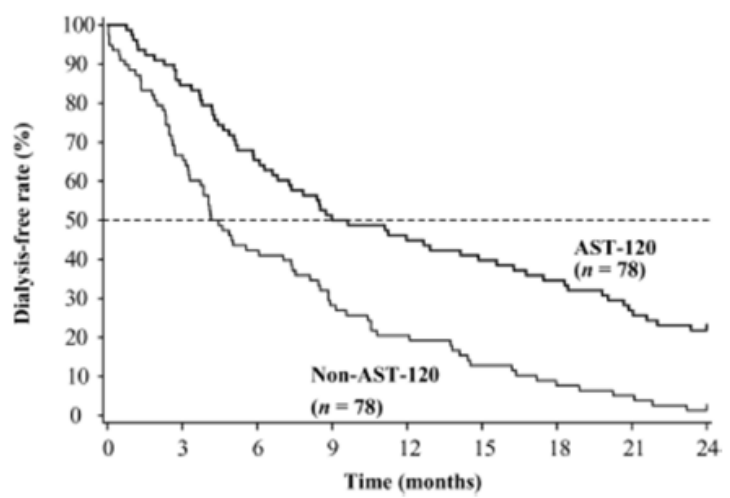
- Oral carbon adsorbent
- Prevent indoxyl sulfate production
- Promote its excretion in faeces





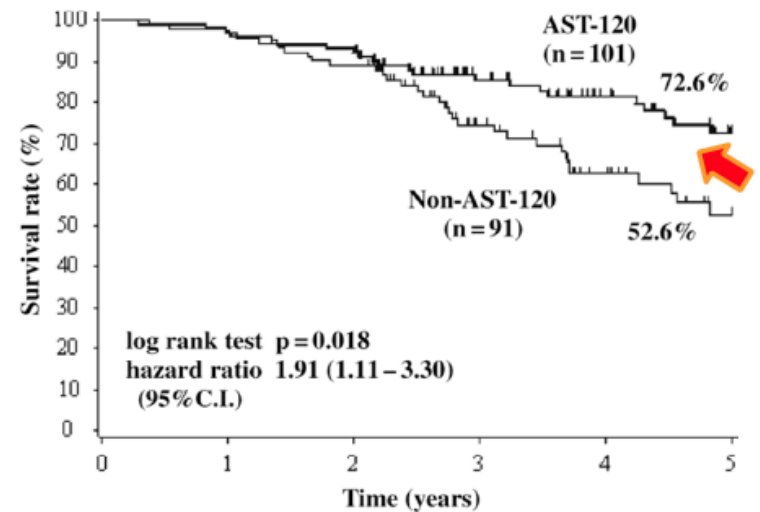
AST-120 IMPROVES OUTCOME OF DIALYSIS PATIENTS

AST-120 DELAYS INITIATION OF DIALYSIS



P<0.001

Ueda et al, Ren Fail, 30, 856-860, 2008



Ueda H. et al, Ther Apher and Dial; 11: 189-195; 2007

Outcome of Global Phase III (EPPIC) Studies

Approx. 2,000 patients were enrolled in the studies which were conducted in Europe and the Americas.

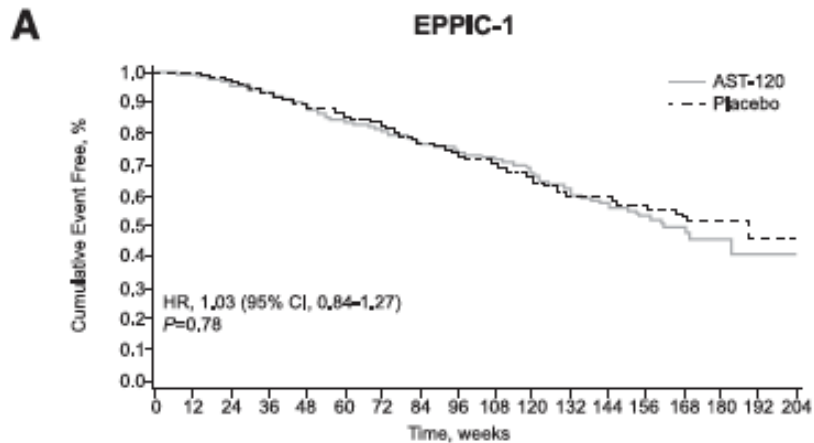
The studies were randomized, double-blind, placebo-controlled trials to evaluate the efficacy and tolerability of AST-120 at a dose of 9 g per day added to standard-of-care therapy in patients with moderate to severe CKD.

The primary endpoint was:

- time to initiation of dialysis
- kidney transplantation or
- doubling of serum creatinine.

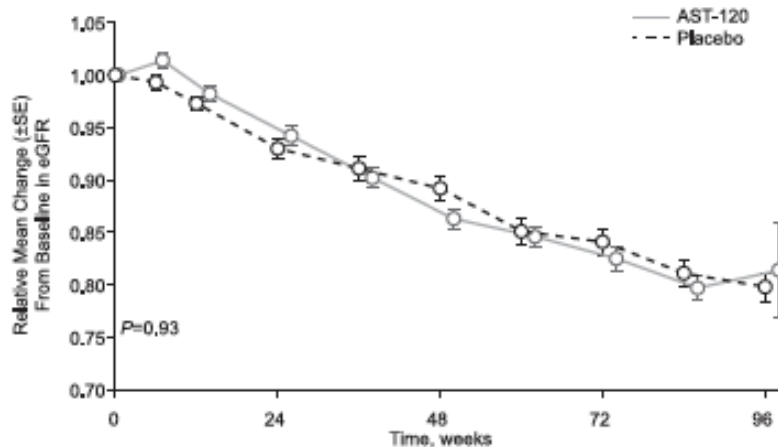
Statistically-significant difference was not shown according to an analysis conducted on the primary endpoint.

Randomized controlled trial of AST-120



Patients at risk, n

AST-120	500	494	470	451	416	386	365	343	326	269	191	135	91	49	29	14	6	1
Placebo	502	500	472	441	416	392	373	342	318	263	205	142	93	63	38	19	8	3



Patients with eGFR data, n

AST-120	500	436	374	317	263
Placebo	502	429	362	317	268

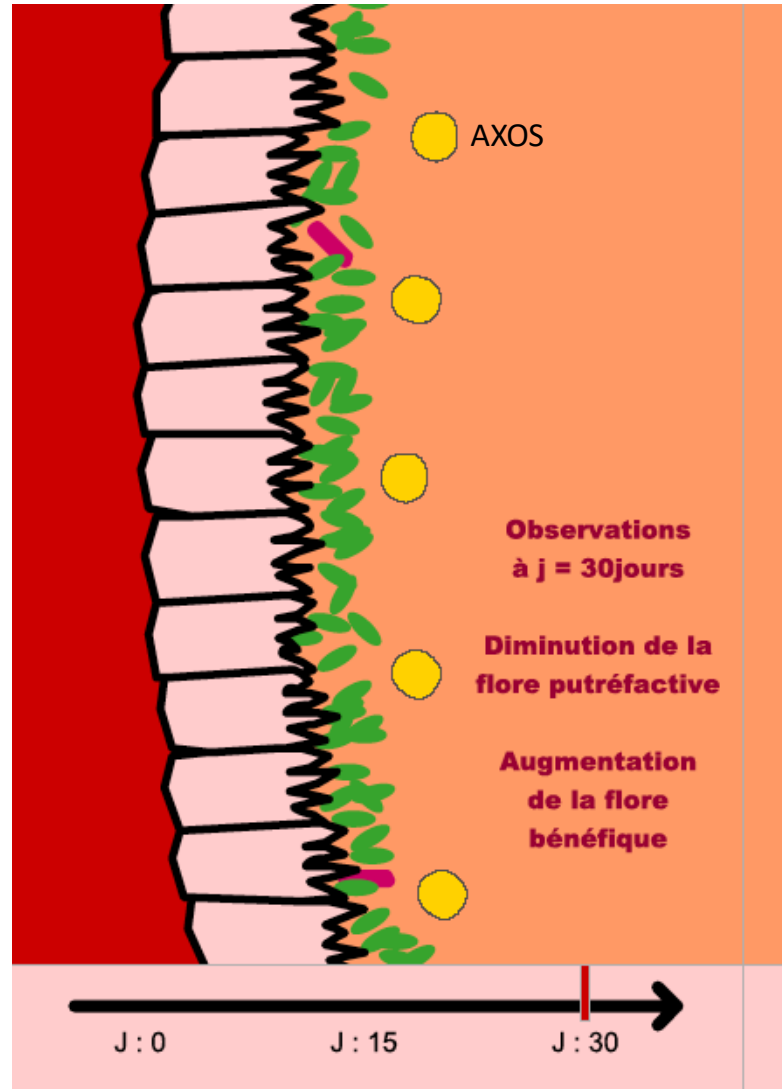
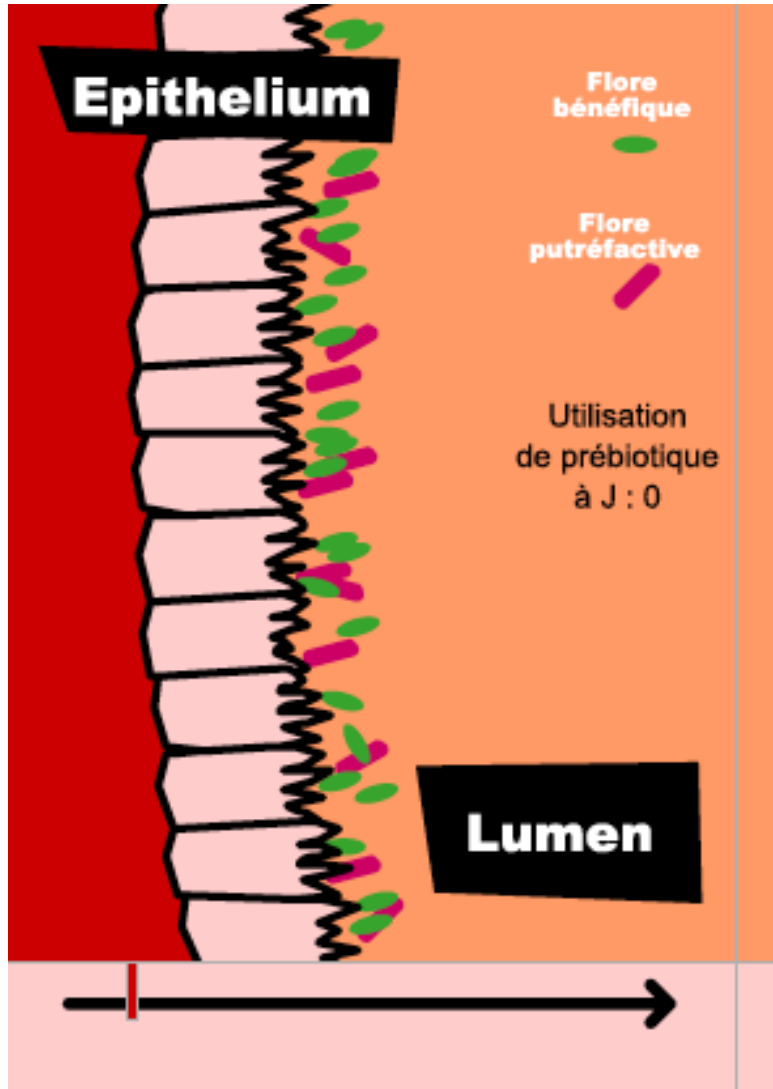
HR 1.03 (95%CI 0.84-1.27)
P=0.78

P=0.93

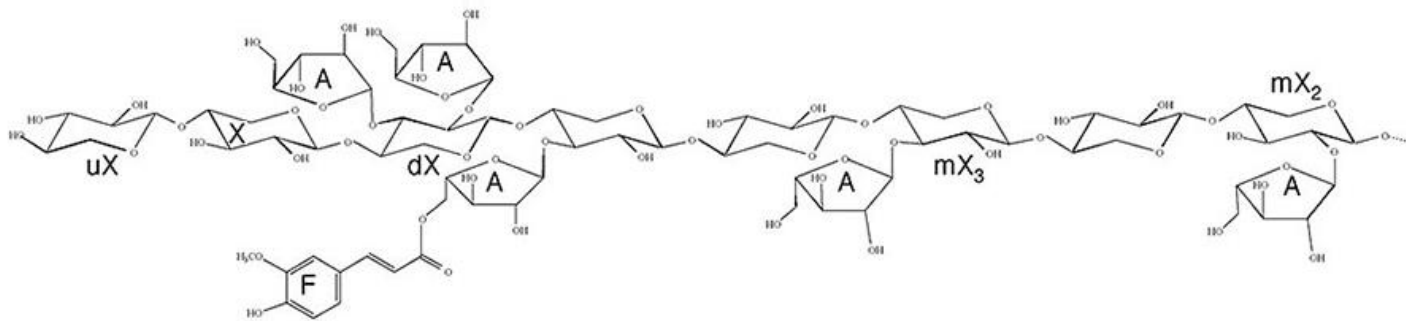
ECHEC !!!

Schulman et al. Randomized Placebo-Controlled EPPIC Trials of AST-120 in CKD. *Journal of the American Society of Nephrology*. 2015 Jul 1;26(7):1732-46.

L'effet « prébiotique »



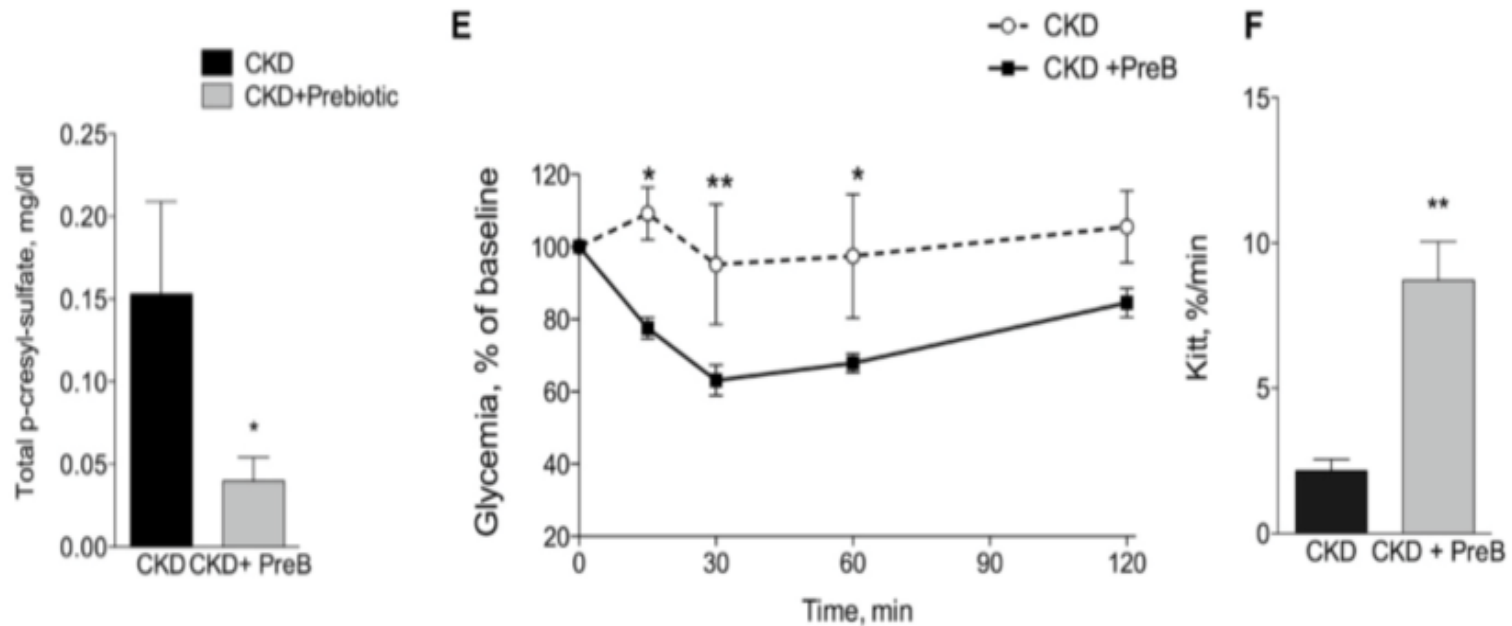
Normalisation du profil métabolique des souris IRC par supplémentation avec un prébiotique



Arabino Xylo Oligo Saccharides (AXOS)

Paramètre plasmatiques	Contrôle			IRC			Variation	AXOS			Variation
		±			±				±		
Glucose mg/dL	84	±	3	98	±	5	+16%*	76	±	9	-10%
Insuline, pg/ml	11	±	6	61	±	11	+550%***	38	±	17	+245%**
Triacylglycérols, mg/dL	70	±	5	118	±	12	+68%*	76	±	9	+8%
Cholestérol, mg/dL	64	±	4	123	±	11	+92%*	80	±	10	+25%**

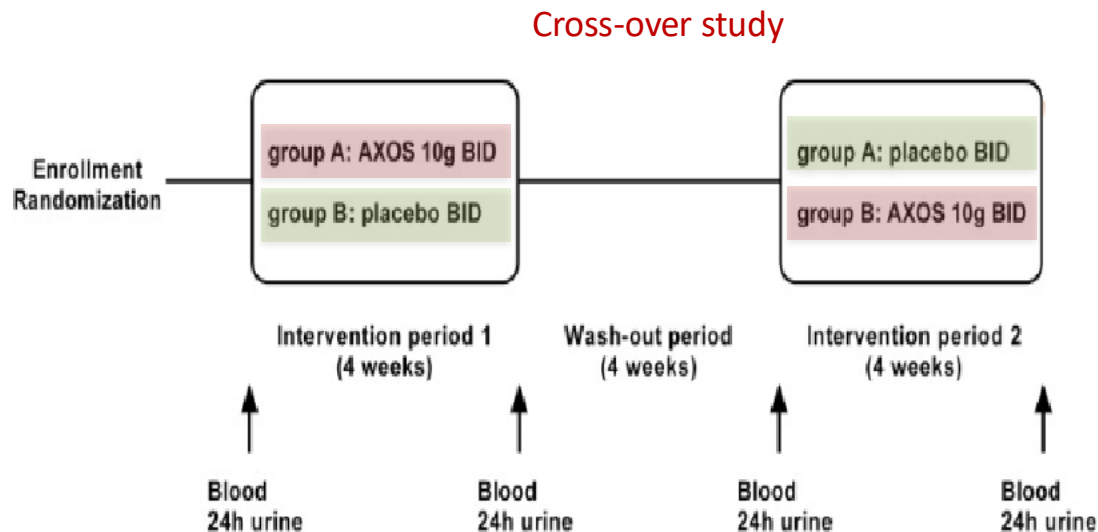
Normalisation du profil métabolique des souris IRC par supplémentation avec un prébiotique: correction de l'insulino-résistance



Pre- and probiotics

The Influence of Prebiotic Arabinoxylan Oligosaccharides on Microbiota Derived Uremic Retention Solutes in Patients with Chronic Kidney Disease: A Randomized Controlled Trial

[Ruben Poesen](#),¹ [Pieter Evenepoel](#),¹ [Henriette de Loor](#),¹ [Jan A. Delcour](#),^{2,3} [Christophe M. Courtin](#),^{2,3}
[Dirk Kuypers](#),¹ [Patrick Augustijns](#),⁴ [Kristin Verbeke](#),^{3,5} and [Björn Meijers](#)^{1,*}



The Influence of Prebiotic Arabinoxylan Oligosaccharides on Microbiota Derived Uremic Retention Solutes in Patients with Chronic Kidney Disease: A Randomized Controlled Trial, PLOS one, 2016

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Table 2

Influence of arabinoxylan oligosaccharides (AXOS) on serum levels of microbial metabolites.

Solute	Treatment effect (AXOS vs. placebo) (95% confidence interval)	P
Urea (Ln)	0.035 (- 0.056–0.127)	0.44
p-Cresyl sulfate (Ln)	- 0.115 (- 0.401–0.171)	0.42
p-Cresyl glucuronide (Ln)	- 0.105 (- 0.496–0.286)	0.59
Indoxyl sulfate (Ln)	- 0.031 (- 0.198–0.136)	0.70
Trimethylamine N-oxide (Ln)(Ln)(Ln)	- 0.237 (- 0.464–0.010)	0.04
Phenylacetylglutamine (Ln)	0.080 (- 0.115–0.275)	0.41

Le génie génétique à la rescousse....

Cell Host & Microbe
Brief Report

CellPress

Modulation of a Circulating Uremic Solute via Rational Genetic Manipulation of the Gut Microbiota

A. Sloan Devlin,^{1,7} Angela Marcobal,³ Dylan Dodd,^{2,3} Stephen Nayfach,^{4,5} Natalie Plummer,⁶ Tim Meyer,⁶ Katherine S. Pollard,^{4,5} Justin L. Sonnenburg,^{3,*} and Michael A. Fischbach^{1,8,*}

¹Department of Bioengineering and Therapeutic Sciences and California Institute for Quantitative Biosciences, University of California, San Francisco, San Francisco, CA 94143, USA

²Department of Pathology

³Department of Microbiology and Immunology
Stanford University School of Medicine, Stanford, CA 94305, USA

⁴Integrative Program in Quantitative Biology, Gladstone Institutes

⁵Division of Biostatistics

University of California, San Francisco, San Francisco, CA 94143, USA

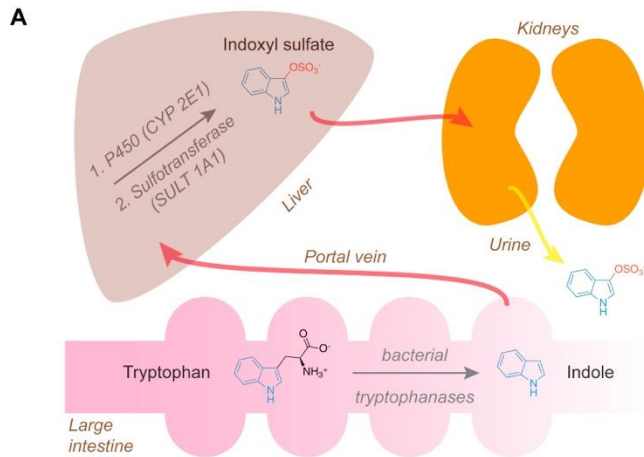
⁶Department of Medicine, VA Palo Alto HCS and Stanford University, Palo Alto, CA 94304, USA

⁷Present address: Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA

⁸Lead Contact

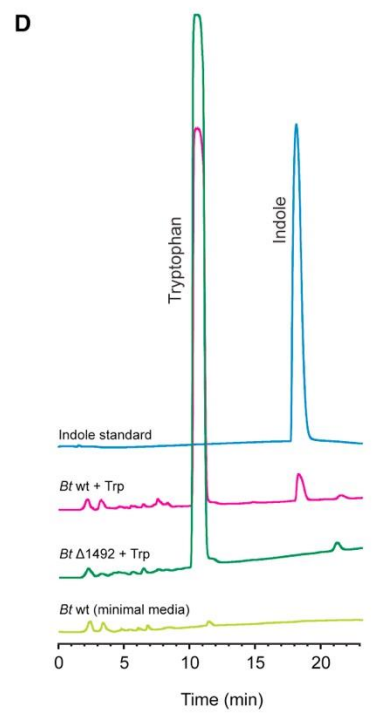
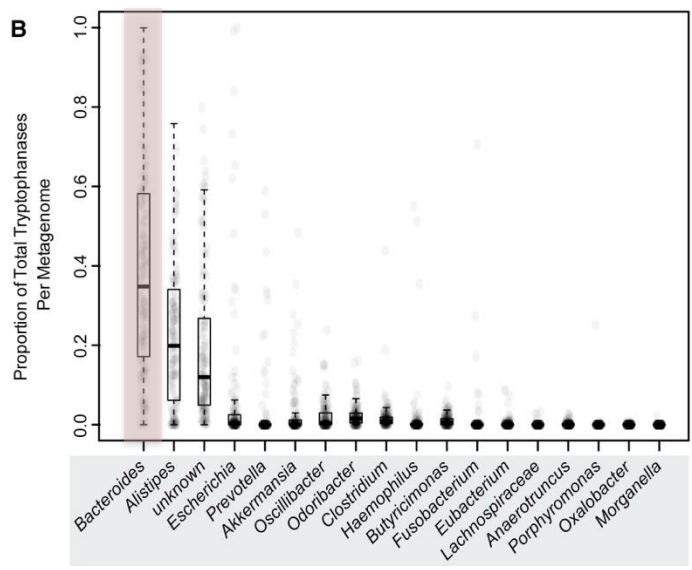
*Correspondence: jsonnenburg@stanford.edu (J.L.S.), fischbach@fischbachgroup.org (M.A.F.)

<http://dx.doi.org/10.1016/j.chom.2016.10.021>

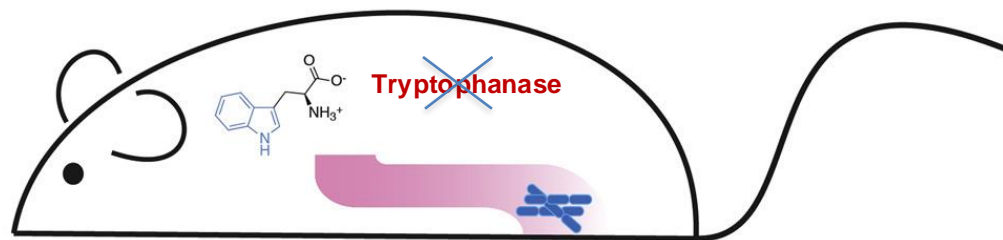
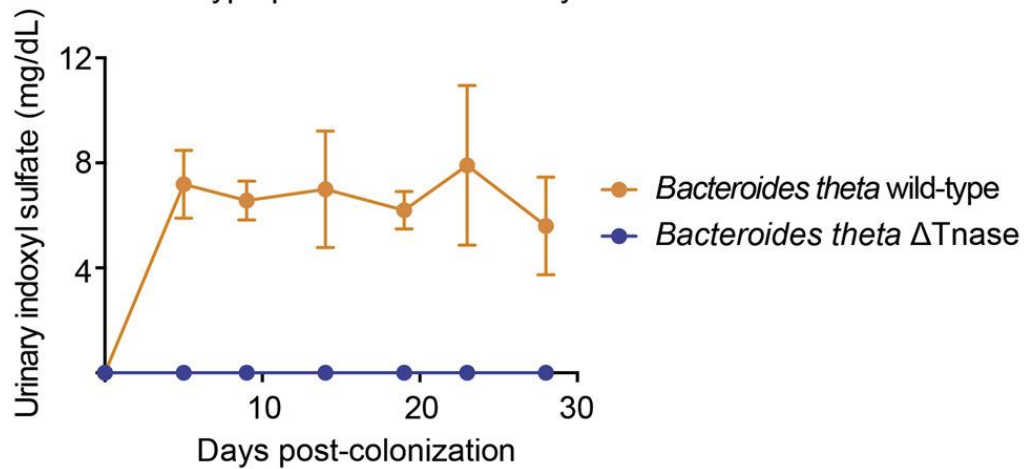
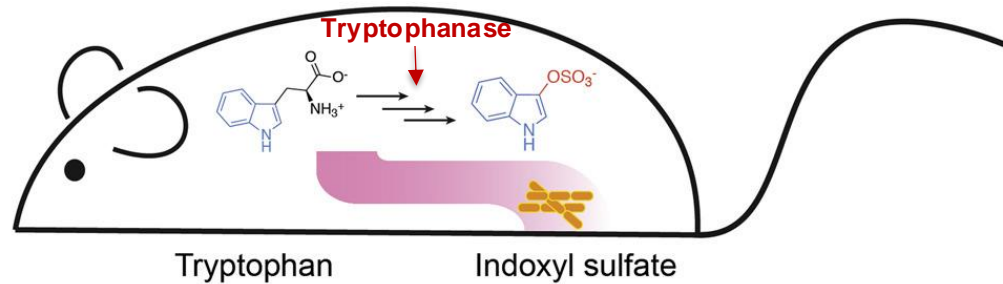


C

	<i>B. caccae</i>	<i>B. fragilis</i>	<i>B. ovalis</i>	<i>B. theta</i>	<i>B. vulgatus</i>
Tryptophanase	+	+	+	+	+
In vitro indole production	+	+	+	+	+

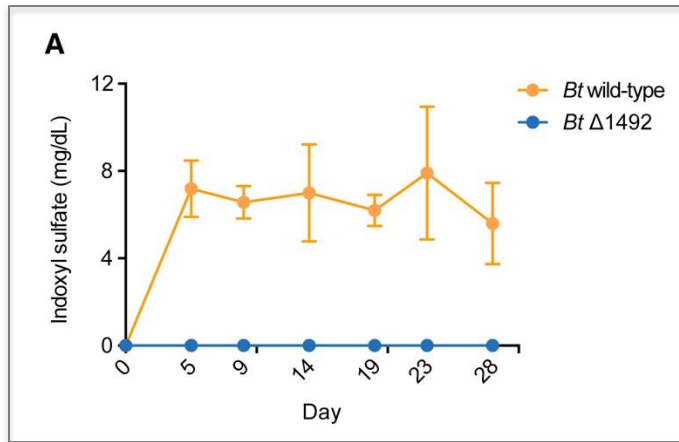


Seules certaines bactéries expriment des tryptophanases
Création de mutants sans tryptophanases

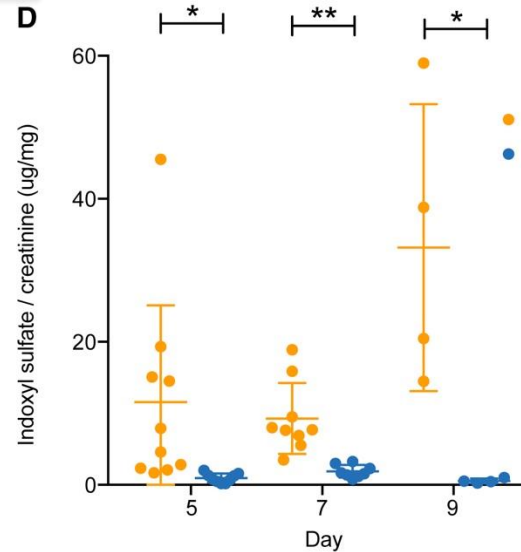
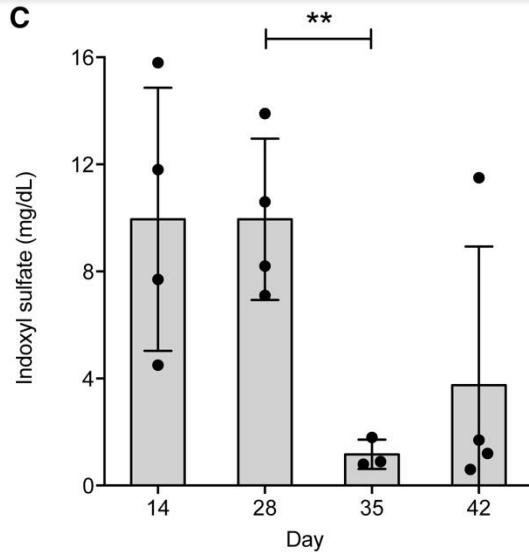
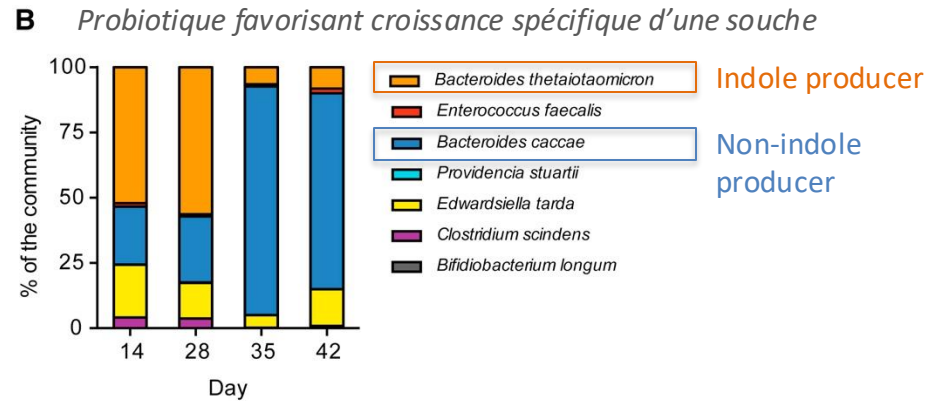


Animaux gnotobiotiques = 1 seule espèce bactérienne

A: Souris « gnotobiotiques): 1 seule espèce bactérienne



C-D: Souris conventionnelles



Gène de résistance à l'érythromycine

Permet par ajout d'érythromycine à l'eau de boisson de sélectionner la croissance de populations bactériennes concernées

Probiotique favorisant croissance spécifique d'une souche
















Traitement antibiotique

Correcting dysbiosis by fecal microbiota transplantation ?



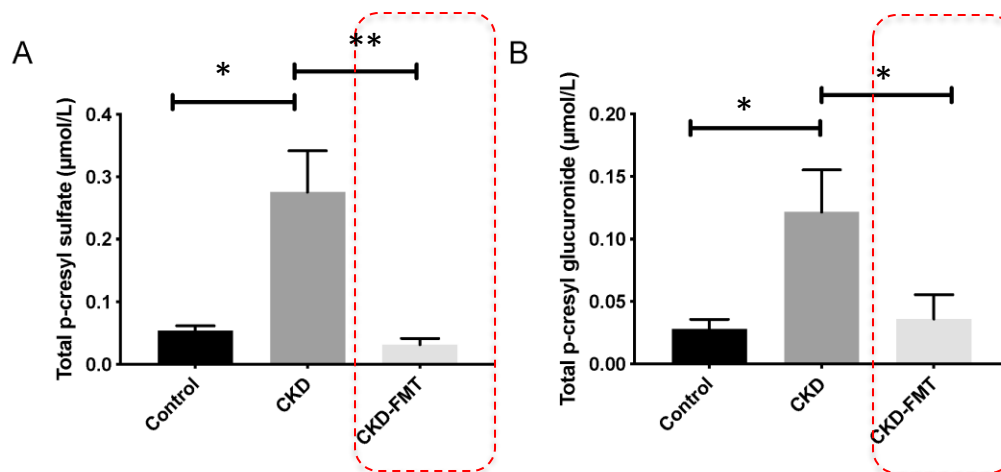
Aucune donnée clinique pour l'instant....

Effects of Fecal Microbiota Transplantation on Composition in Mice with CKD

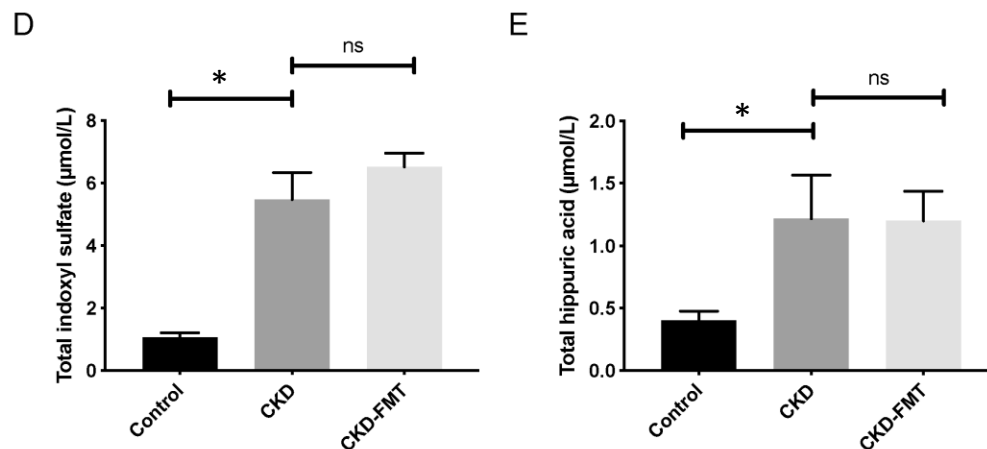
by  Christophe Barba ^{1,2}  ,  Christophe O. Soulage ¹ ,  Gianvito Caggiano ³ ,
 Griet Glorieux ⁴  ,  Denis Fouque ^{1,2}  and  Laetitia Koppe ^{1,2,*}  

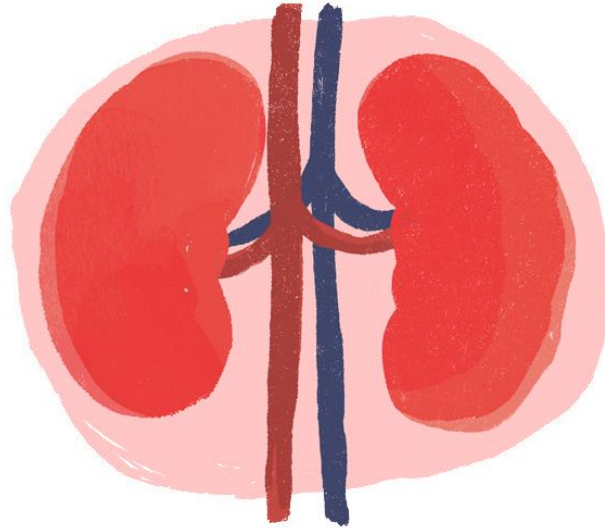
Toxins 2020, 12(12), 741; <https://doi.org/10.3390/toxins12120741>

Toxines issues du métabolisme du p-crésol (Tyr)



Toxines issues du métabolisme des indoles (Trp)

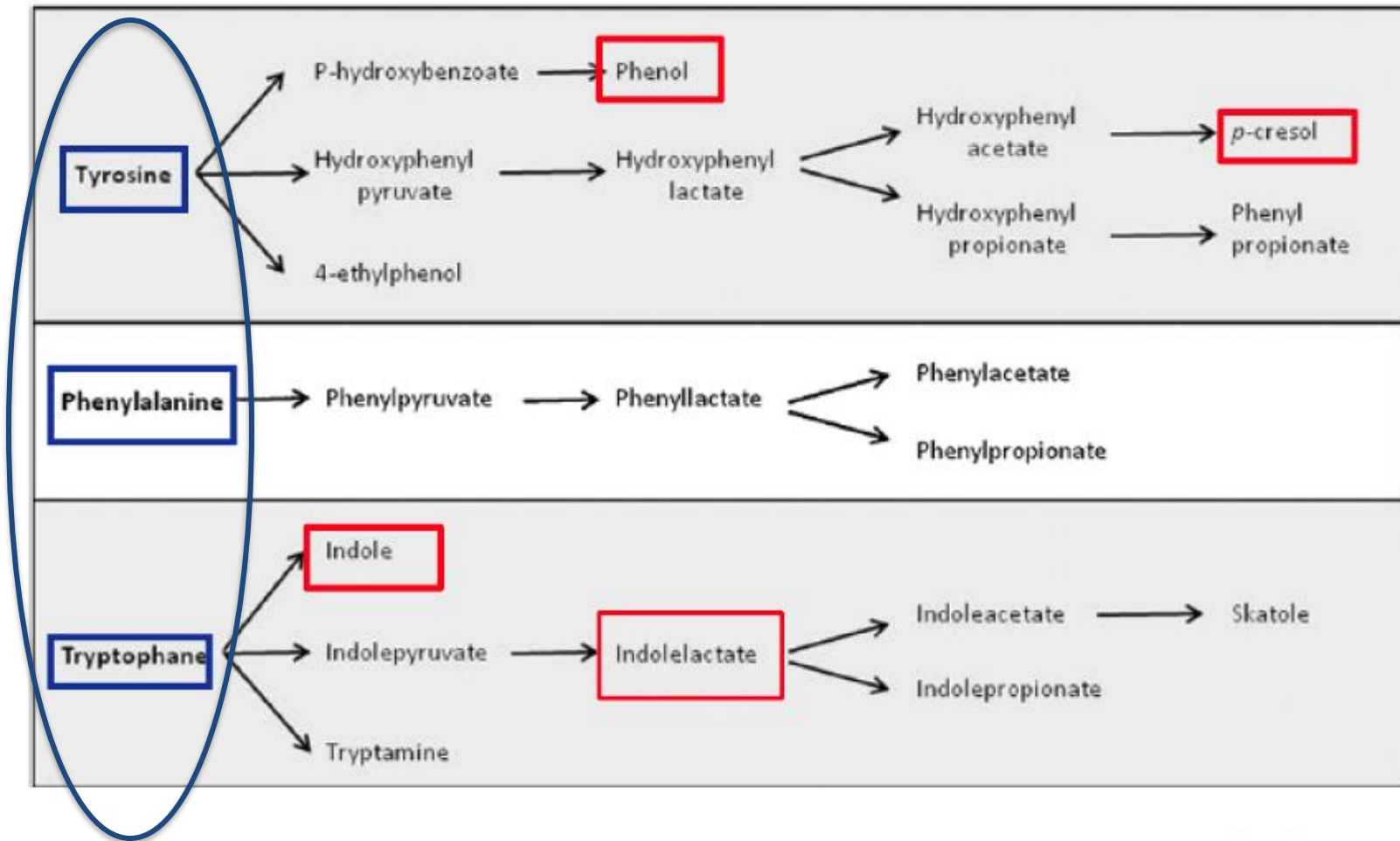




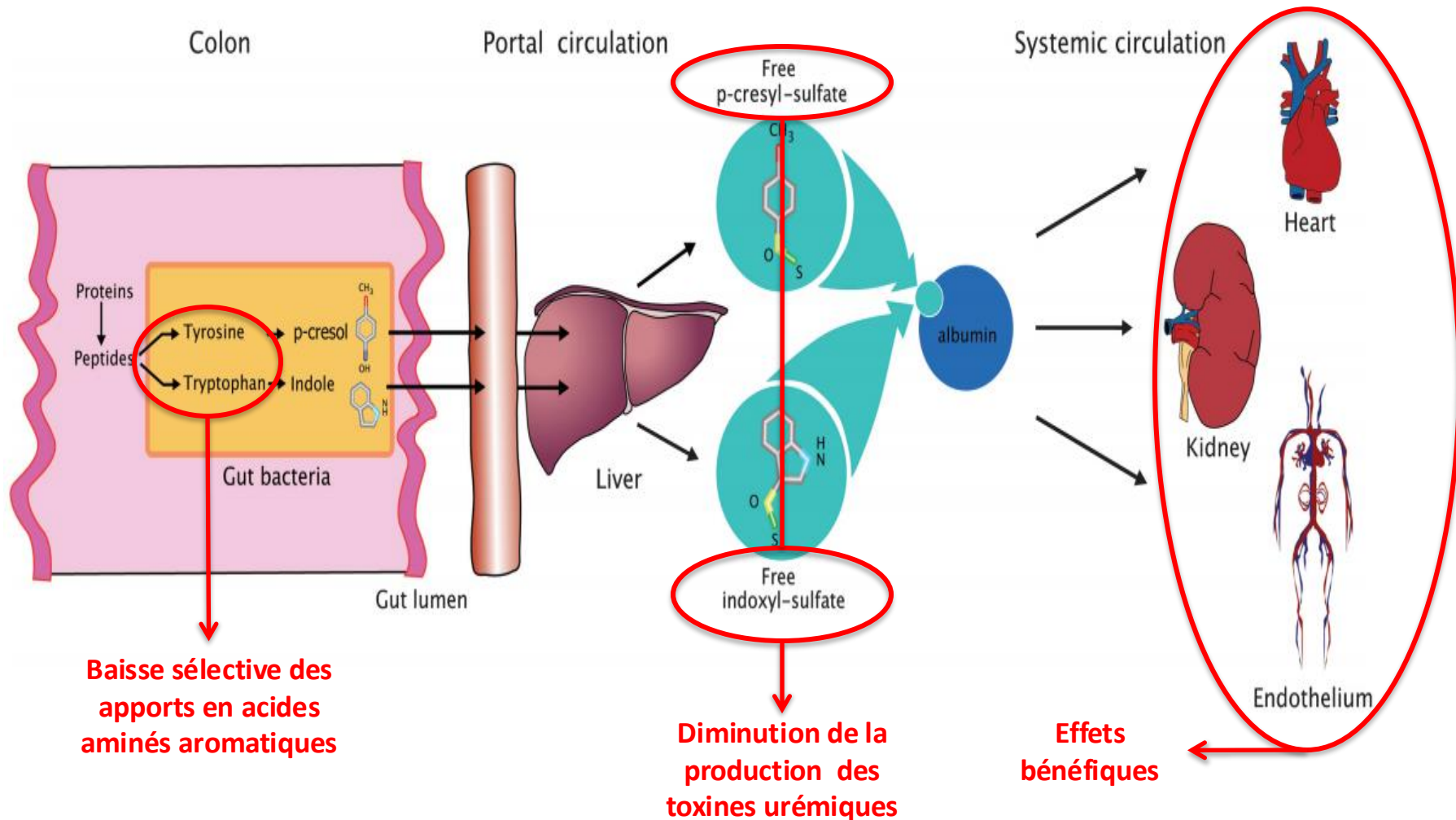
Exemple de nouvelle stratégie thérapeutique:
Préserver la fonction rénale en diminuant l'apport en acides
aminés aromatiques
Etude expérimentale chez la souris

Conserver le bénéfice des régimes LPD... sans en avoir les inconvénients

Many protein-bound uremic toxins are issued from gut microbiota metabolism



Origine des toxines urémiques



Design expérimental

But: diminuer l'apport en **acides aminés aromatiques** (tyrosine, tryptophane, phénylalanine) sans limiter l'apport protéique

Induction de l'IRC

4 semaines
régime adénine
0,25%

2 semaines
washout



Régime pauvre en AA
aromatiques (14% protéines)



Régime pauvre en
protéines
(5% protéines)

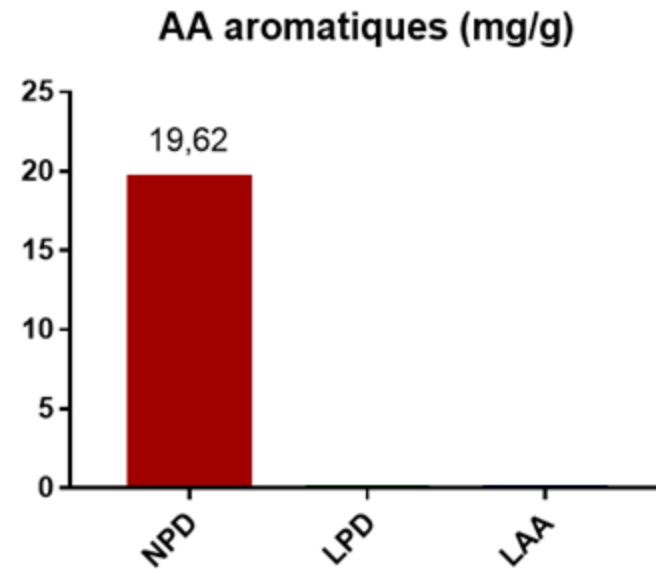
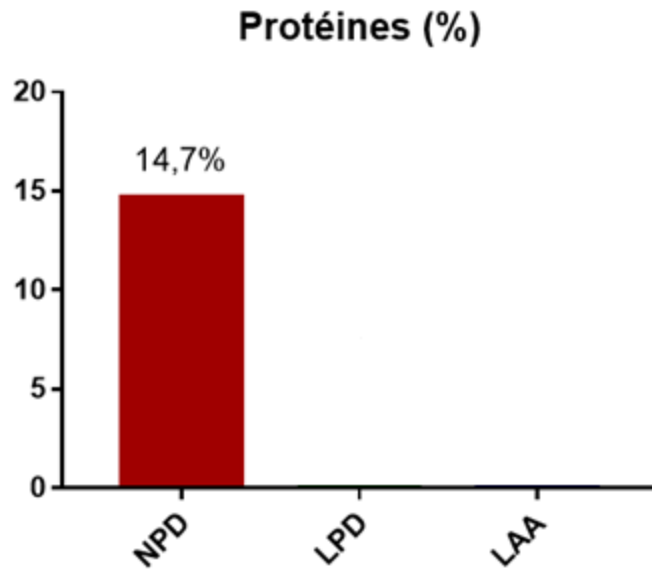


Régime standard
(14,7% protéines)

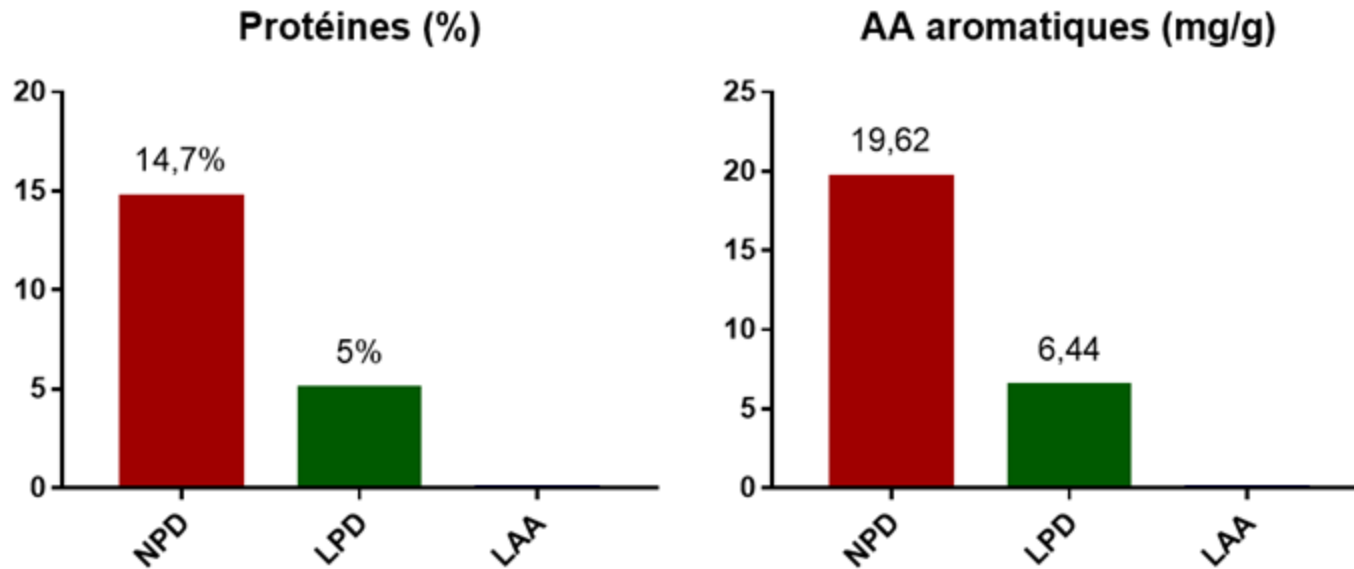
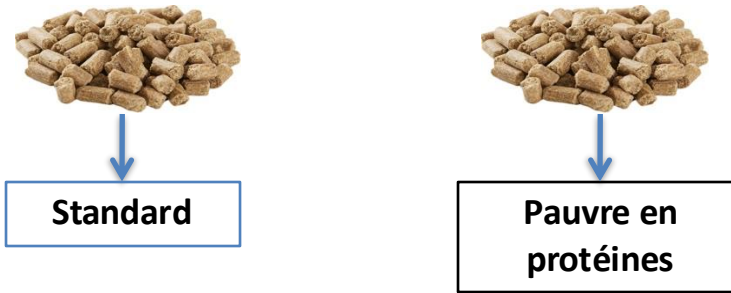
-Euthanasie et nécropsie
-Analyses biochimiques
-Analyses histologiques
-Dosage des toxines
urémiques

6 semaines de
régime spécifique

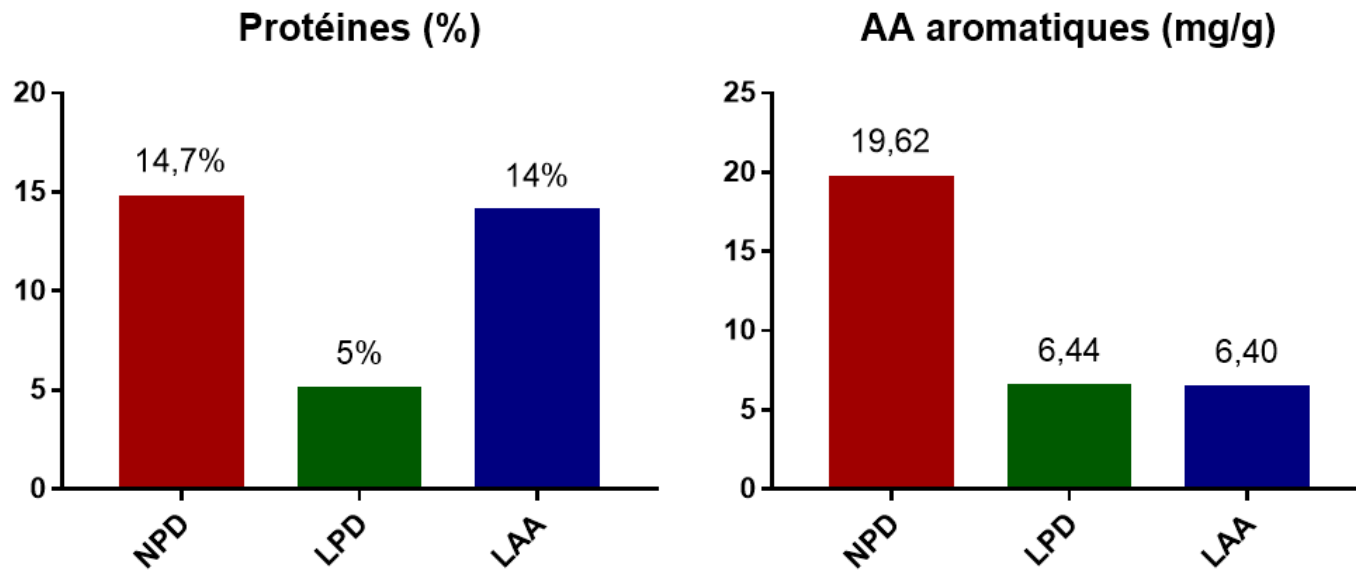
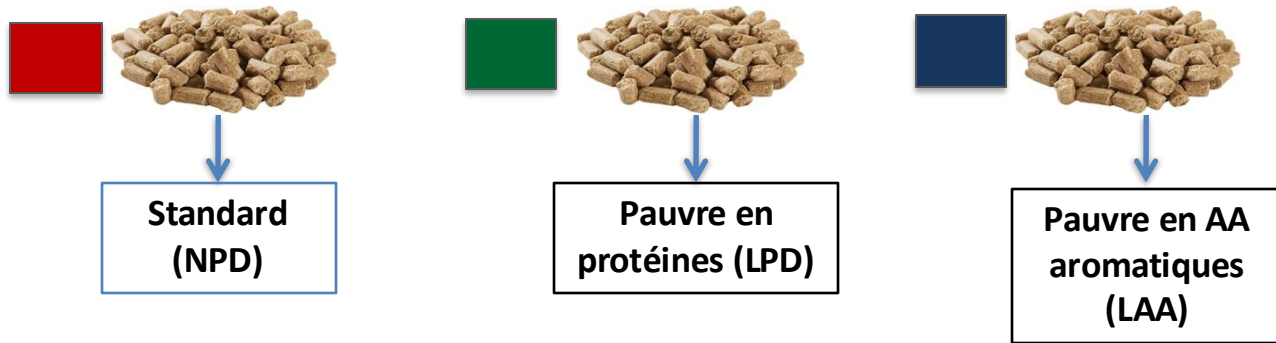
Composition des régimes utilisés



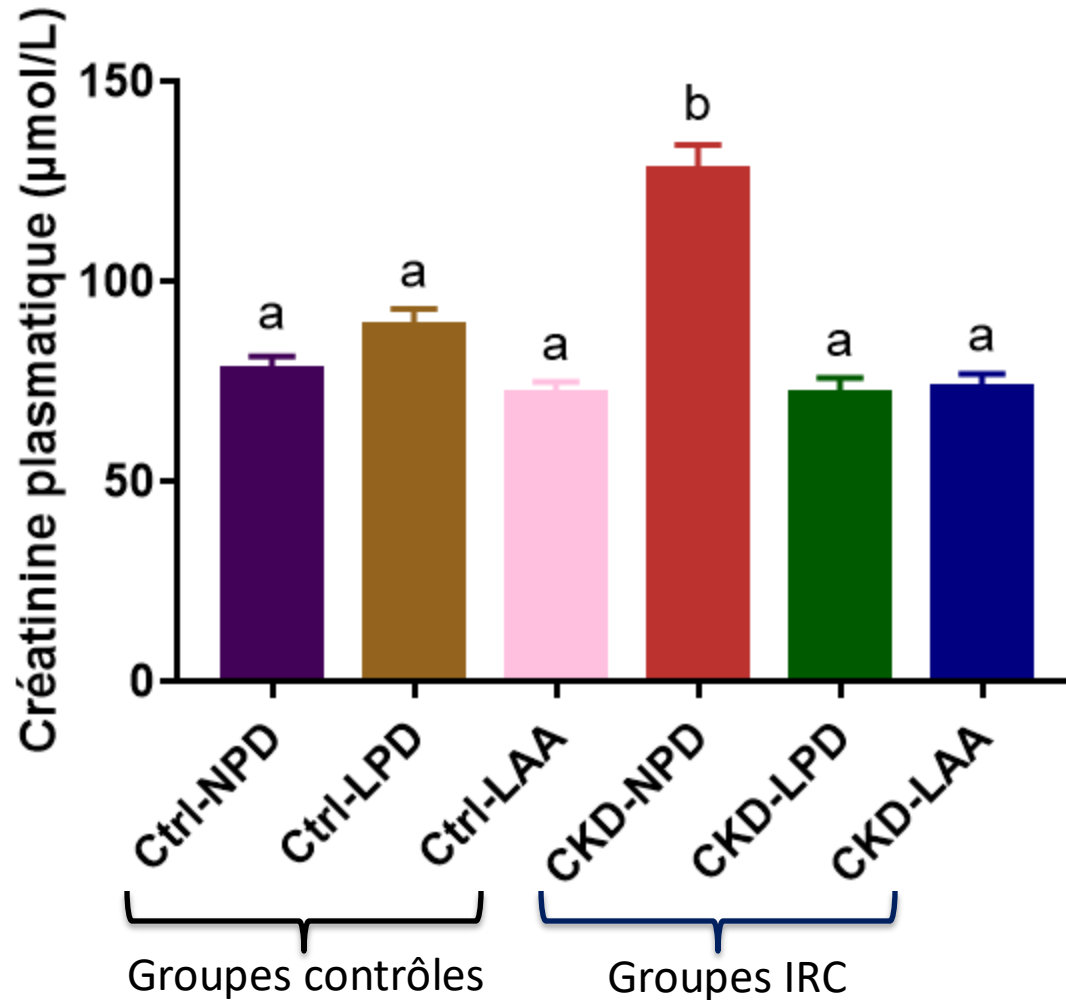
Composition des régimes utilisés



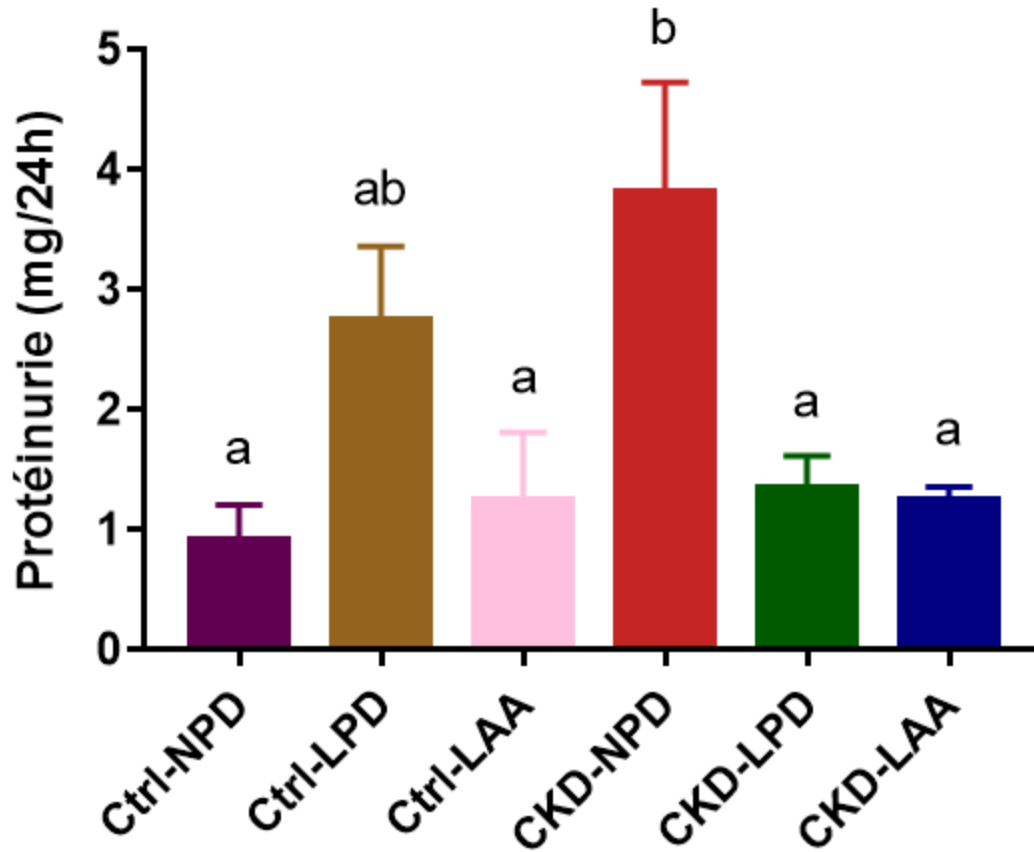
Composition des régimes utilisés



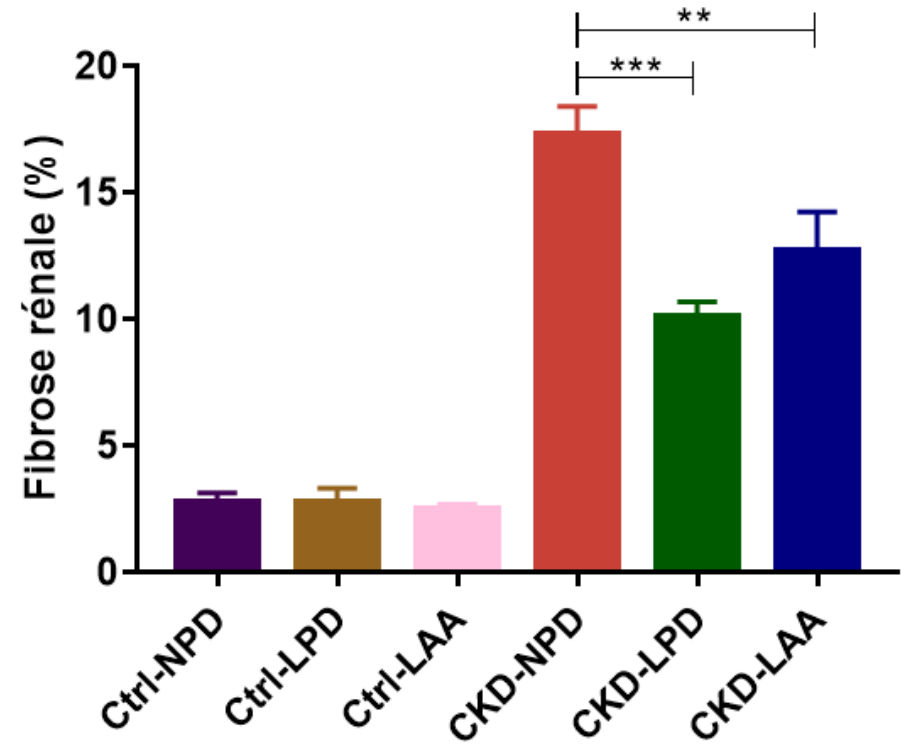
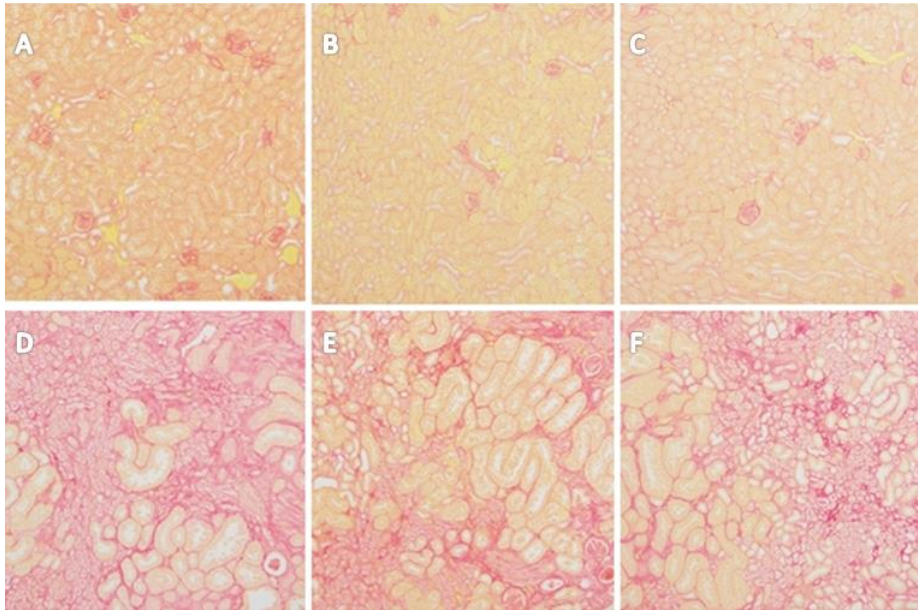
Effets sur la fonction rénale



Effets sur la protéinurie

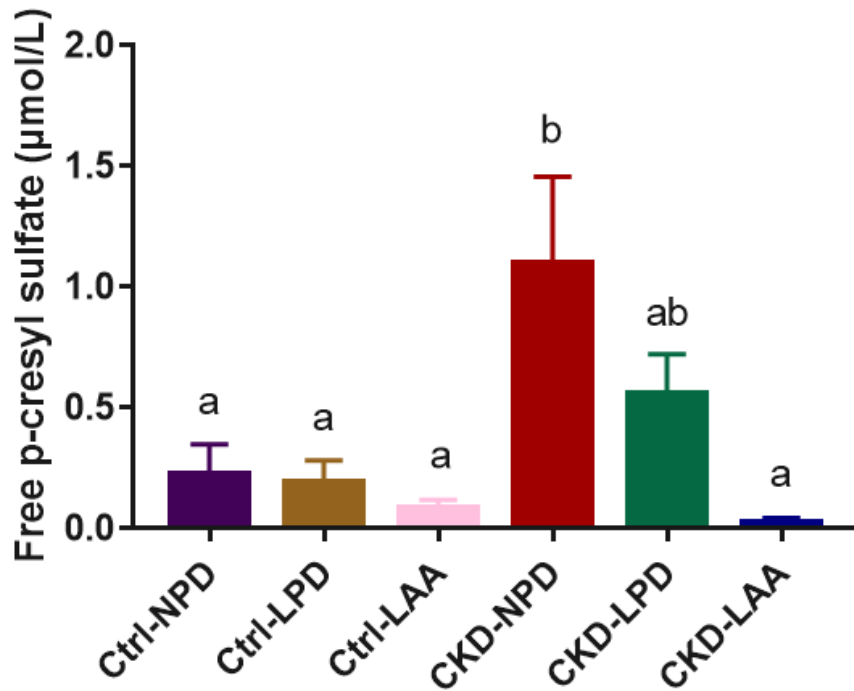


Effets sur la fibrose rénale

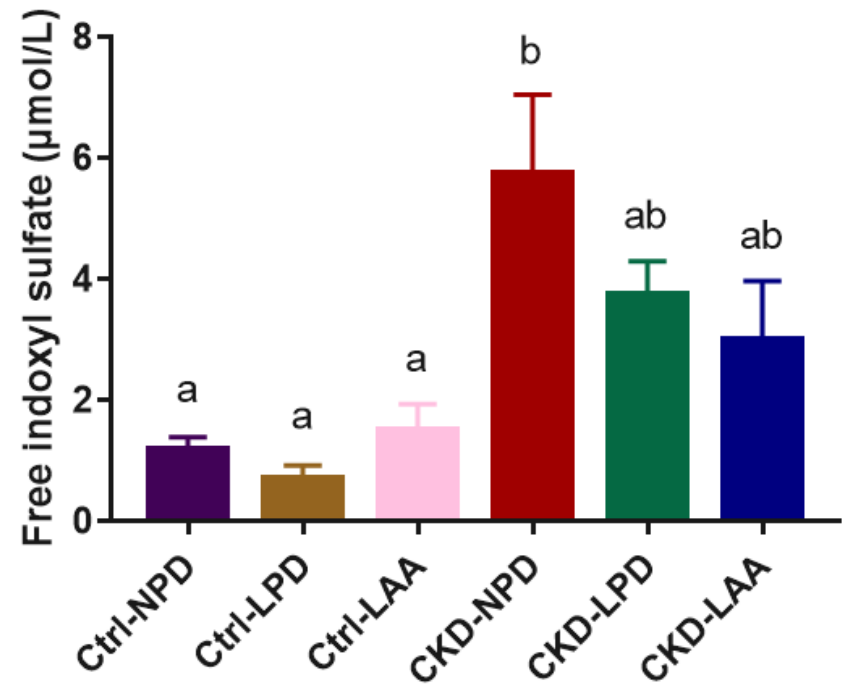


Fraction libre des toxines urémiques

P-cresyl sulfate libre



Indoxyl sulfate libre












Conclusion de l'étude expérimentale



- Preuve de concept que la modification **qualitative** (et non uniquement quantitative) de l'apport protéique peut préserver la fonction rénale
- Etudes complémentaires nécessaires pour en comprendre les déterminants

Les 8 acides aminés essentiels pour l'adulte*

	Leucine	Isoleucine	Valine	Phénylalanine	Thréonine	Méthionine	Lysine	Tryptophane
 Oeufs	✓	✓	✓	✓	✓	✓	✓	✓
 Volaille	✓	✓	✓	✓	✓	✓	✓	
 Viande rouge	✓	✓	✓	✓	✓	✓	✓	
 Poisson	✓	✓	✓		✓	✓	✓	✓
 Fromage	✓		✓	✓	✓	✓		✓
 Soja	✓	✓	✓	✓			✓	
 Céréales (blé, riz, maïs)		✓				✓	✓	✓
 Légumineuses (haricots, lentilles)		✓	✓	✓		✓	✓	
 Fruits à coque (amande, noix)		✓		✓				✓

Attention aux carences...

Solution ? apport exclusivement parentéral donc pas de transformation par le microbiote intestinal

* Ajouter l'arginine et la glutamine chez l'enfant

Quelles applications pratiques ?

Compléments alimentaires pour patients en hémodialyse



- Hyper calorique
- Hyper protidique
- **mais pauvre en AA aromatiques**

Lutter contre la dénutrition protéino-énergétique chez les patients hémodialysés ...

Protéines synthétiques pauvres ou dépourvues d'aromatiques (ex: collagène)

En conclusion: les toxines urémiques

- **Toxicité démontrée** tant en pré-clinique qu'en clinique...
- Grande difficulté à améliorer la clairance en hémodialyse des composés liés aux protéines
 - Technique de dilution, augmentation de la force ionique, colonnes d'affinité/échangeuses d'ions, déplaceurs... Pb de coûts et de disponibilité des équipements
- Moduler leur production ou leur absorption...
 - Low protein diet, régimes végétariens,...
 - Transplantation de flore pour corriger la dysbiose urémique
 - Pré/probiotiques
- **Beaucoup de stratégies prometteuses en pré-Clinique n'ont données que des résultats plutôt décevants en Clinique....**



Merci pour votre attention