Què sabem de nou de les toxines urèmiques?

On behalf of the European Uraemic Toxin Group

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Directeur de recherche
Professeur Associé

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Què en sabíem i ara ho considerem vell de les toxines urèmiques?

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Quines són les noves vies d'exploració de la importància de les toxines urèmiques?

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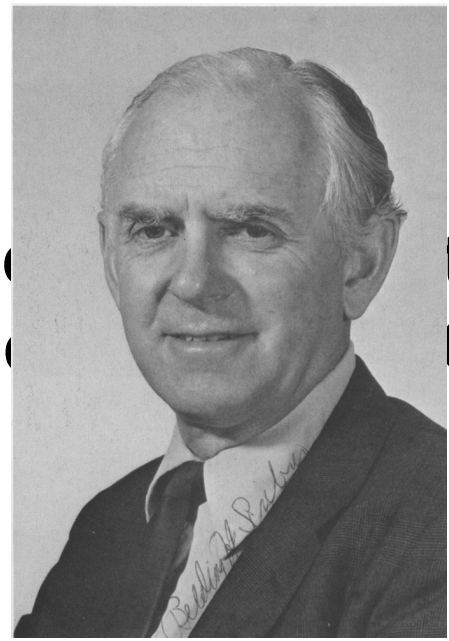
TRANSACTIONS



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VOLUME VI
1960

THE TREATMENT OF CHRONIC UREMIA BY MEANS OF INTERMITTENT HEMODIALYSIS: A PRELIMINARY REPORT

B. H. Scribner, R. Buri, J. E. Z. Caner, R. Hegstrom, and J. M. Burnell

Ever since the introduction of the first artificial kidney by Abel, Rountree and Turner in 1913[1] and its development into a clinically useful apparatus by Kolff[2], it has seemed possible that life could be prolonged in patients dying of chronic renal failure. Yet, the technical problems associated with intermittent hemodialysis have been so great that it has not been possible to effectively treat such patients. The chief efforts to study the role of dialysis in chronic renal failure have emerged from the laboratories of Alwall[3], Merrill[4] and Schreiner[5,6]. The results to date have not been encouraging. In general, significant prolongation of life has been limited to those patients who have acute reversible extrarenal factors jeopardizing their minimal renal function. Dialysis has also been of limited benefit to those patients who have very slowly progressive renal disease and considerable remaining function at the time dialysis was undertaken. The best results have been obtained in patients with polycystic disease with remissions from the uremic syndrome of as long as six months or more.

The discouraging results and those that bear on the cases to be reported herein are those obtained in cases of subacute nephritis. These patients remained oliguric or anuric throughout the course of therapy. The case reported in this issue of the Transactions by Maher, Schreiner and Waters[6] is typical and demonstrates that a major problem is inability to maintain the nutritional status of the patient despite repeated dialyses. Merrill also emphasizes that he has been unable to prevent mental deterioration for more than a few weeks in patients with complete loss of renal function[7].

The development of a technique for permanent indwelling teflon cannulation of radial artery and forearm vein has made it possible to perform an unlimited number of dialyses on patients with chronic renal failure[8]. As a result, a study was undertaken to evaluate the effect of frequent intermittent hemodialyses in two patients who were dying from uremia due to chronic glomerulonephritis. The techniques of cannulation and hemodialysis are reported elsewhere in this journal[8,9]. Essentially, they consisted of placement of indwelling teflon cannulae in the radial artery and a forearm vein. When not in use for dialysis, a small arterio-venous teflon bypass shunts blood from artery to vein. The patients have become ambulatory. They eat well, a diet containing 1/2 gm. of protein per Kg. of body weight, and except during the period of dialysis are home or at work part time. They are not strong enough to work full time. Each dialysis

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Supported by grants from the Boeing Employees' Research Fund, The Kidney Foundation, The National Institutes of Health, and Washington State Heart Association.

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Case I: C. S., a 39-year-old machinist, was told he had kidney disease in 1953 on the basis of an accidental discovery of 4+ proteinuria. He had had an episode diagnosed as rheumatic fever at the age of 19. C. S. remained relatively well and asymptomatic. In February, 1959, his BUN was normal, but he was excreting 10 grams of protein per 24 hours and had red cells and oval fat bodies in his urine. In November, 1959, his BUN was 70 mg.% and serum creatinine 6.5 mg.%. In December, 1959, he was forced to stop work because of weakness with vomiting and headaches. At that time, his BUN was 125 mg.% and his serum creatinine 10.7 mg.%. His blood pressure was 160/90. Following a period of hospitalization, he improved slightly after liberalization of sodium intake and increase protein restriction. His creatinine fell to 8.7 mg.%. However, his blood pressure rose to 230/140 by the end of January, 1960.

On admission to the hospital early in March, he was barely able to walk to the bathroom. His speech was thick and his sensorium clouded. He had uremic tremors and exudates and hemorrhages in his fundi. Physical examination was otherwise negative.

After the first dialysis of 76 hours, C. S. was much improved. He was fully ambulatory for the first time in weeks. He stopped vomiting, and he felt generally well. However, during the next three days, he developed severe headache, weakness and moderate shortness of breath, associated with a gain in

The General Picture of Uremia

Cyrielle Almeras* and Àngel Argilés*†‡

Seminars in Dialysis **22**:329-333, 2009

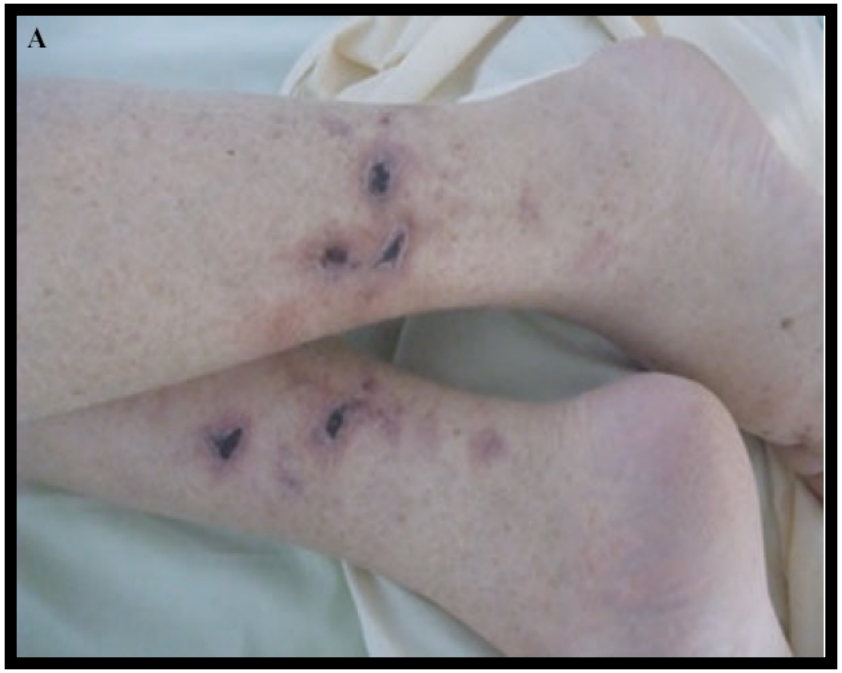

TABLE 1. Principal clinical features of uremia

Central nervous system	Diurnal somnolence, night insomnia, disorders of the memory and the concentration, asthenia, headache, confusion…
Peripheral nervous system	Polyneuritis, restless legs, cramps
Gastrointestinal	Anorexia, nausea, gastroparesia, parotiditis, stomatitis
Hematologic	Anemia, haemostasis disorders
Cardiovascular	Hypertension, atherosclerosis, coronary artery disease
Skin	Itching, skin dryness, calciphylaxis
Endocrinology	Growth impairment, impotence, infertility, sterility
Osteoarticular	Secondary hyperparathyroidism, osteomalacia, β2-microglobulin amyloidosis
Nutrition	Malnutrition, weight loss, muscular catabolism
Immunity	Low response rate to vaccination, increased sensitivity to infectious diseases
Biochemical	Metabolic acidosis, hyperphosphatemia, hyperkaliemia

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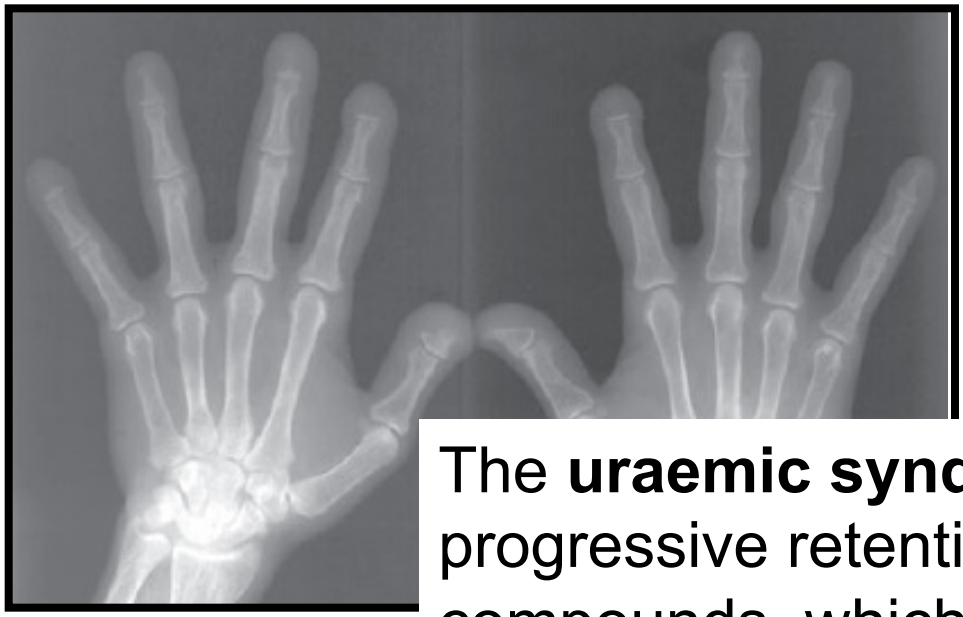

The **uraemic syndrome** is attributed to the progressive retention of a large number of compounds, which under normal conditions are excreted by the kidneys (« **uraemic retention solutes** »), which when they interact negatively with biologic functions are called « **uraemic toxins** ».

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Kidney International, Vol. 63 (2003), pp. 1934–1943

Review on uremic toxins: Classification, concentration, and interindividual variability

RAYMOND VANHOLDER, *et al*

EUTox data base
(http://www.nephro-leipzig.de/eutoxdb/index.php)

Table 1. Free water-soluble low-molecular-weight solutes ($N = 45$)

Solute	C_N	C_U	C_{MAX}	MW	Ref	Group
1-methyladenosine *μg/L*	17.1 ± 5.1/10	104.0 ± 56.2/17	216.4	281	[12]	Ribonucleosides
1-methylguanosine *μg/L*	13.7 ± 16.9/10	41.6 ± 23.8/17	89.2	297	[12]	Ribonucleosides
1-methylinosine *μg/L*	13.5 ± 3.9/10	620.4 ± 203.4/14	1027.2	282	[12]	Ribonucleosides
ADMA *mg/L*	0.2 ± 0.06/6	1.6 ± 1.2/10	7.3[a]	202	[13, 14]	Guanidines
α-keto-δ-guanidinovaleric acid *μg/L*	<30.2/66	—	140.4[a]	151	[15]	Guanidines
α-*N*-acetylarginine *μg/L*	18.1 ± 24.8/16	328.3 ± 142.6/13	4580.0[a]	216	[16, 17]	Guanidines
Arab(in)itol *mg/L*	<0.6/33	15.0 ± 9.0/12	33.0	152	[18, 19]	Polyols
Argininic acid *μg/L*	<77.0/66	80.5 ± 56.0/11	197.8[a]	175	[15, 16]	Guanidines
Benzylalcohol *mg/L*	—	27.0 ± 50.7/17	187.9[a]	108	[20]	
β-guanidinopropionic acid *μg/L*	<3.3/24	28.8 ± 18.3/29	65.4	131	[21]	Guanidines
β-lipotropin *ng/L*	<55.3/10	62.7/22	108.8[a]	461	[22]	Peptides
Creatine *mg/L*	9.7 ± 3.3/24	134.0 ± 30.3/29	235.8[a]	131	[21]	Guanidines
Creatinine *mg/L*	<12.0/23	136.0 ± 46.0/19746	240.0[a]	113	[23, 24]	Guanidines
Cytidine *μg/L*	<468.0	683.3 ± 287.8/7	1263.6[a]	234	[25]	Purines
Dimethylglycine *μg/L*	<381.1/33	576.8/18	1040.3[a]	103	[26]	
Erythritol *mg/L*	<0.7/33	9.8 ± 14.0/12	37.0[a]	122	[18, 19]	Polyols
γ-guanidinobutyric acid *μg/L*	<3.6/24	33.3 ± 16.0/30	1750.0[a]	145	[27, 17]	Guanidines
Guanidine *μg/L*	<11.8/16	172.9 ± 83.8/13	800.0[a]	59	[16, 17]	Guanidines
Guanidinoacetic acid *μg/L*	222.3 ± 79.6/24	383.8 ± 143.9/29	693.8[a]	117	[21]	Guanidines
Guanidonosuccinic acid *mg/L*	0.03 ± 0.01/16	6.5 ± 3.4/13	47.0[a]	175	[16, 17]	Guanidines
Hypoxanthine *mg/L*	1.5 ± 0.5/145	2.0 ± 1.6/65	5.3	136	[28, 29]	Purines
Malondialdehyde *μg/L*	257.7 ± 81.7/30	428.8 ± 170.4/16	769.6	71	[30]	
Mannitol *mg/L*	<1.3/33	26.0 ± 25.0/12	76.0	182	[18, 19]	Polyols
Methylguanidine *μg/L*	<7.3/24	773.8 ± 508.8/5	1820.0[a]	73	[21, 17]	Guanidines
Myoinositol *mg/L*	<10.0/8	94.0 ± 69.0/12	232.0	180	[18]	Polyols
N^2,N^2-dimethylguanosine *μg/L*	9.0 ± 4.7/10	236.4 ± 89.7/14	415.8	311	[12]	Ribonucleosides
N^4-acetylcytidine *μg/L*	57.0 ± 17.1/10	159.6 ± 30.8/14	221.2	285	[12]	Ribonucleosides
N^6-methyladenosine *μg/L*	18.5 ± 8.4/10	70.3 ± 53.3/17	176.9	281	[12]	Ribonucleosides
N^6-threonylcarbamoyladenosine *μg/L*	35.5 ± 27.2/10	378.0 ± 151.2/17	680.4	378	[12]	Ribonucleosides
Orotic acid *mg/L*	0.5 ± 1.4/30	6.7 ± 16.0/22	38.7	174	[31]	Pyrimidines
Orotidine *mg/L*	1.2 ± 1.6/30	20.2 ± 13.5/22	47.2	288	[31]	Pyrimidines
Oxalate *mg/L*	0.3 ± 0.1/8	4.9 ± 1.4/8	7.6	90	[32]	
Phenylacetylglutamine *mg/L*	<4.7	53.3 ± 44.7/6	120.6[a]	264	[33]	
Pseudouridine *mg/L*	0.5 ± 5.8/30	13.1 ± 21.4/7	86.6[a]	244	[25, 31]	Ribonucleosides
SDMA *μg/L*	76.1 ± 21.0/66	640.3 ± 212.1/38	1232.2[a]	202	[15]	Guanidines
Sorbitol *mg/L*	<0.4/33	3.1 ± 2.1/12	7.3	182	[18, 19]	Polyols
Taurocyamine *μg/L*	<52.2/24	—	121.8[a]	174	[17]	Guanidines
Threitol *μg/L*	<319.6/33	990.0 ± 920.0/12	5697.4[a]	122	[18, 19]	Polyols
Thymine *mg/L*	—	2.8 ± 4.2/22	11.2	126	[31]	Pyrimidines
Uracil *μg/L*	<224.0	252.0 ± 154.6/7	448.0[a]	112	[25]	Purines
Urea *g/L*	<0.4/23	2.3 ± 1.1/16	4.6[a]	60	[24]	
Uric acid *mg/L*	<67.2	83.4 ± 44.5/7	146.7[a]	168	[25]	Purines
Uridine *mg/L*	1.5 ± 1.3/30	9.8 ± 11.4/22	32.6	244	[31]	Pyrimidines
Xanthine *mg/L*	0.5 ± 1.4/180	1.5 ± 0.8/65	3.0	152	[28, 29]	Purines
Xanthosine *μg/L*	23.9 ± 12.8/10	96.6 ± 62.9/11	222.4	284	[12]	Ribonucleosides

Abbreviations are: C_N, normal concentration; C_U, mean/median uremic concentration; C_{MAX}, maximal uremic concentration; MW, molecular weight; ref, reference; ADMA, asymmetrical dimethylarginine; SDMA, symmetrical dimethylarginine. The underlined numbers behind the slash point to the number of data on which the means or medians have been obtained. No underlined number indicates that no data about the number of samples were available. Normal values are reported as means ± SD, or in the case of a single value as a maximum (accompanied by <); uremic values are reported as means ± SD or, in the case of a single value, as a median.

[a] C_{MAX} values are original data (all other values were calculated as mean + 2 SD based on C_U)

Kidney International, Vol. 63 (2003), pp. 1934–1943

Review on uremic toxins: Classification, concentration, and interindividual variability

RAYMOND VANHOLDER, *et al*

EUTox data base
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Table 2. Protein-bound solutes ($N = 25$)

Solute	C_N	C_U	C_{MAX}	MW	Ref	Group
2-methoxyresorcinol *μg/L*	—	19.6 ± 81.2/17	322.0[a]	140	[20]	Phenols
3-deoxyglucosone *mg/L*	0.3 ± 0.1/30	1.7 ± 1.0/27	3.5	162	[34]	AGE
CMPF *mg/L*	7.7 ± 3.3/7	61.0 ± 16.5/15	94.0[a]	240	[35]	
Fructoselysine *mg/L*	—	58.1 ± 10.8/10	79.7	308	[10]	AGE
Glyoxal *μg/L*	67.0 ± 20.0	221.0 ± 28.0/20	277.0	58	[36]	AGE
Hippuric acid *mg/L*	<5.0	247.0 ± 112.0/7	471.0	179	[37]	Hippurates
Homocysteine *mg/L*	<1.7/24	8.1 ± 1.6/7	26.4[a]	135	[38–40]	
Hydroquinone *μg/L*	—	50.6 ± 84.7/17	286.0[a]	110	[20]	Phenols
Indole-3-acetic acid *μg/L*	17.5 ± 17.5/7	875.0 ± 560.0/42	9076.9[a]	175	[41,42]	Indoles
Indoxyl sulfate *mg/L*	0.6 ± 5.4/40	53.0 ± 91.5/20	236.0	251	[35]	Indoles
Kinurenine *μg/L*	<391/7	686.4 ± 178.9/21	952.6	208	[43]	Indoles
Kynurenic acid *mg/L*	<1.0	—	9.5[a]	189	[44]	Indoles
Leptin *μg/L*	8.4 ± 6.7/56	72.0 ± 60.6/8	490.0[a]	16000	[45, 46]	Peptides
Melatonin *ng/L*	26.5 ± 7.1/35	175.8 ± 130.2/13	436.2	126	[47]	Indoles
Methylglyoxal *μg/L*	47.0 ± 12.0/15	110.0 ± 18.0/20	146.0	72	[36]	AGE
N^{ε}–(carboxymethyl)lysine *mg/L*	1.1 ± 0.3/24	4.3 ± 1.3/44	6.9	204	[11]	AGE
p-cresol *mg/L*	0.6 ± 1.0/12	20.1 ± 10.3/20	40.7	108	[48]	Phenols
Pentosidine *μg/L*	51.6 ± 18.8/19	896.0 ± 448.0/24	2964.0[a]	342	[49]	AGE
Phenol *mg/L*	0.6 ± 0.2/12	2.7 ± 3.9/10	10.5	94	[48]	Phenols
P-OHhippuric axid *mg/L*	—	18.3 ± 6.6/13	31.5	195	[50]	Hippurates
Putrescine *μg/L*	21.1 ± 7.9/10	77.4 ± 27.3/25	132.0	88	[51]	Polyamines
Quinolinic acid *mg/L*	0.1 ± 0.05/10	1.5 ± 0.9/54	3.3	167	[52]	Indoles
Retinol-binding protein *mg/L*	<80	192.0 ± 78.0/112	369.2[a]	21200	[53]	Peptides
Spermidine *μg/L*	—	97.2 ± 45.0/25	187.2	145	[51]	Polyamines
Spermine *μg/L*	—	18.2 ± 16.2/25	66.7[a]	202	[51]	Polyamines

Abbreviations are: C_N, normal concentration; C_U, mean/median uremic concentration; C_{MAX}, maximal uremic concentration; MW, molecular weight; ref, reference; CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid; AGE, advanced glycation end products. The underlined numbers behind the slash point to the number of data on which the means or medians have been obtained. No underlined number indicates that no data about the number of samples were available. Normal values are reported as means ± SD, or in the case of a single value as a maximum (accompanied by <); uremic values are reported as means ± SD.

[a] C_{MAX} values are original data (all other values were calculated as mean + 2 SD based on C_U).

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Table 3. Middle molecules ($N = 22$)

Solute	C_N	C_U	C_{MAX}	MW	Ref	Group
Adrenomedullin *ng/L*	13.2 ± 4.6/17	41.8 ± 19.7/29	81.2	5729	[54]	Peptides
Atrial natriuretic peptide *ng/L*	28.0 ± 12.2/23	202.0 ± 117.3/27	436.6	3080	[55]	Peptides
β_2-microglobulin *mg/L*	<2.0	55.0 ± 7.9/10	100.0[a]	11818	[53, 56]	Peptides
β-endorphin *ng/L*	<173.3/10	301.5/22	492.0[a]	3465	[22]	Peptides
Cholecystokinin *ng/L*	<20.0	45.9 ± 32.3/38	131.5[a]	3866	[57]	Peptides
Clara cell protein (CC16) *mg/L*	<0.1	3.3 ± 2.0/112	12.5[a]	15800	[53]	Peptides
Complement factor D *mg/L*	1.9 ± 0.5/5	19.8 ± 4.1/5	26.0[a]	23750	[58]	
Cystatin C *mg/L*	<1.6	11.8 ± 3.0/112	20.0[a]	13300	[53]	Peptides
Degranulation inhibiting protein I[c] *μg/L*	321.7 ± 59.7/23	713.7 ± 390.0/125	1631.4[a]	14100	[59][b]	Peptides
Delta-sleep inducing peptide *μg/L*	—	1.5 ± 0.9/7	3.3	848	[60]	Peptides
Endothelin *ng/L*	20.8 ± 3.8/23	63.0 ± 33.2/12	129.4	4283	[55]	Peptides
Hyaluronic acid *μg/L*	<124.0/86	215.0 ± 257.0/184	1843.0[a]	25000	[61]	Peptides
Interleukin-1β *ng/L*	<160.0/15	428.0 ± 134.0/29	1700.0	32000	[62]	Cytokines
Interleukin-6 *ng/L*	13.3 ± 3.1/28	92.3 ± 117.9/230	328.1	24500	[63]	Cytokines
κ-Ig light chain *mg/L*	34.0 ± 15.0/15	70.0 ± 60.9/104	287.0[a]	25000	[64]	Peptides
λ-Ig light chain *mg/L*	31.0 ± 11.2/15	87.0 ± 60.9/104	328.0[a]	25000	[64]	Peptides
Leptin *μg/L*	8.4 ± 6.7/56	72.0 ± 60.6/8	490.0[a]	16000	[45, 46]	Peptides
Methionine-enkephalin *ng/L*	<18.3/10	32.2/22	75.5[a]	555	[22]	Peptides
Neuropeptide Y *ng/L*	<80.0	64.9 ± 25.5/19	115.9	4272	[57]	Peptides
Parathyroid hormone *μg/L*	<0.06	1.2 ± 0.6/10	2.4	9225	[65]	Peptides
Retinol-binding protein *mg/L*	<80	192.0 ± 78.0/112	369.2[a]	21200	[53]	Peptides
Tumor necrosis factor-α *ng/L*	13.3 ± 3.0/28	114.0 ± 147.0/230	408.0	26000	[63, 66]	Cytokines

Abbreviations are: C_N, normal concentration; C_U, mean/median uremic concentration; C_{MAX}, maximal uremic concentration; MW, molecular weight; ref, reference. The underlined numbers behind the slash point to the number of data on which the means or medians have been obtained. No underlined number indicates that no data about the number of samples were available. No number indicates that no n value was given. Normal values are reported as mean ± SD, or in the case of a single value as a maximum (accompanied by <); uremic values are reported as mean ± SD or, in the case of a single value, as a median.

[a] C_{MAX} values are original data (all other values were calculated as mean + 2 SD based on C_U)

[b] S Schmaldienst, Vienna: personal communication

[c] Degranulation inhibiting protein I corresponds to angiogenin

Normal and Pathologic Concentrations of Uremic Toxins

Flore Duranton,* Gerald Cohen,† Rita De Smet,‡ Mariano Rodriguez,§ Joachim Jankowski,‖ Raymond Vanholder,‡ and Angel Argiles,* on behalf of the European Uremic Toxin Work Group

Table 1. Contingency table of uremic retention solutes depending on solute classification, solute status, and number of records retrieved for each solute

Solute Classification	Solute Status		Total (Count)
	Known Retention Solute (Count)	Newly Identified Retention Solute (Count)	
Free water-soluble low molecular weight molecules			
results based on one report (count)	8	25	33
results based on several reports (count)	3	4	7
total	11	29	40 (46%)
Protein-bound solutes			
results based on one report (count)	3	4	7
results based on several reports (count)	8	8	16
total	11	12	23 (25%)
Middle molecules			
results based on one report (count)	2	10	12
results based on several reports (count)	8	5	13
total	10	15	25 (28%)
Total (count)	32 (37%)	56 (63%)	88 (100%)

Solutes that had been presented in the previous reviews[1,2] were considered as known retention solutes.

J Am Soc Nephrol 23: 1258–1270, 2012. doi: 10.1681/ASN.2011121175

Complexity of uraemic toxicity and uraemic toxin research

Highly divers group of uraemic retention solutes

Post-translational modifications by exposure to the uraemic milieu

Wide variety of affected cell systems

Blood cells (leukocytes and monocytes, but also platelets)

Endothelial cells

Cells of a variety of different systems
(nervous system, heart, renal tissue, joints and bones, …)

Different aspects of the uraemic syndrome

and

Participation of different uraemic retention solutes

Water Soluble Compounds:

Urea

Van Helmont (1577 – 1644),
Alchimist
Painting by Mary Beale (c1674)

Jean Baptiste van Helmont, a Brussels born chemist and physician, was the founder of the iatrochemical school which looked to chemical explanations of vital phenomena. He was a man of great intellectual curiosity and studied philosophy at Louvain. His description of the salt of urine is the first evidence of the urinary content of urea.

History of Urea

Description of physico-chemical characteristics of urinary UREA

"salt of urine that never occurs outside man's body which is bred in the course of digestion from a substance not a salt".
"It differs from sea-salt, also present in urine, by remaining unchanged in its course through the body and on putrefaction of urine".

"The sea-salt in its cooling, adheres to a wooden vessel even while it is separated from saltpeter, but the salt of urine grows together in the bottom of the liquor".

Van Helmont JB. Van Helmont's Works. Translated into English by J Chandler. London : L. Lloyd, 1664.

Contribution from chemistry:

Urea is the diamide carbonic acid (H_2NCONH_2)

Discovered in urine as a salt different from the sea salt in 1727 (Hermann Boerhaave., Leyden, The Netherlands)

Purified by FOURCROIY and VAUQUELIN and further by F William Prout

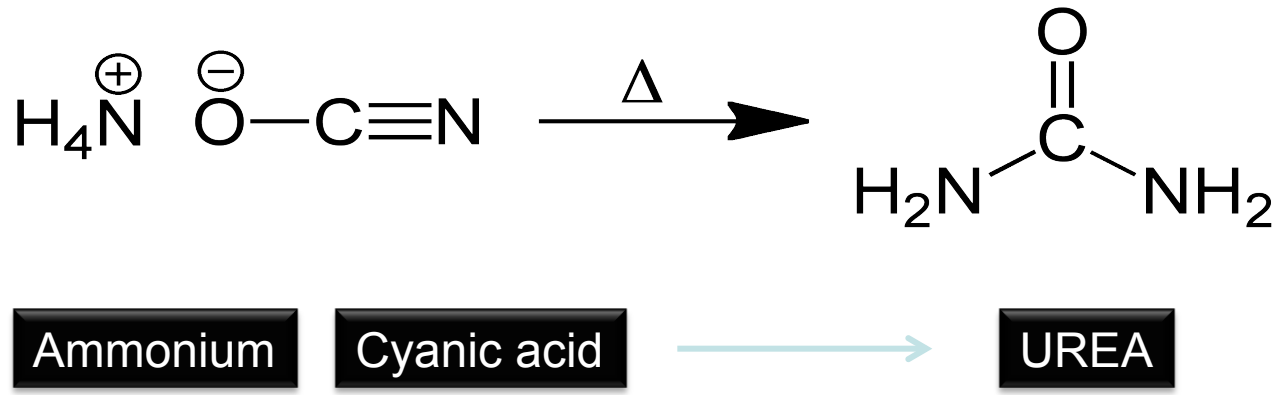
FOURCROY and VAUQUELIN *Annales du Museum d'Histoire Naturelle. T. 11*. cah: 63. 226 (180 8)

PROUT, W., *Med. Chir. Transactions,* 7, 527 (1817) [reprinted in *Thomson's Annals of Philosophy,* 11, 352 (1818); *Ann. chim. phys.,* /2/, 10, 369 (1817).

Bérard, M J E, *Annal Chim. Phys.,* 5, 290 (1817)

Universiteit Leiden

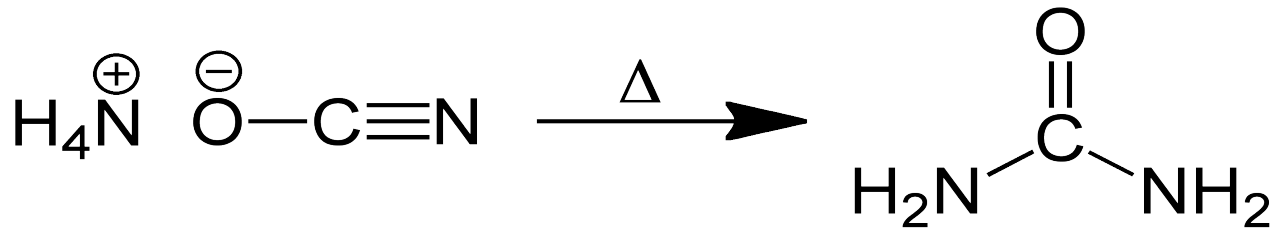
Friedrich Wöhler



WOOHLER F: Über küdnstlische Bildung des Harnstoffs. Annalen des Physics und Chemie, tome 12, 253, 1828.

French translation in Ann Chimie et Physique, 1828, tome 37, pp. 330—334

Friedrich Wöhler



Ammonium + Cyanic acid → UREA

"I can make urea without needing a kidney of man or dog. The ammonium salt of cyanic acid is UREA"

Letter to Berzelius in Stockholm, 1828

Origin of organic chemistry, since it was the first report showing that urea can be synthesized outside the body with inorganic substances

Growing evidence for an indirect toxicity

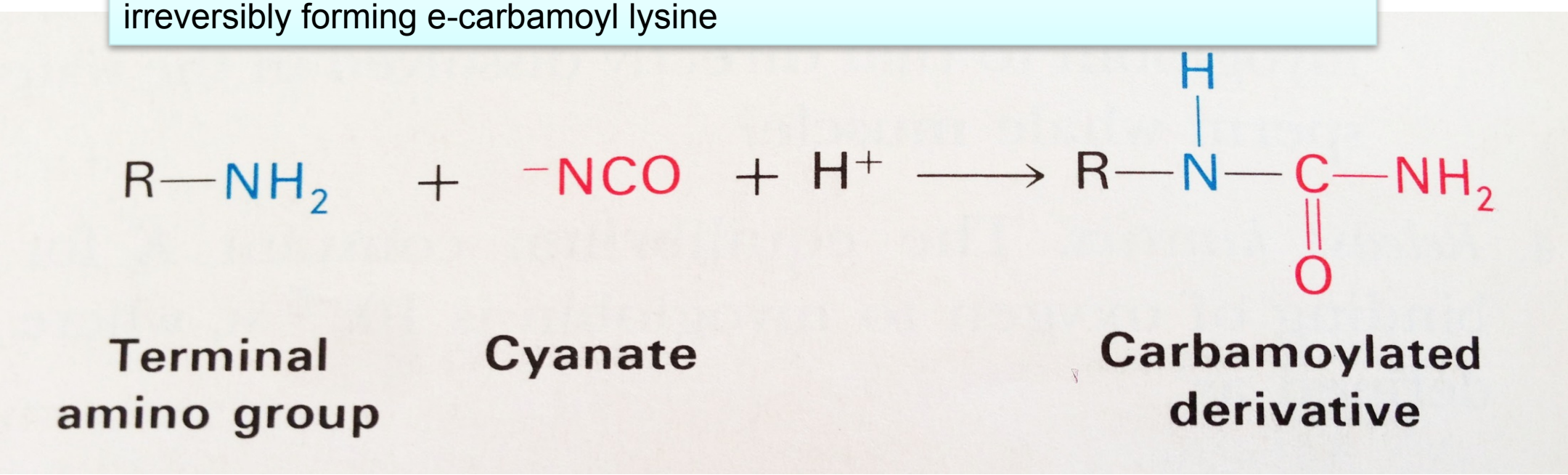
Urea, a uraemic toxin?

Spontaneous generation of Cyanate from UREA at equilibrium

$$H_2N\text{-}CO\text{-}NH_2 \leftrightarrows NCO^- + NH^+_4$$

UREA ⇆ Cyanic acid + Ammonium

Isocyanic acid reaction with free aminoacids and lysine residues of proteins irreversibly forming e-carbamoyl lysine



BRIEF COMMUNICATION

J Am Soc Nephrol 21:1852-57, 2010

Apostolov *et al*

Chronic Uremia Stimulates LDL Carbamylation and Atherosclerosis

Methods and groups of mice

	Diet		Urea level
CRF	Chow / High-Fat Diet (HFD)		↑
Uni-Nephrectomy	Chow / High-Fat Diet (HFD)	Non-Urea Consuming (-UC)	—
		Urea Consuming (+UC) (20 mg/mL supplemented Urea in the drinking water)	↑

BRIEF COMMUNICATION

J Am Soc Nephrol 21:1852-57, 2010

Apostolov *et al*

Chronic Uremia Stimulates LDL Carbamylation and Atherosclerosis

Aortic atherosclerotic lesions develop in CRF mice and in non CRF mice supplemented with Urea (both fed with High Fat Diet)

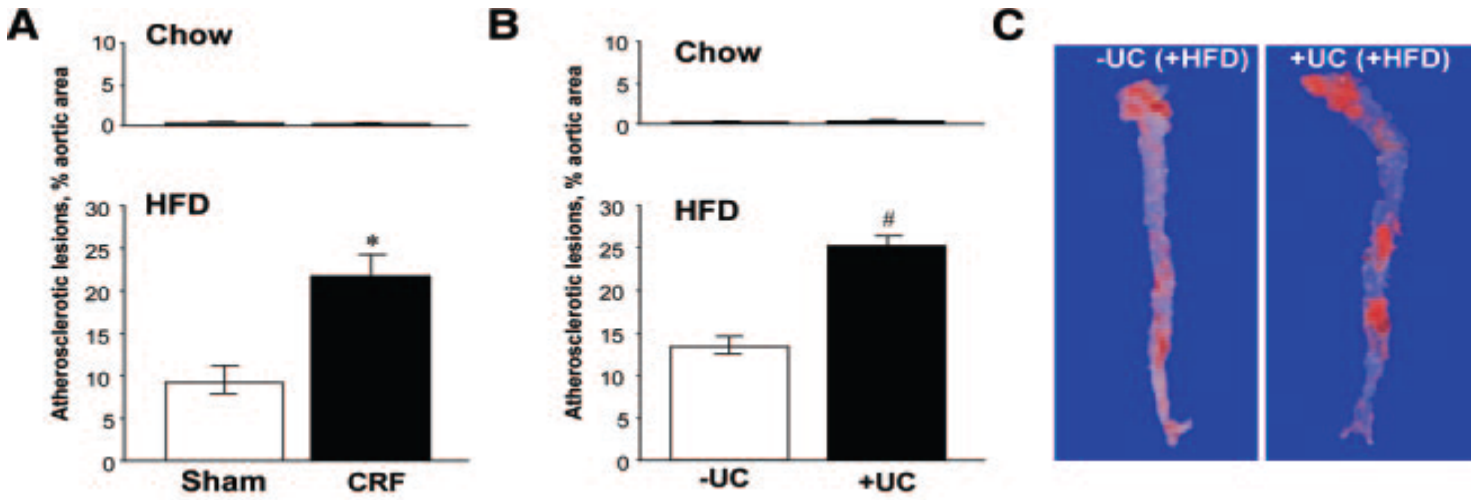


Figure 3. CRF (A) and UC (B and C) mice develop atherosclerotic plaques and lipid deposits in aortas as detected by Sudan IV *en face* staining: measurements (A and B) and representative images (C). *P 0.05 compared with sham-operated mice fed with HFD. #P 0.05 compared with UC control mice fed with HFD.

Science Translational Medicine
AAAS

Carbamylation of Serum Albumin as a Risk Factor for Mortality in Patients with Kidney Failure

Anders H. Berg *et al.*

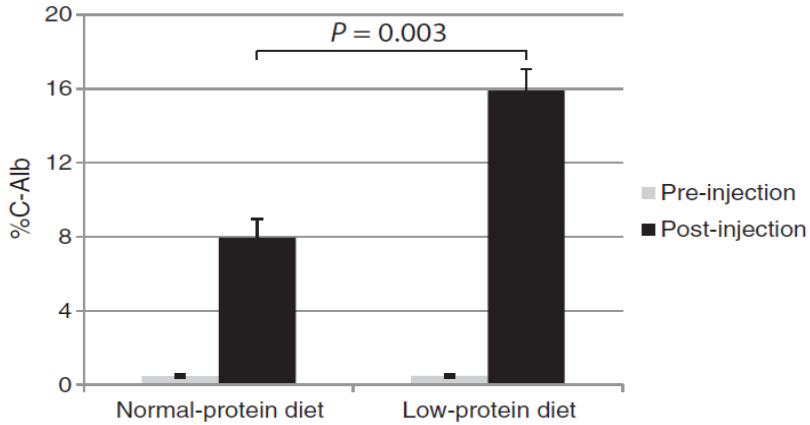
DOI: 10.1126/scitranslmed.3005218
Sci Transl Med **5**, 175ra29 (2013)

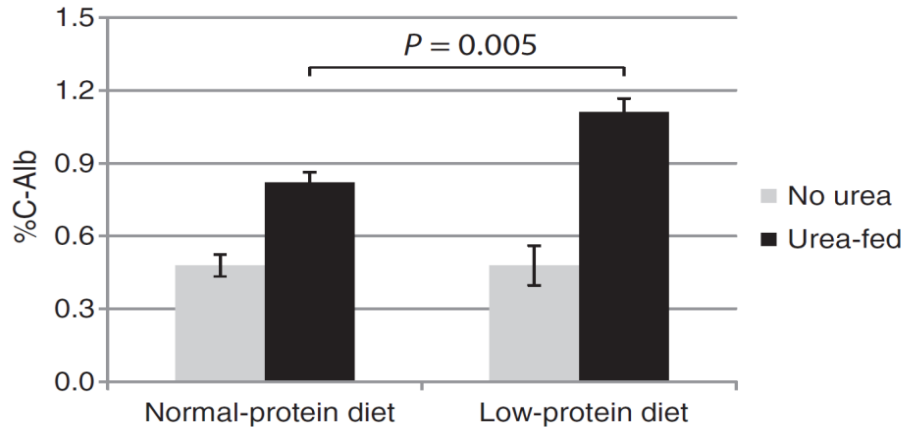
C57BL/6J mice (N= 6)
male 10-week-old

Given low / normal protein diet for 15 days

± Cyanate injection

± Urea feeding





Injection of cyanate increases carbamylated albumin concentration in mice

Feeding urea also increases carbamylated albumin concentration in mice

Science Translational Medicine
AAAS

Carbamylation of Serum Albumin as a Risk Factor for Mortality in Patients with Kidney Failure

Anders H. Berg *et al.*

DOI: 10.1126/scitranslmed.3005218
Sci Transl Med **5**, 175ra29 (2013)

Carbamylated albumin increases in CKD and dialysis

Carbamylated albumin is a risk factor for mortality

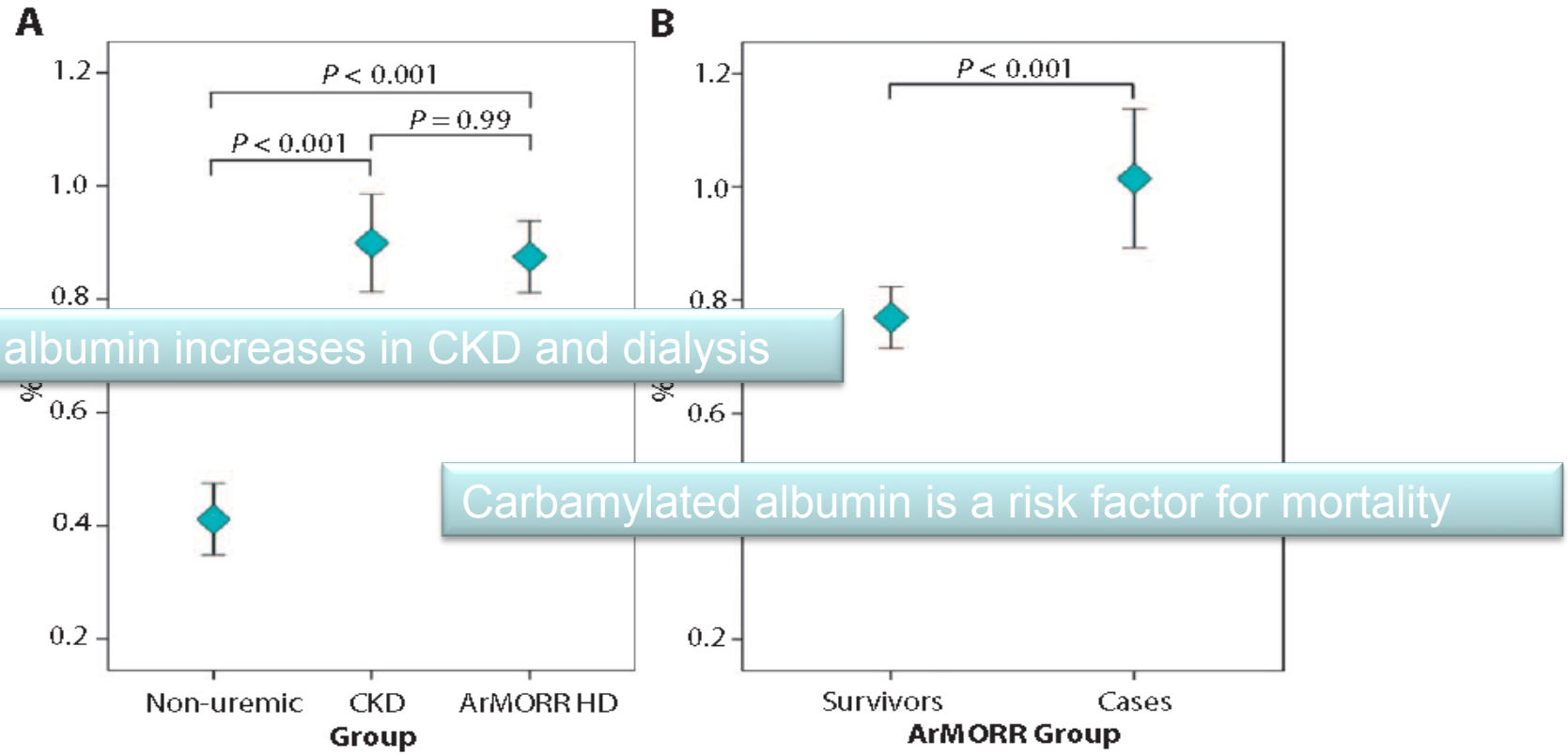


Fig. 2. Average carbamylated albumin values in uremic and non-uremic patients. (A) Average %C-Alb values in non-uremic subjects (n = 20) and in patients with stage 3 or 4 CKD (n = 122) and ArMORR HD subjects (n = 187). (B) Average %C-Alb in ArMORR survivors who lived longer than 12 months (n = 106) and in ArMORR cases who died during the 12-month study period (n = 81). Individual %C-Alb values for each group are shown in table S2. Data are expressed as average carbamylated albumin as a percentage of total; error bars, 95% confidence intervals (CIs) of the mean; Student's t test P values are shown.

Science Translational Medicine
AAAS

Carbamylation of Serum Albumin as a Risk Factor for Mortality in Patients with Kidney Failure

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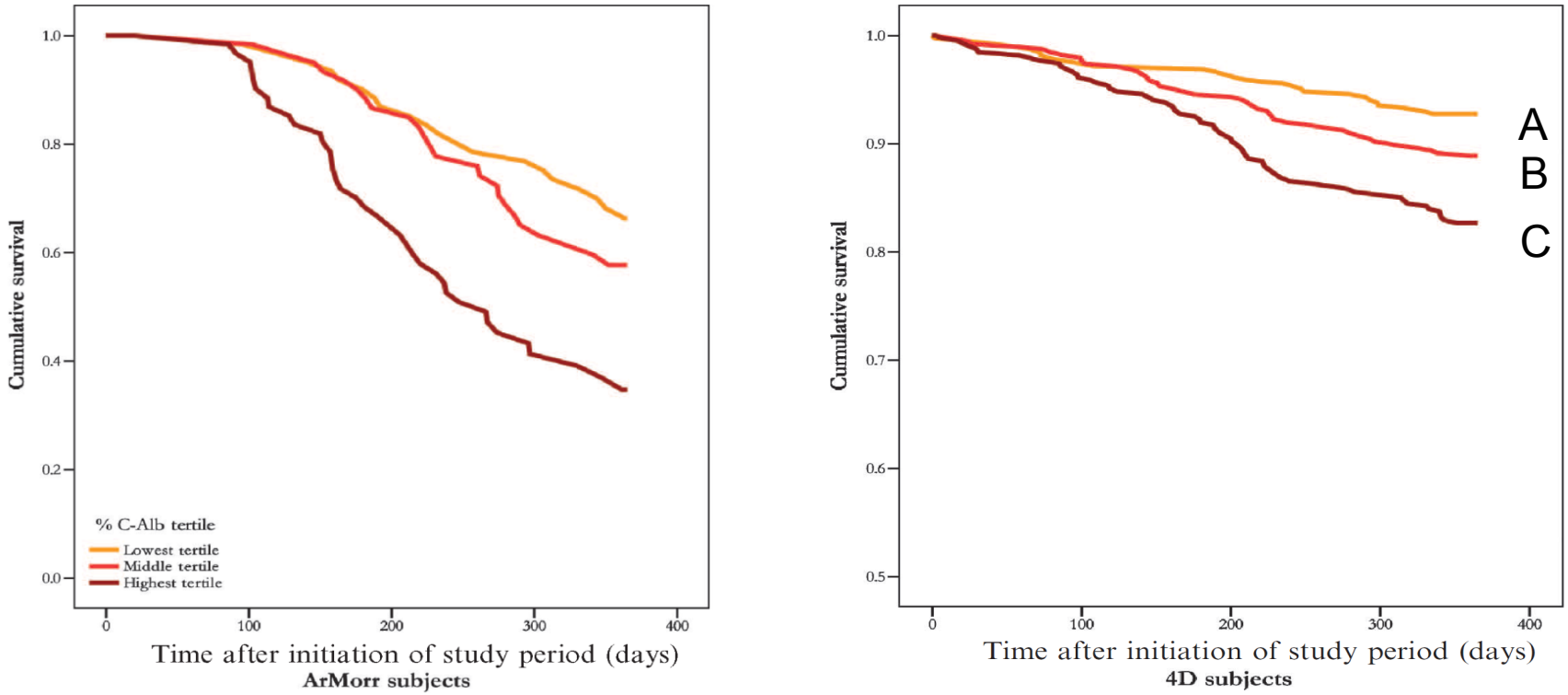
Sci Transl Med **5**, 175ra29 (2013)

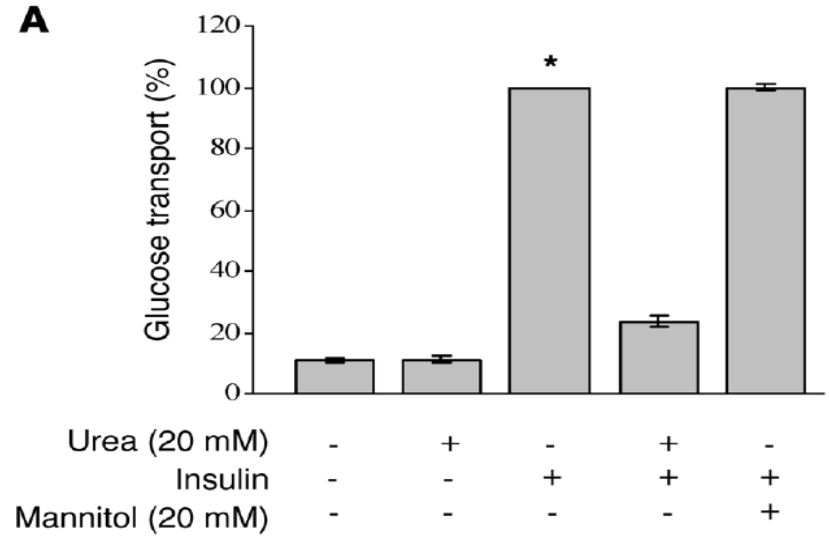


Kaplan-Meier curve estimates of the incidence of all-cause mortality in ESRD patients. Subjects were categorized into lower (A), middle (B), and upper © tertiles according to serum %C-Alb values measured at the outset of the study. Twelve-month survival rates in 4D study subjects (modified from AH Berg *et al*, *Sci Transl Med* 5:175ra29 (2013))

J. Clin. Invest. **120**:203–213 (2010). doi:10.1172/JCI37672

Urea – induced ROS generation causes insulin resistance in mice with chronic renal failure

D'Apolito Maria *et al*

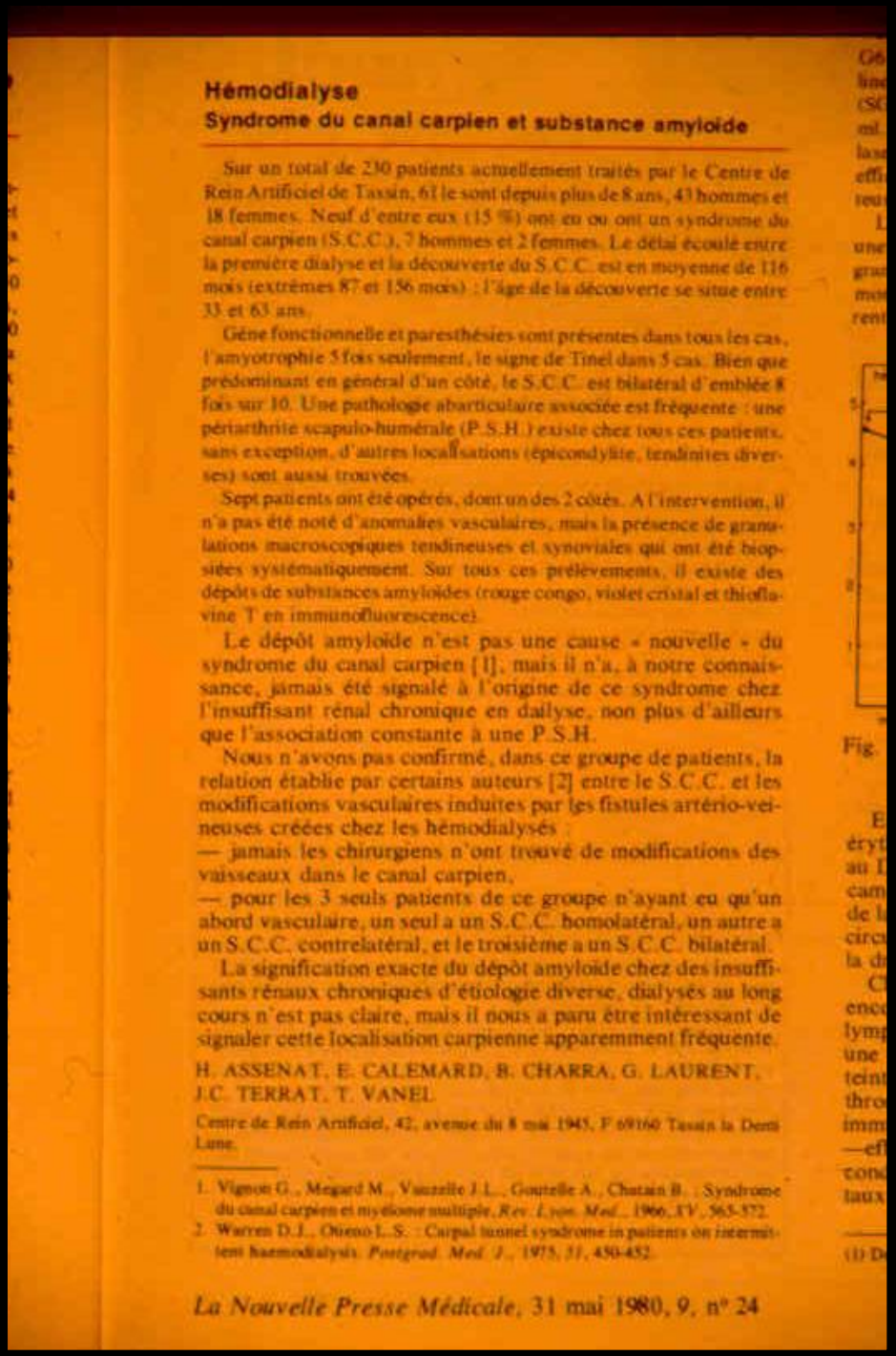


Urea decreases Glucose transport

Urea cause[s] [decreased] glucose transport. (**A**) Effect of urea on insulin-stimulated glucose uptake in differentiated 3T3L1 cells. (**B**) Immunoblot analysis of insulin-induced phosphorylation of IRS-1 tyrosine and Ser636 in urea-treated T3L1 cells and controls. (**C**) Immunoblot analysis of insulin-induced AKT phosphorylation in urea-treated 3T3L1 cells and their controls. Maximum levels of IRS-1 and AKT phosphorylation are shown as 100% in bar graphs.IP, immunoprecipitation. *n* = 5; **P* < 0.01 compared with controls. Data represent mean ± SEM.

Middle mol wt compounds:

β2 microglobulin



Hémodialyse

Syndrome du canal carpien et substance amyloïde

Sur un total de 230 patients actuellement traités par le Centre de Rein Artificiel de Tassin, 61 le sont depuis plus de 8 ans, 43 hommes et 18 femmes. Neuf d'entre eux (15 %) ont eu ou ont un syndrome du canal carpien (S.C.C.), 7 hommes et 2 femmes. Le délai écoulé entre la première dialyse et la découverte du S.C.C. est en moyenne de 116 mois (extrêmes 87 et 156 mois) ; l'âge de la découverte se situe entre 33 et 63 ans.

Gêne fonctionnelle et paresthésies sont présentes dans tous les cas, l'amyotrophie 5 fois seulement, le signe de Tinel dans 5 cas. Bien que prédominant en général d'un côté, le S.C.C. est bilatéral d'emblée 8 fois sur 10. Une pathologie abarticulaire associée est fréquente : une périarthrite scapulo-humérale (P.S.H.) existe chez tous ces patients, sans exception, d'autres localisations (épicondylite, tendinites diverses) sont aussi trouvées.

Sept patients ont été opérés, dont un des 2 côtés. A l'intervention, il n'a pas été noté d'anomalies vasculaires, mais la présence de granulations macroscopiques tendineuses et synoviales qui ont été biopsiées systématiquement. Sur tous ces prélèvements, il existe des dépôts de substances amyloïdes (rouge congo, violet cristal et thioflavine T en immunofluorescence).

Le dépôt amyloïde n'est pas une cause « nouvelle » du syndrome du canal carpien [1], mais il n'a, à notre connaissance, jamais été signalé à l'origine de ce syndrome chez l'insuffisant rénal chronique en dialyse, non plus d'ailleurs que l'association constante à une P.S.H.

Nous n'avons pas confirmé, dans ce groupe de patients, la relation établie par certains auteurs [2] entre le S.C.C. et les modifications vasculaires induites par les fistules artério-veineuses créées chez les hémodialysés :
— jamais les chirurgiens n'ont trouvé de modifications des vaisseaux dans le canal carpien,
— pour les 3 seuls patients de ce groupe n'ayant eu qu'un abord vasculaire, un seul a un S.C.C. homolatéral, un autre a un S.C.C. controlatéral, et le troisième a un S.C.C. bilatéral.

La signification exacte du dépôt amyloïde chez des insuffisants rénaux chroniques d'étiologie diverse, dialysés au long cours n'est pas claire, mais il nous a paru être intéressant de signaler cette localisation carpienne apparemment fréquente.

H. ASSENAT, E. CALEMARD, B. CHARRA, G. LAURENT, J.C. TERRAT, T. VANEL

Centre de Rein Artificiel, 42, avenue du 8 mai 1945, F 69160 Tassin la Demi Lune.

1. Vignon G., Megard M., Vauzelle J.L., Goutelle A., Chatain B. : Syndrome du canal carpien et myélome multiple. *Rev. Lyon. Med.*, 1966, *XV*, 565-572.
2. Warren D.J., Otieno L.S. : Carpal tunnel syndrome in patients on intermittent haemodialysis. *Postgrad. Med. J.*, 1975, *51*, 450-452.

La Nouvelle Presse Médicale, 31 mai 1980, 9 N°24

First report involving amyloid deposits in dialysis patients

Study involving 230 dialysis patients.

Carpal Tunnel Syndrome = 9 patients

Elapsed time from dialysis onset = 116 months

Bilateral

Associated to Scapulo-humeral periarthritis

Vol. 129, No. 3, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 28, 1985 Pages 701-706

A NEW FORM OF AMYLOID PROTEIN ASSOCIATED WITH CHRONIC HEMODIALYSIS WAS IDENTIFIED AS β_2-MICROGLOBULIN

F. Gejyo[a], T. Yamada[a], S. Odani[b], Y. Nakagawa[a], M. Arakawa[a], T. Kunitomo[c], H. Kataoka[c], M. Suzuki[d], Y. Hirasawa[d], I. Shirahama[e], A.S. Cohen[e] and K. Schmid[f]

Departments of [a]Internal Medicine and [b]Biochemistry,
Niigata University School of Medicine, Niigata 951, Japan

[c]Basic Research Laboratories, Toray Industries, Inc., Kamakura 248, Japan

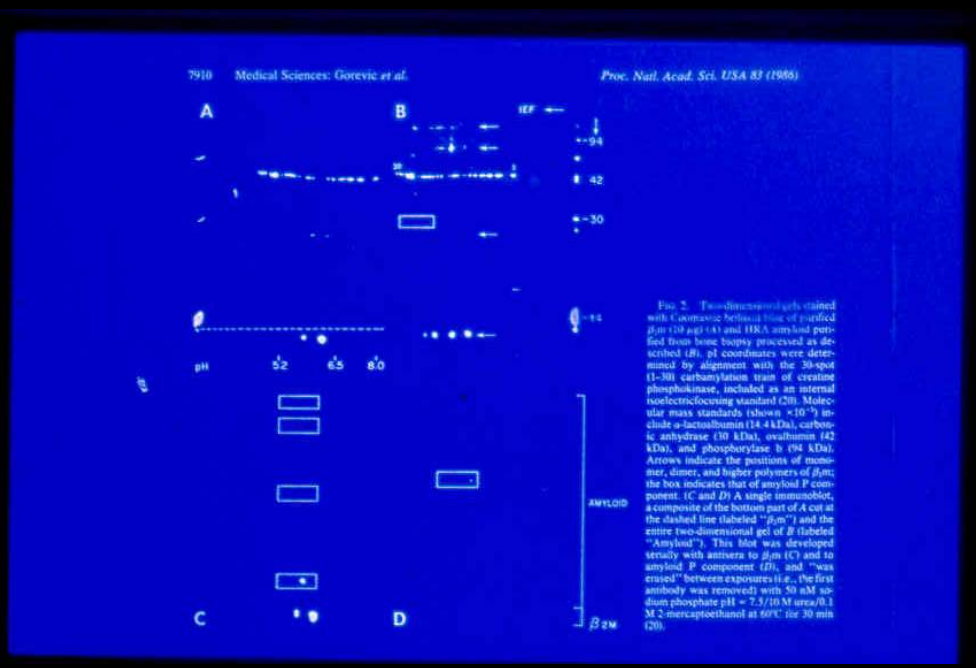
[d]Shinrakuen Hospital, Niigata 950-21, Japan

[e]Arthritis Center and [f]Department of Biochemistry,
Boston University School of Medicine, Boston, MA 02118

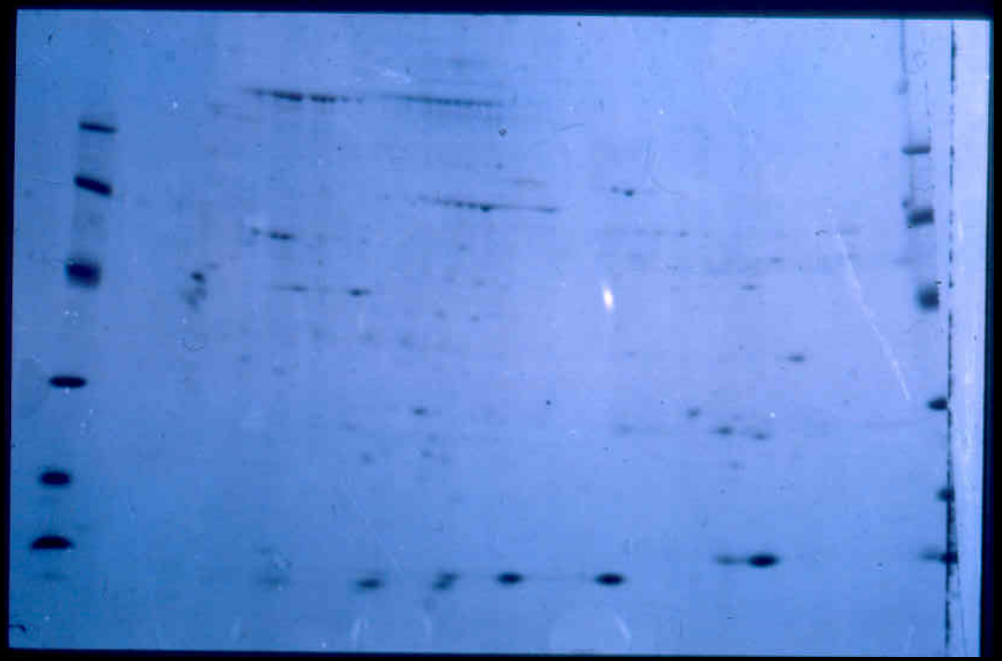
Received May 10, 1985

Amyloid fibrils were isolated from amyloid-laden tissue obtained from a chronic hemodialysis patient with carpal tunnel syndrome. After solubilization in guanidine HCl, a significant amount of the protein was located in a

β2m from amyloid deposits consists of more acidic isoforms



Gorevic *et al* PNAS 1986



Argilés *et al* Néphrologie 1987

Cleavage hypothesis

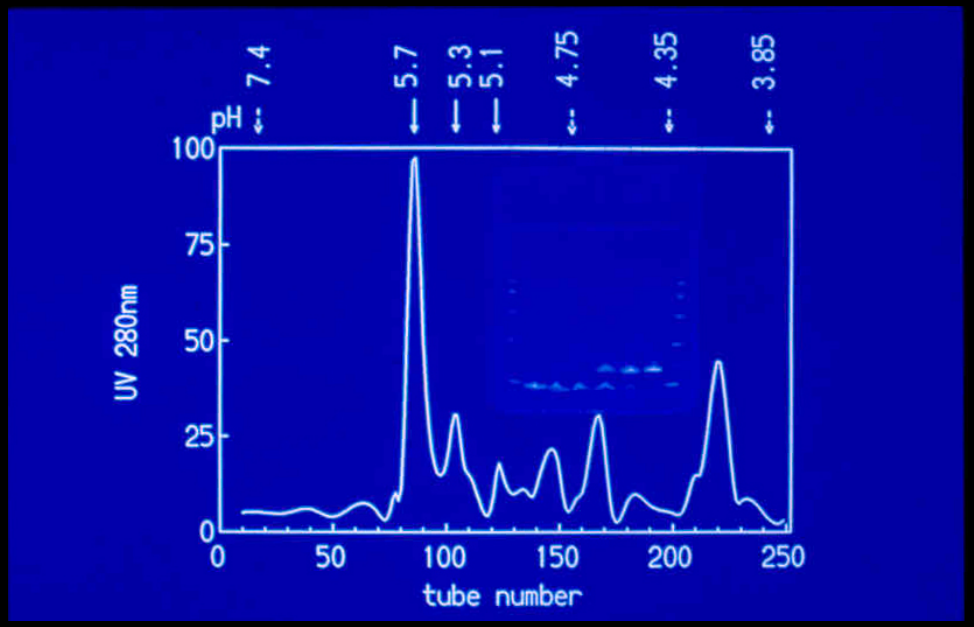
Kidney International, 36:675—681, 1989

Lysine-specific cleavage of β_2-microglobulin in amyloid deposits associated with hemodialysis

REINHOLD P. LINKE, HANNELORE HAMPL, HARTMUT LOBECK, EBERHARD RITZ, JÜRGEN BOMMER, RÜDIGER WALDHERR, and MANFRED EULITZ

Sequencing of the more acidic isoforms of β2m found an intact N-terminus

Argilés *et al Nephrol Dial Transpl* 7:1106-10, 1992



sequence analysis
(B2M isoforms)

urinary B2M

5.7 pI (1) Ile - Gln - Arg - Thr - Pro
5.3 pI (1) Ile - Gln - Arg - Thr - Pro

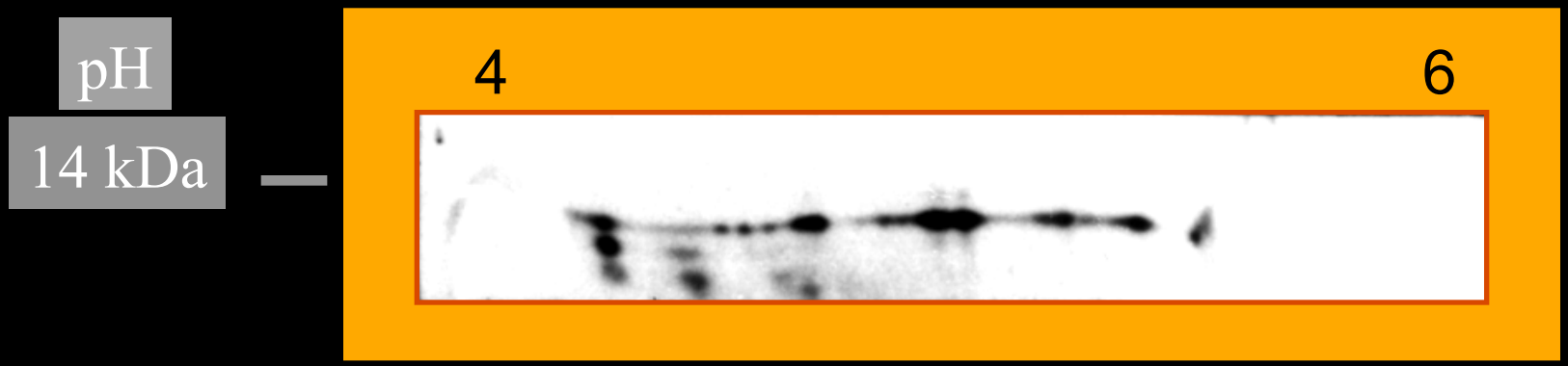
serum B2M

5.7 pI (1) Ile - Gln - Arg - Thr - Pro
5.3 pI (1) Ile - Gln - Arg - Thr - Pro

5.1 pI (1) Ile - Gln - Arg - Thr - Pro
(6) Lys - Ile - Gln - Val - Tyr
(11) Ser - Arg - His - Pro - Ala
(16) Glu - Asn - Gly - Lys - Ser

β2-microglobulin from amyloid fibrils consists of a variety of isoforms with lower pI

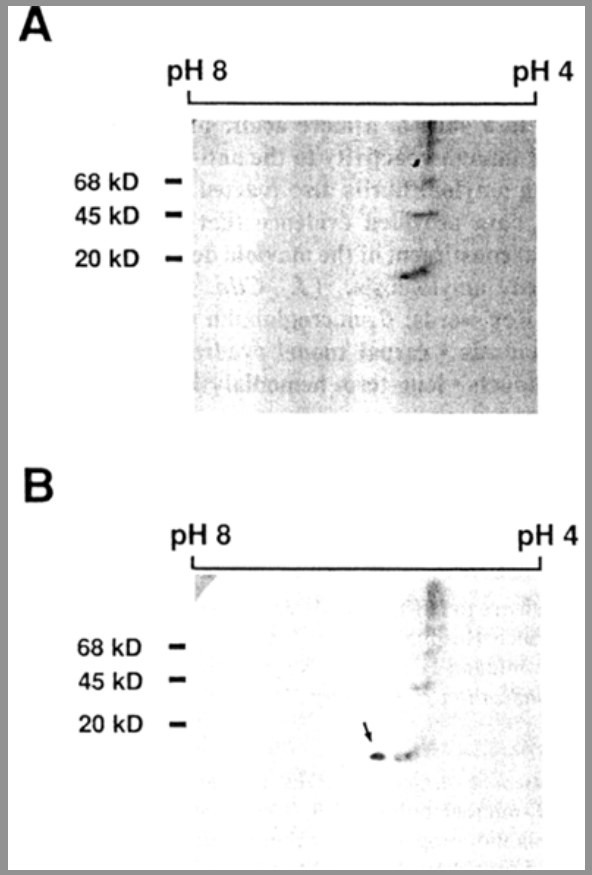
Western blotting analysis of the 2D-SDS-PAGE using anti β2 microglobulin Ab



Sequencing of the more acidic isforms β2 microglobulin from amyloid deposits revealed an intact N-terminus

Argilés *et al*, Kidney Int, 1995

β2-microglobulin modified with advanced glycation end products is a major component of hemodialysis-associated amyloidosis



Western blotting of 2D-gels

A amyloid fibril proteins

B amyloid fibril proteins + "normal" b2-microglobulin

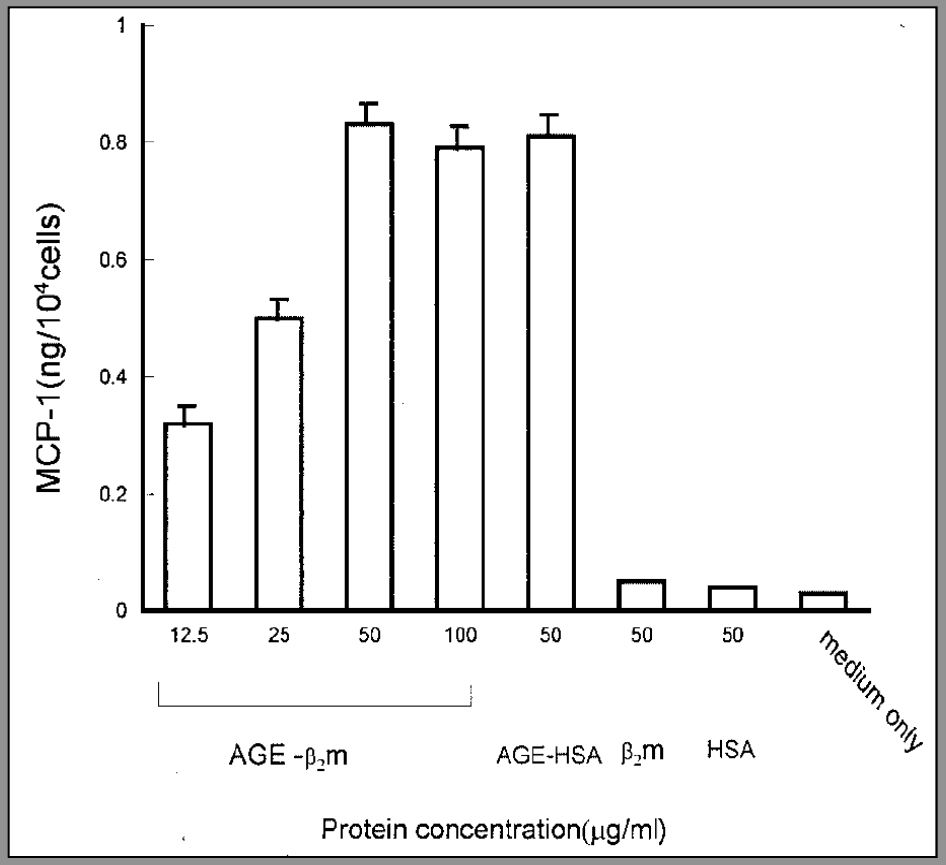
→ acidic β2-m is recognised by anti-AGEP Ab

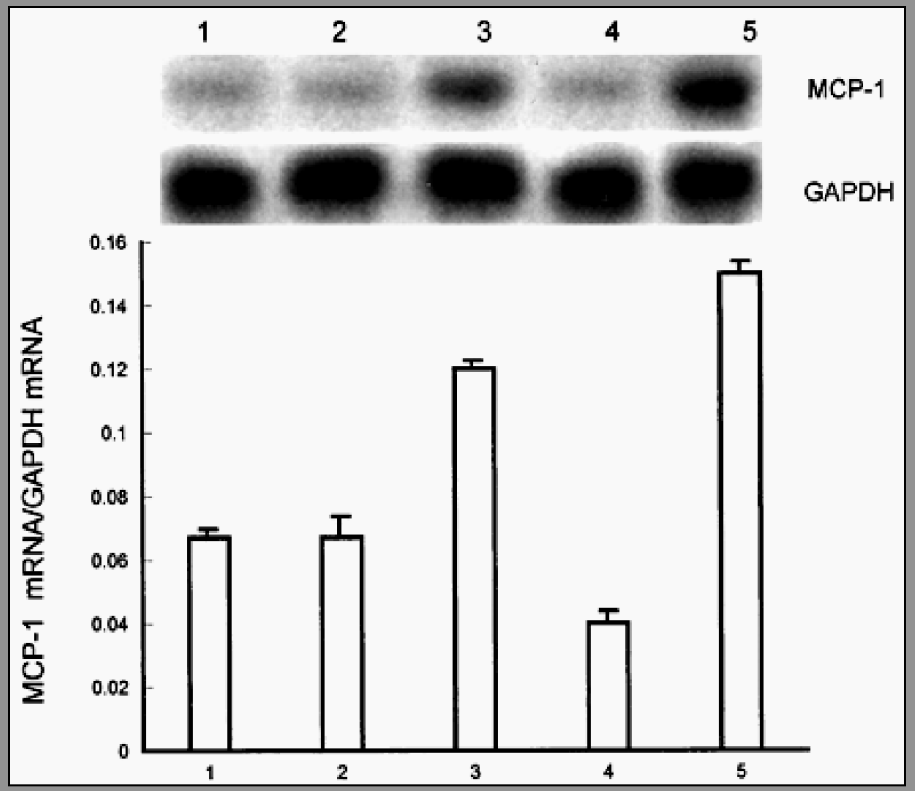
→ glucose incubation of normal β2-m modifies the protein which migrates to the acidic β2-m region

Miyata *et al*, J Clin Invest 1993

Receptor for advanced glycation end products on human synovial fibroblasts: role in the pathogenesis of dialysis-related amyloidosis

Monocyte chemoatractant protein 1: synthesis and protein level



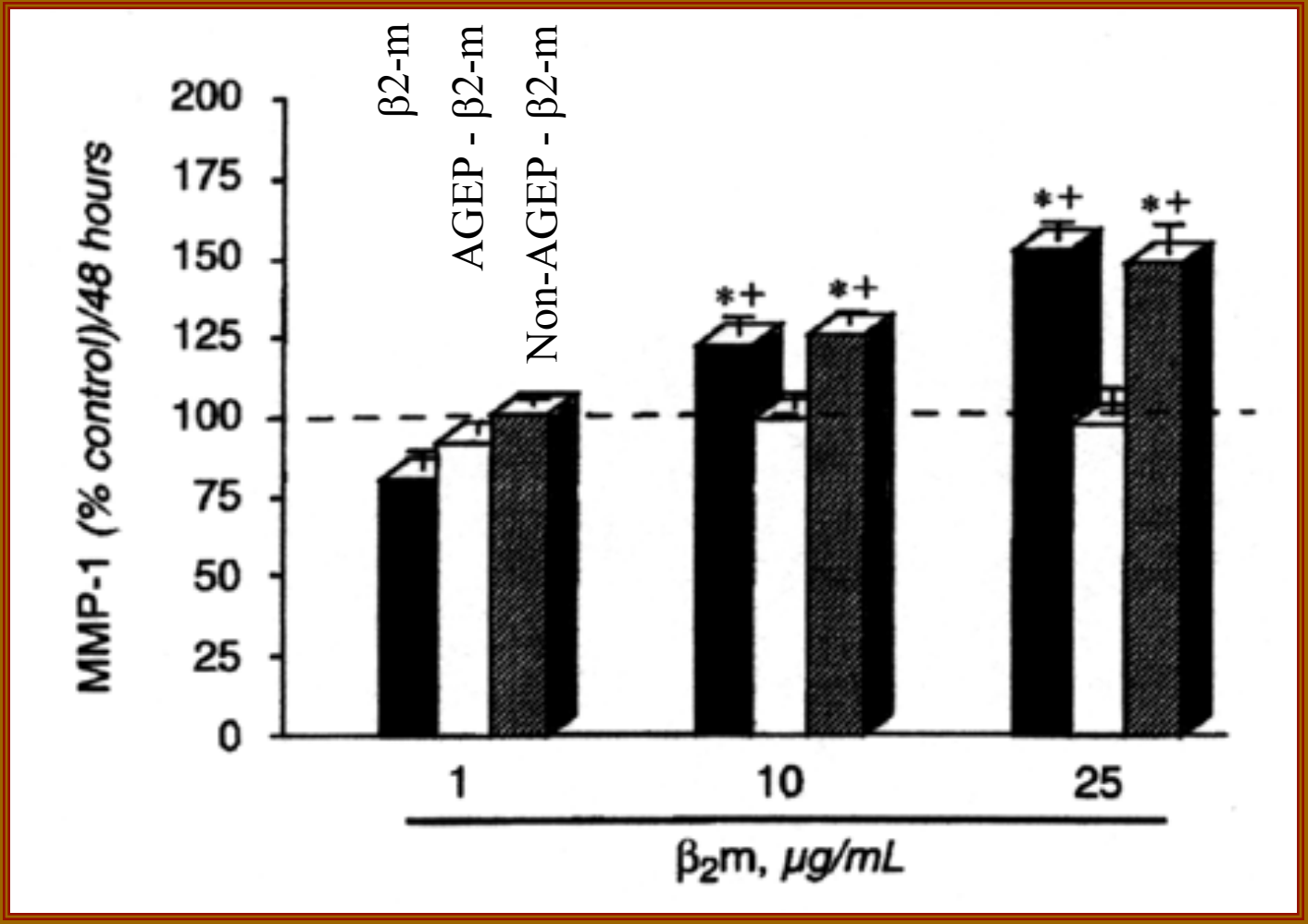


Hou FF *et al* J Am Soc Nephrol 13:1296-306, 2002

Kidney International, Vol. 57 (2000), pp. 2023-2034

β2-microglobulin induces MMP-1 but not TIMP-1 expression in human synovial fibroblasts

SHARON M. MOE, GURINDER K. SINGH, and ANNA M. BAILEY



Native β2-m increases collagenase synthesis by synovial fibroblasts by 48 hours

AGEP - β2-m does not have this effect

MMP-1 : matrix metalloproteinase - 1 interstitial collagenase

Kidney International, Vol. 39 (1991), pp. 1012—1019

Effect of dialysis membrane and patient's age on signs of dialysis-related amyloidosis

C. VAN YPERSELE DE STRIHOU, M JADOUL, J MALGHEM, B. MALDAGUE, J. JAMART, and the Working party on dialysis amyloidosis

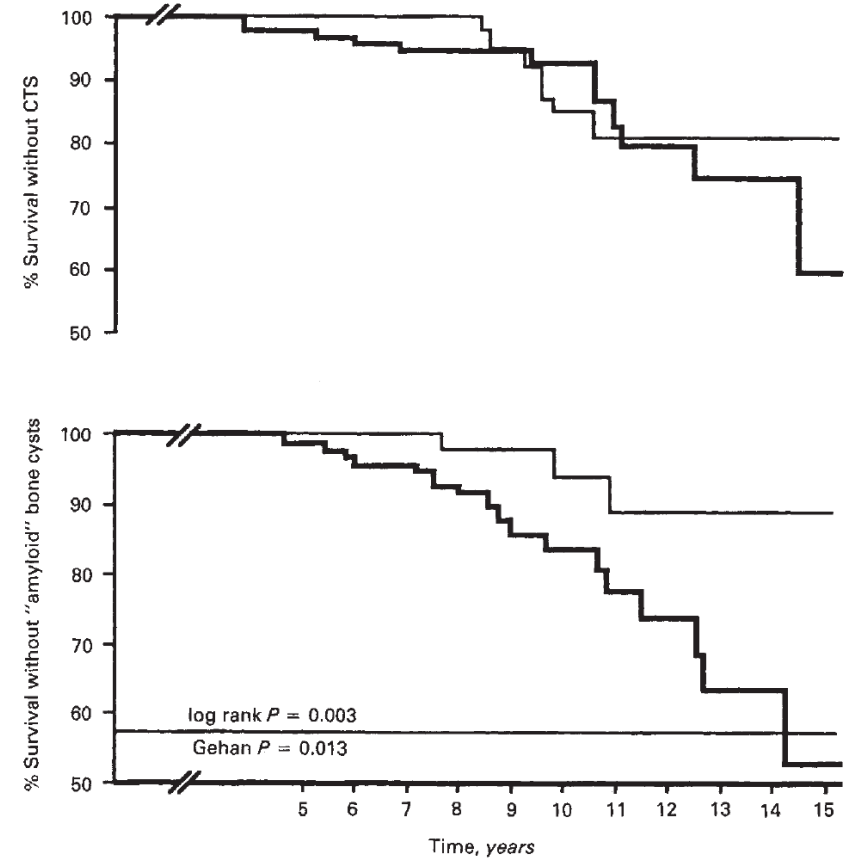


Fig. 5. Kaplan-Meier survival curves without carpal tunnel syndrome (A) or without amyloid bone cysts (B) in patients treated exclusively with either AN69 (—) or cellulosic membranes (). Survival without amyloid bone cysts is significantly different for the 2 groups.

Treatment with cellulosic membranes is associated with a higher incidence of bone cysts

Kidney International, Vol. 52 (1997), pp. 1077-83

β2-microglobulin associated amyloidosis: a vanishing complication of long-term hemodialysis?

S. SCHWALBE, HOLZHAUER M, SCHAEFFER J. *et al*

Study of the prevalence of β2m amyloidosis

Comparison 1988 versus 1996 in a single unit

Randomly included patients matched for time in dialysis and age

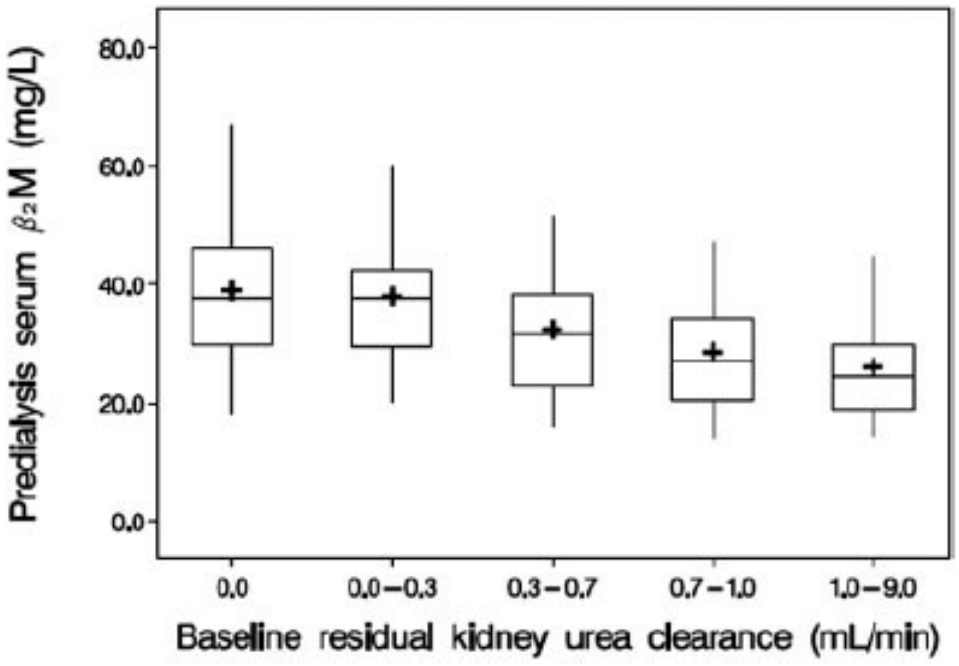
Decrease of 80% in β2m amyloidosis prevalence

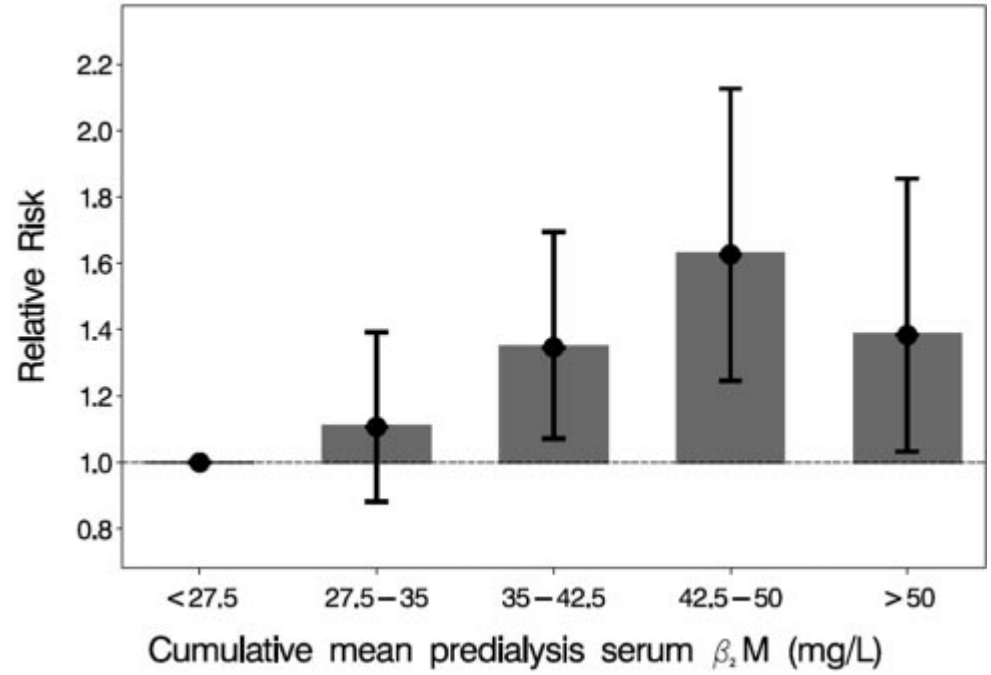
« Increased removal of β2m is unlikely to account for this phenomenon. Rather other factors, for example, dialysate composition and purity, may be involved ».

J Am Soc Nephrol 17: 546-555, 2006

Serum β-2 Microglobulin Levels Predict Mortality in Dialysis Patients: Results of the HEMO Study

Alfred K Cheung *et al*





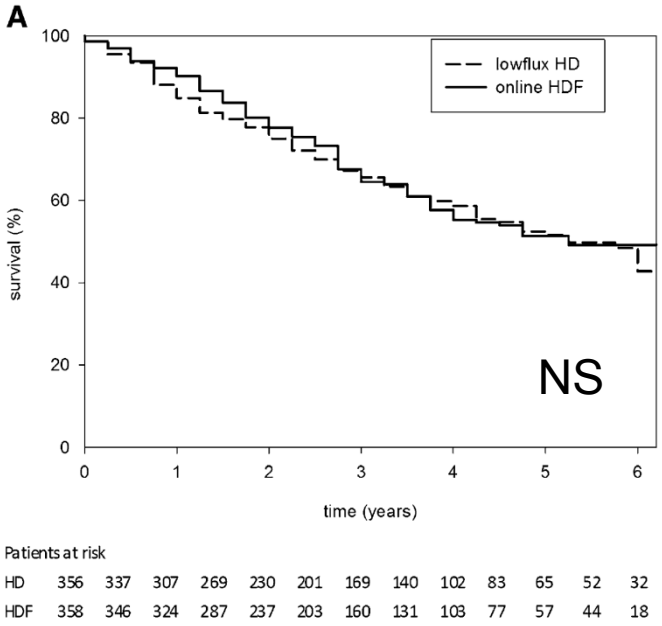
Residual function reduction is associated with an increase in serum β2m

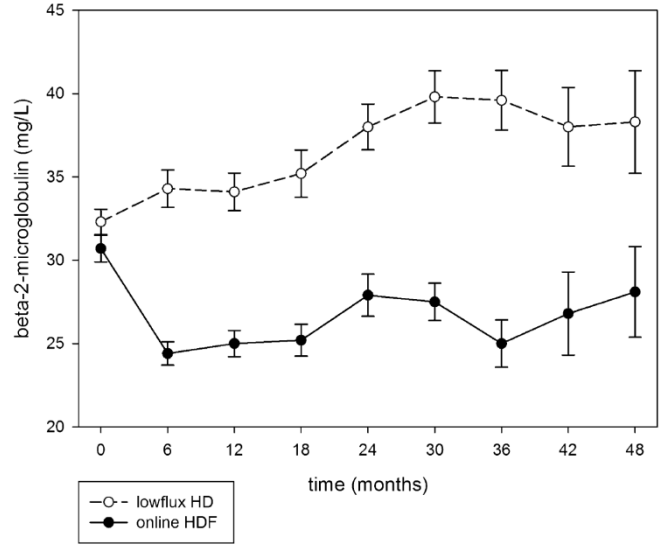
Increased serum β2m is associated with an increase in mortality

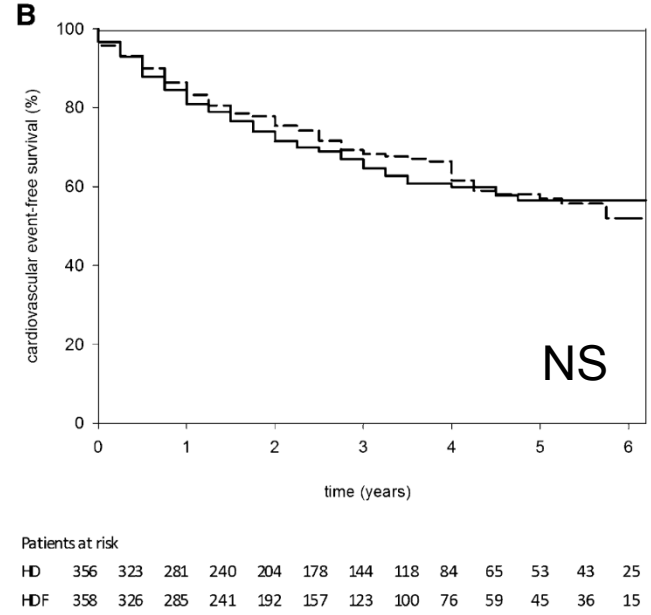
J Am Soc Nephrol 23: 1087–1096, 2012. doi: 10.1681/ASN.2011121140

Effect of Online Hemodiafiltration on All-Cause Mortality and Cardiovascular Outcomes

Muriel P.C. Grooteman,*,† Marinus A. van den Dorpel,‡ Michiel L. Bots,§ E. Lars Penne,*,‖ Neelke C. van der Weerd,* Albert H.A. Mazairac,‖ Claire H. den Hoedt,‡‖ Ingeborg van der Tweel,§ Renée Lévesque,¶ Menso J. Nubé,*,† Piet M. ter Wee,*,† and Peter J. Blankestijn,‖ for the CONTRAST Investigators







OL-HDF significantly decreased serum level of β2μ

Survival was similar in low Flux HD and in OL - HDF

Protein bound uraemic retention solutes:

Indoxyl Sulfate
P-cresyl sulfate

BRIEF REVIEW

J Am Soc Nephrol 25: 1897- 1907, 2014.

The Uremic Toxicity of Indoxyl Sulfate and p-Cresyl Sulfate: A Systematic Review

Raymond Vanholder *et al*

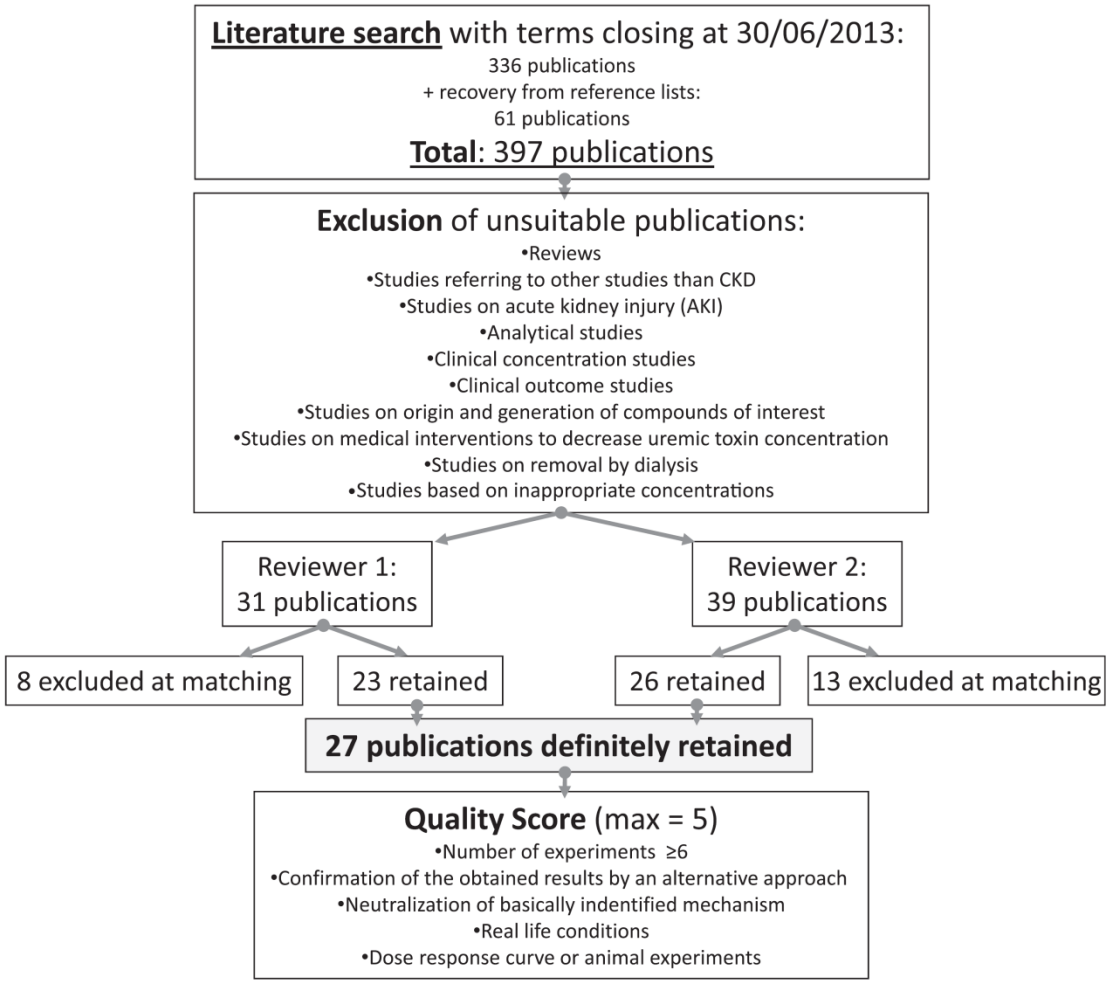


Figure 1. Flowchart of the review procedure and quality scoring.

We conclude that our systematic approach allowed the retrieval of methodologically correct studies unbiased by erroneous conditions related to albumin binding.

Our data seem to confirm the toxicity of indoxyl sulfate and p-cresyl sulfate and support their roles in vascular and renal disease progression.

CLINICAL RESEARCH

Colonic Contribution to Uremic Solutes

Pavel A. Aronov et al. JASN 22:1769-76, 2011

Table 1. Patient characteristics

Characteristics	Intact Colon ($n = 9$)	Colectomy ($n = 6$)
Women/men	6/3	3/3
Age (years)	56 ± 7	72 ± 14
Body mass index	26 ± 5	26 ± 4
Duration on dialysis (years)	9 ± 6	8 ± 6
Duration colectomy (years)	–	20 ± 19
Kt/V	1.77 ± 0.42	1.60 ± 0.21
Duration of dialysis session (minutes)	188 ± 13	203 ± 29
Diabetes	5 of 9	3 of 6

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	–

Indoxyl Sulfate and Paracresyl Sulfate are not anymore increased in dialysis patients having had a colectomy.

Methylamine (MMA) and Dimethylamine (DMA) are still increased in patients having had a colectomy.

JASN

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.

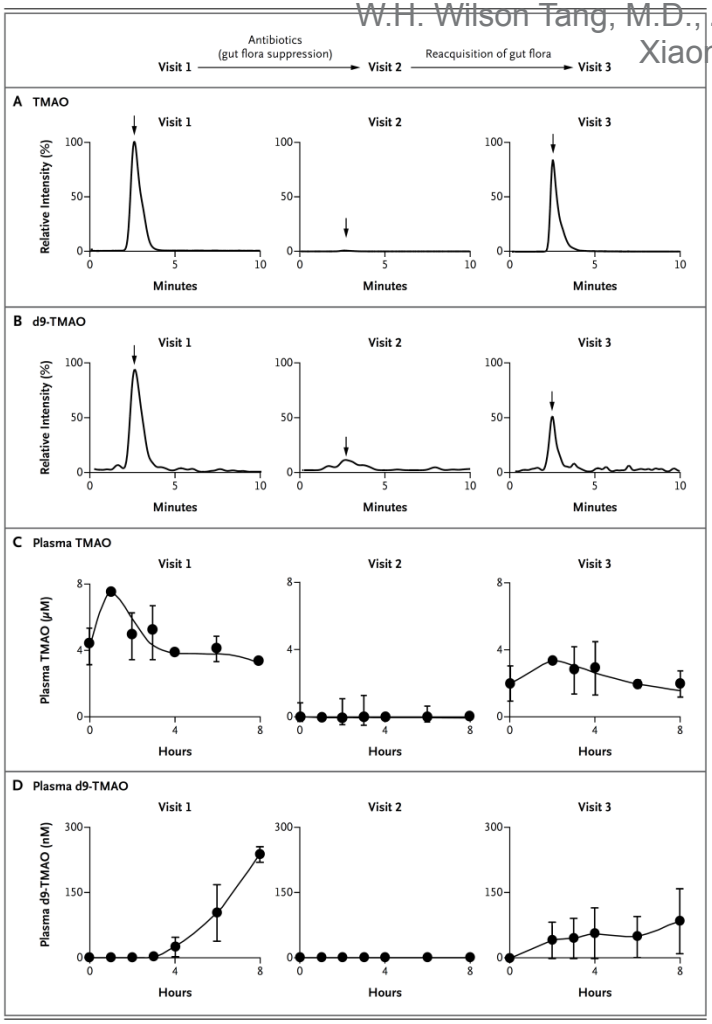


Figure 1 (facing page). Effects of a Phosphatidylcholine Challenge and Administration of Antibiotics on Mean Levels of Trimethylamine-*N*-*Oxide (TMAO) and Its d9* Isotopologue (d9-TMAO).

All 40 study participants underwent the first dietary phosphatidylcholine challenge (visit 1), which consisted of the ingestion of deuterium-labeled phosphatidylcholine (d9-phosphatidylcholine) and two hard-boiled eggs. Six participants then received broad-spectrum antibiotics for 1 week, followed by a second phosphatidylcholine challenge (visit 2). These same participants returned again at least 1 month after discontinuing the antibiotics for a third challenge (visit 3). Shown are the results of assays for TMAO (Panel A) and d9-TMAO (Panel B) after the phosphatidylcholine challenge, before and after the administration of antibiotics, with the intensity of stableisotope- dilution assays measured by means of highperformance liquid chromatography with online electrospray ionization tandem mass spectrometry. The arrows indicate retention time where authentic isotope-labeled TMAO standards elute. Also shown are the plasma levels of TMAO (Panel C) and d9-TMAO (Panel D) at each visit. The plasma levels of TMAO were markedly suppressed after the administration of antibiotics and subsequently reappeared after the cessation of antibiotics, indicating that the production of TMAO from dietary phosphatidylcholine is dependent on metabolism by the intestinal microbiota.

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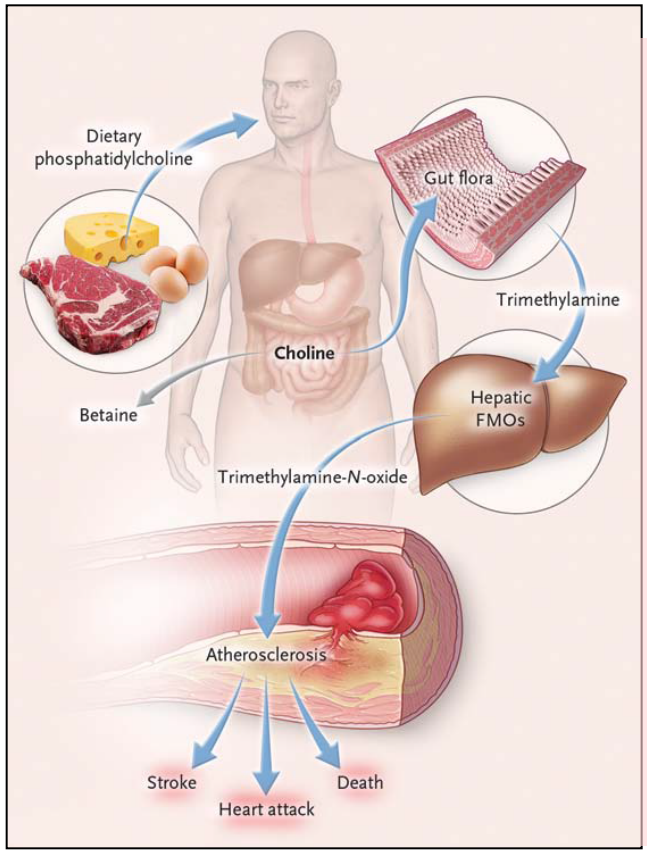
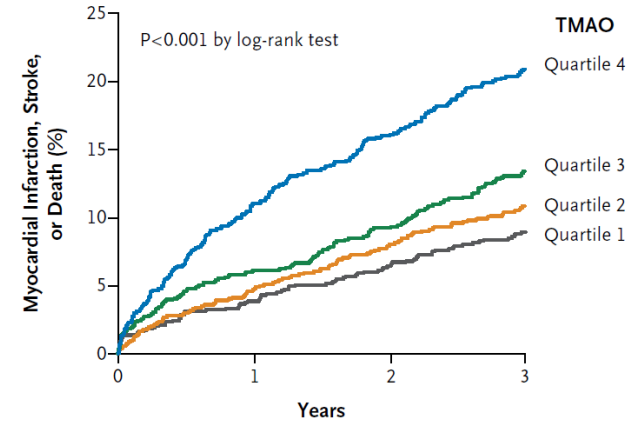


Figure 3. Pathways Linking Dietary Phosphatidylcholine, Intestinal Microbiota, and Incident Adverse Cardiovascular Events. Ingested phosphatidylcholine (lecithin), the major dietary source of total choline, is acted on by intestinal lipases to form a variety of metabolic products, including the choline-containing nutrients glycerophosphocholine, phosphocholine, and choline. Choline-containing nutrients that reach the cecum and large bowel may serve as fuel for intestinal microbiota (gut flora), producing trimethylamine (TMA). TMA is rapidly further oxidized to trimethylamine-*N*-oxide (TMAO) by hepatic flavin-containing monooxygenases (FMOs). TMAO enhances the accumulation of cholesterol in macrophages, the accumulation of foam cells in artery walls, and atherosclerosis,[7] all factors that are associated with an increased risk of heart attack, stroke, and death. Choline can also be oxidized to betaine in both the liver and kidneys.[20] Dietary betaine can serve as a substrate for bacteria to form TMA[21] and presumably TMAO.

trimethylamine-*N*-oxide (TMAO)



No. at Risk				
Quartile 1	1001	933	869	827
Quartile 2	998	940	884	843
Quartile 3	1003	938	888	835
Quartile 4	1005	913	849	791

Figure 2. Kaplan–Meier Estimates of Major Adverse Cardiovascular Events, According to the Quartile of TMAO Level.

Data are shown for 4007 participants in the clinical-outcomes study. The P value is for all comparisons.

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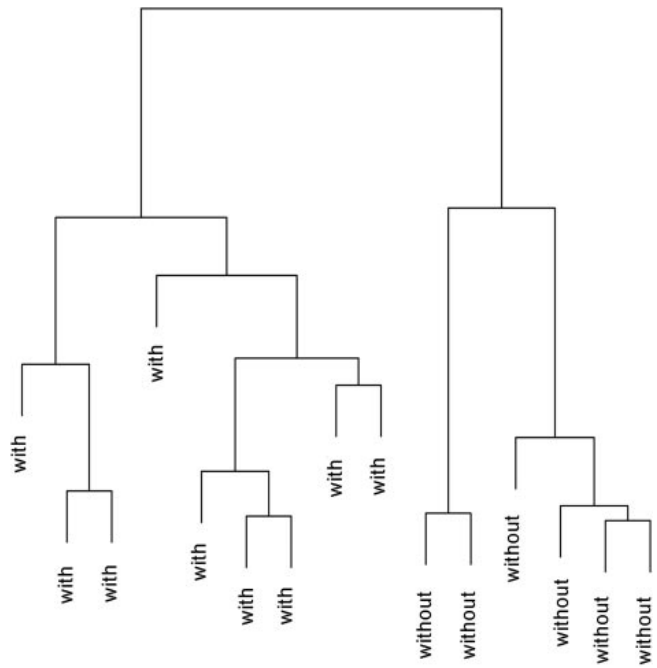

Figure 1. Clustering identified the six patients with colectomies and the nine patients with intact colons as belonging to different groups. Unsupervised hierarchical clustering was performed on the distances calculated from the sum of the squared differences between the log-transformed amplitudes of 1055 features detected
by MS in predialysis plasma samples from the 15 hemodialysis patients. Distances along the vertical axis provide an index of the aggregate differences in feature amplitudes between individual patients and groups of patients.

- 1055 compounds identified that differentiate colectomised from non-colectomised dialysis patients
- 27 compounds were statistically in lower concentration in colectomised dialysis patients
  - It is not a “single solute problem”, it is much more complex
  - 1028 compounds putatively uraemic retention solutes are not from colonic origin showing that microbiota’s role might not be the most important factor in uraemic toxicity.

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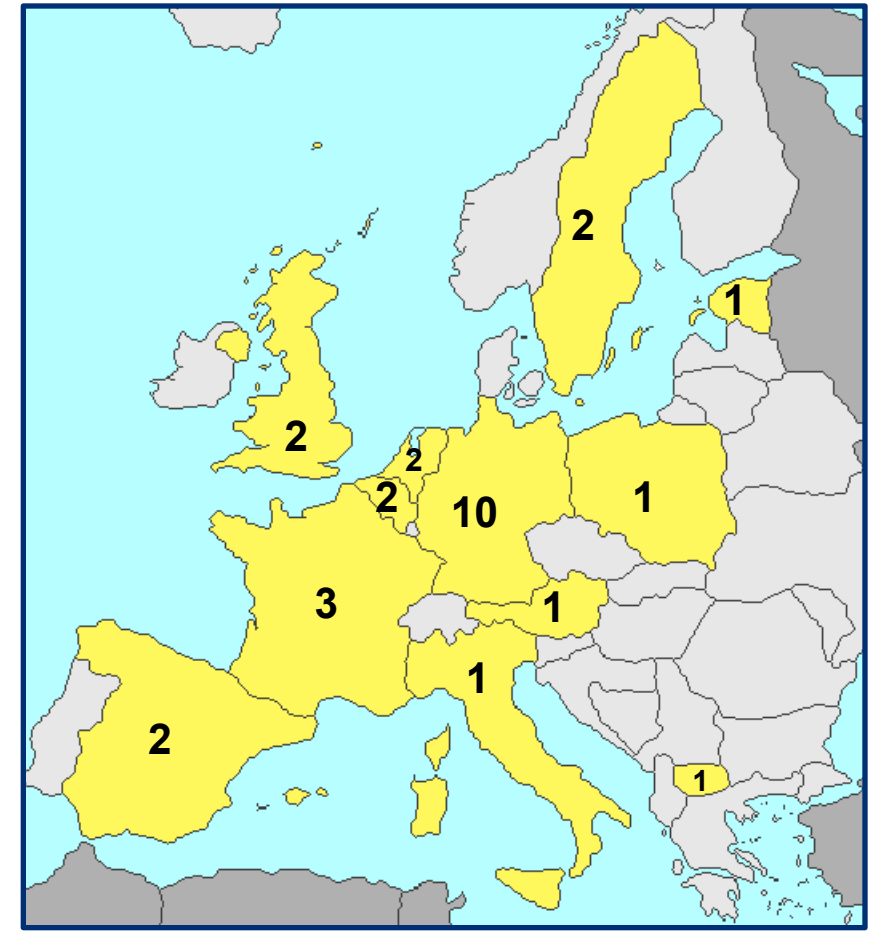
European Uremic Toxin WG of
ESAO, endorsed by ERA-EDTA

EUTox Members

28 Senior academia members (map)

from

26 European research groups



5 Corporate members

Gambro, Fresenius, Bayer, B BRAUN, Vifor-Pharma


Website: http://uremic-toxins.org/

Felluns, Catalunya Nord
Mirant el Canigó

BRIEF COMMUNICATION

J Am Soc Nephrol 21:1852-57, 2010

Apostolov *et al*

Chronic Uremia Stimulates LDL Carbamylation and Atherosclerosis

Lipid deposits increase in Aortas from in CRF mice and from non CRF mice supplemented with Urea

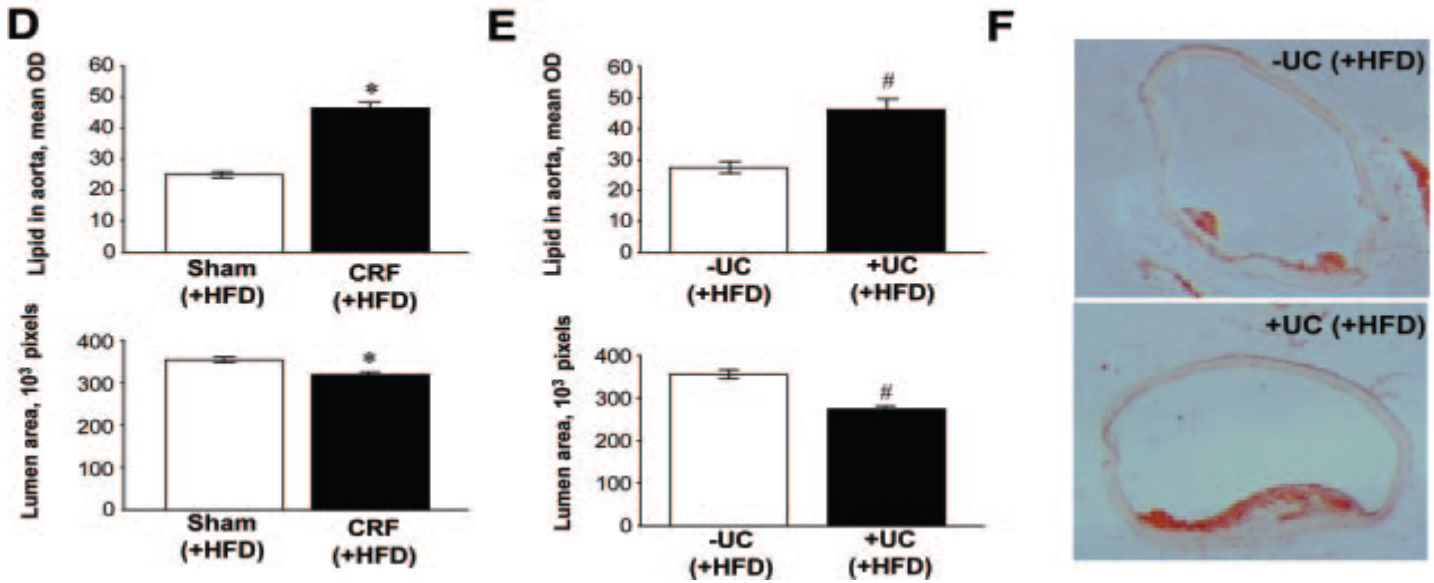


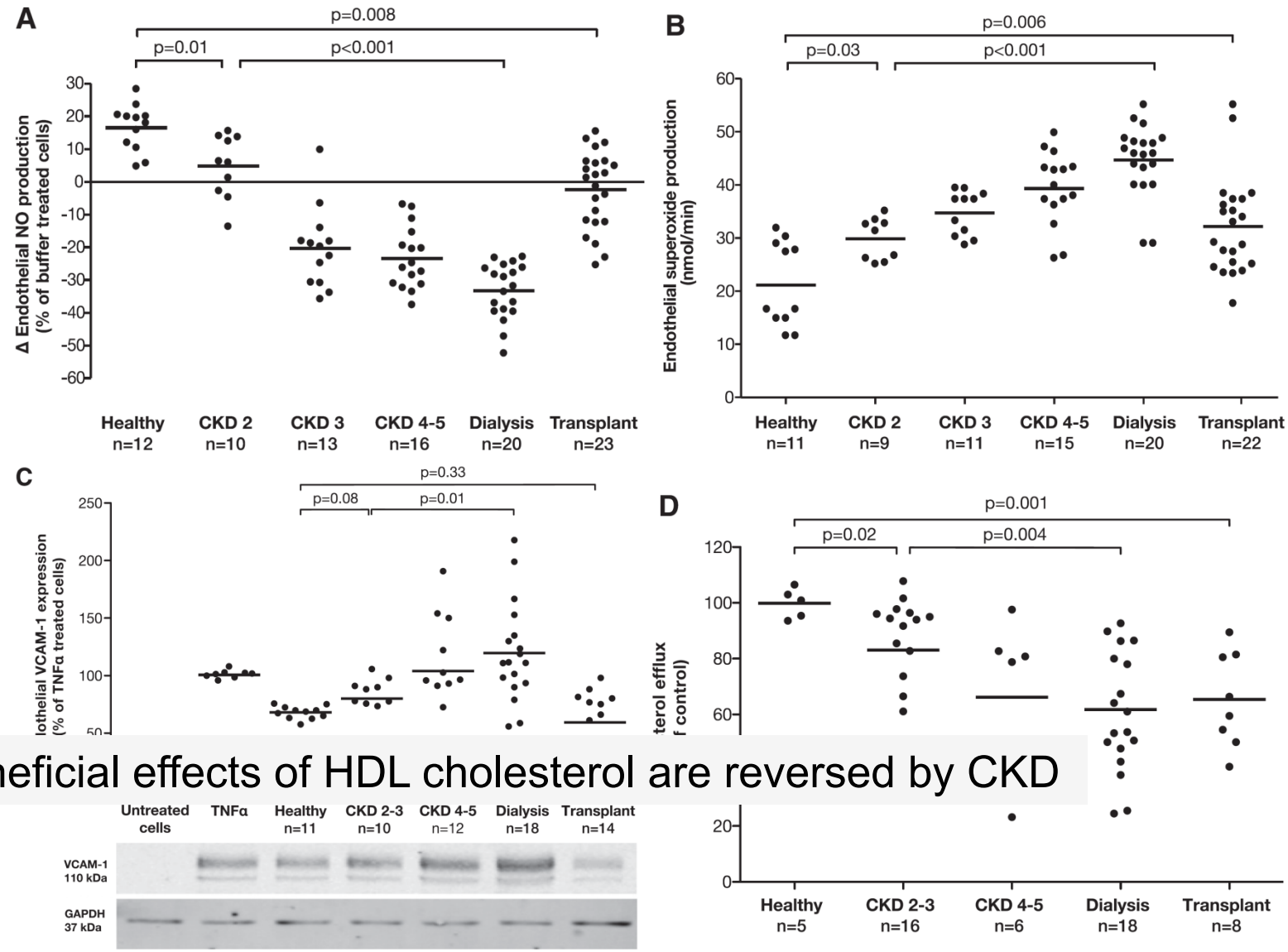
Figure 3. Staining of atherosclerotic plaques and lipid deposits in ascending aortas by Oil red O (D–F) and their quantification in CRF (D) and UC (E and F) mice fed with HFD.*P 0.05 compared with sham-operated mice fed with HFD. #P 0.05 compared with UC control mice fed with HFD.

CLINICAL RESEARCH J Am Soc Nephrol 25: 2658–2668, 2014.

HDL in Children with CKD Promotes Endothelial Dysfunction and an Abnormal Vascular Phenotype

Rukshana Shroff *et al*

Human aortic endothelial cells (HAECs) were incubated with HDL isolated from children with CKD (HDL^{CKD}) and from healthy children ($HDL^{Healthy}$) to measure endothelial properties of HDL. $HDL^{Healthy}$ increased endothelial NO production, but HDL^{CKD} substantially inhibited endothelial NO production (A), promoted basal endothelial production of SO radicals (B), increas VCAM-1 production reduced reverse cholesterol transport capacity (D).



The beneficial effects of HDL cholesterol are reversed by CKD