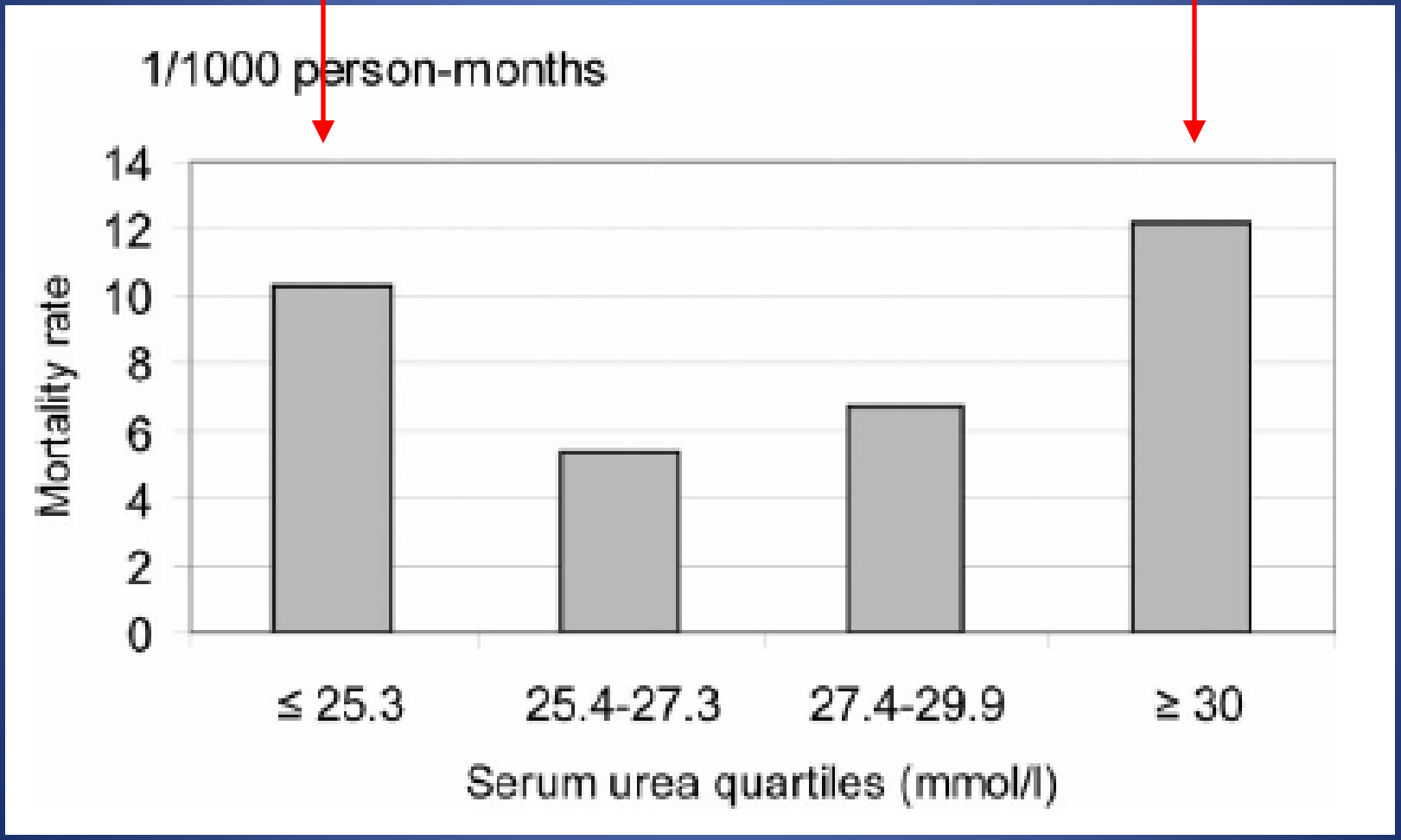
Emerging biomarkers of kidney disease

Anders H. Berg, MD-PhD
Biomarkers of the Cardiorenal Axis Conference
University of Weurzburg
January 22, 2016

Protein energy wasting

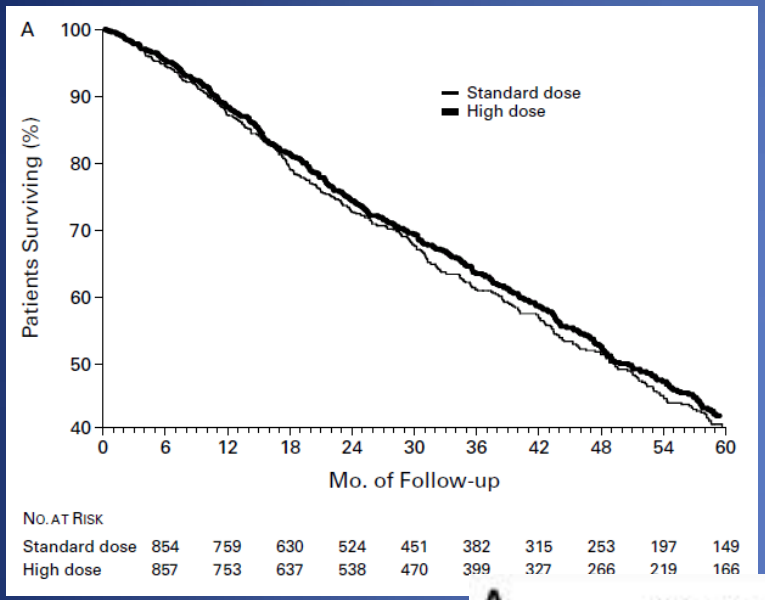
Uremic Toxicity



M. Stosovic et. al., Renal Failure, 31:335-340, 2009.

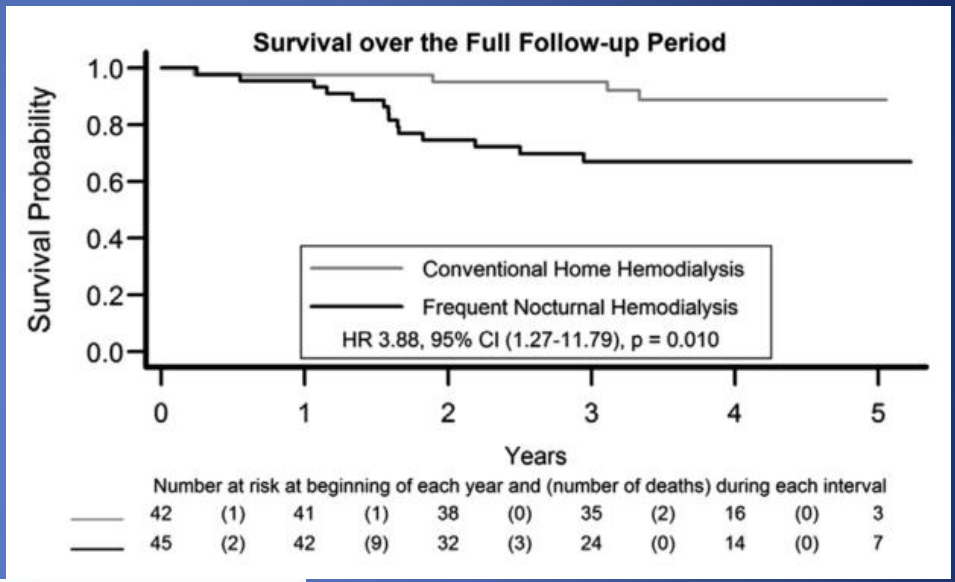
Clinical trials of intensive hemodialysis have produced mixed results

HEMO Trial

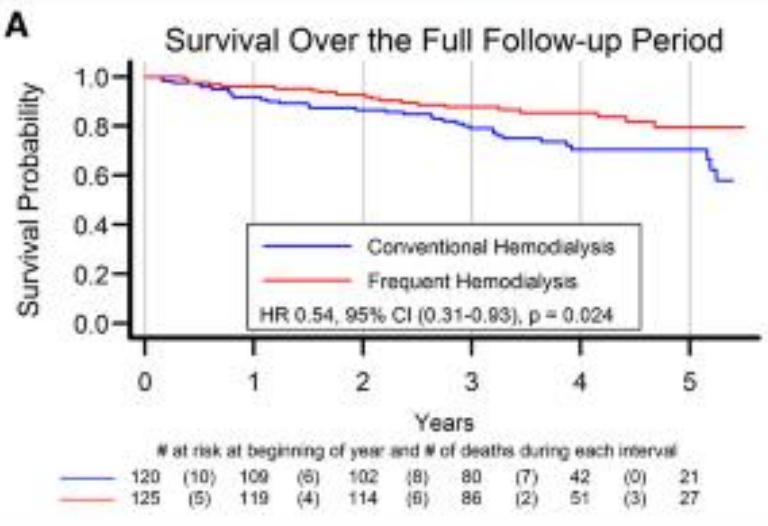


Garabed Eknoyan, M.D.,
N Engl J Med, Vol. 347, No. 25

FHN Nocturnal Trial



FHN In-center Trial



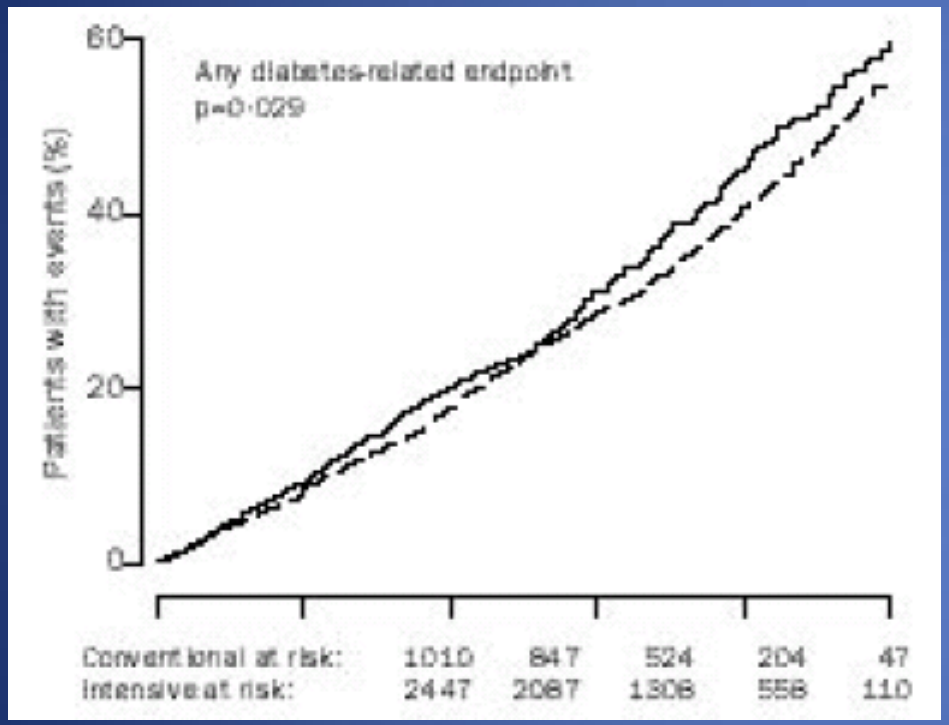
Glenn M. Chertow,* Nathan W. Levin,† Gerald J. Beck,‡ John T. Daugirdas,§ Paul W. Eggers,‖ Alan S. Kliger,¶ Brett Larive,‡ Michael V. Rocco,** and Tom Greene,‡†† for the Frequent Hemodialysis Network (FHN) Trials Group

J Am Soc Nephrol 27: ●●●–●●●, 2015

Rocco et al

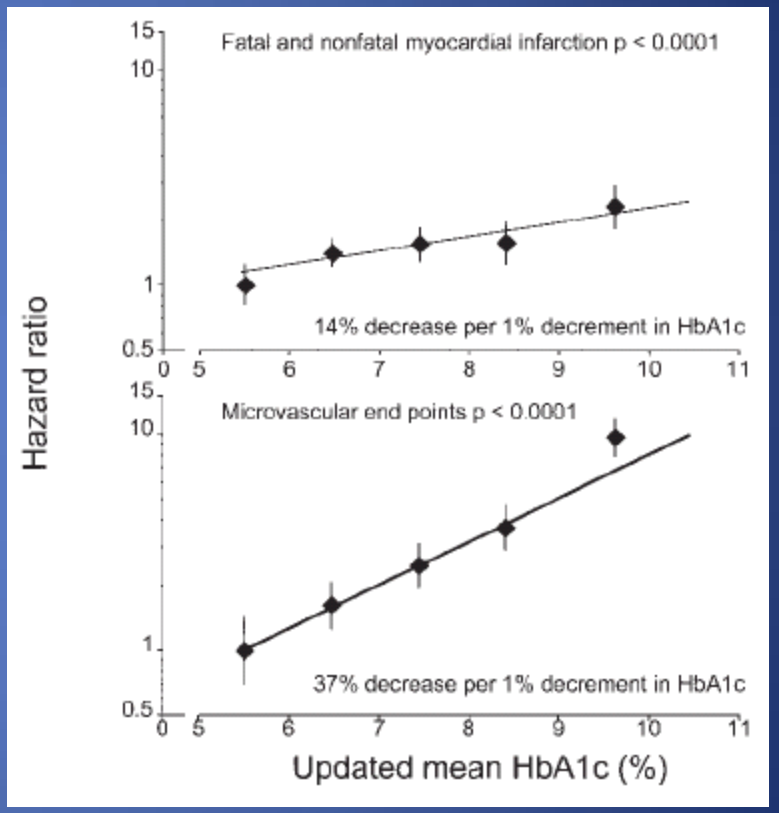
Am J Kidney Dis. 2015;66(3):459-468

Hemoglobin A_{1c} revealed the benefits of blood glucose control



*UK Prospective Diabetes Study (UKPDS) Group**

THE LANCET • Vol 352 • September 12, 1998

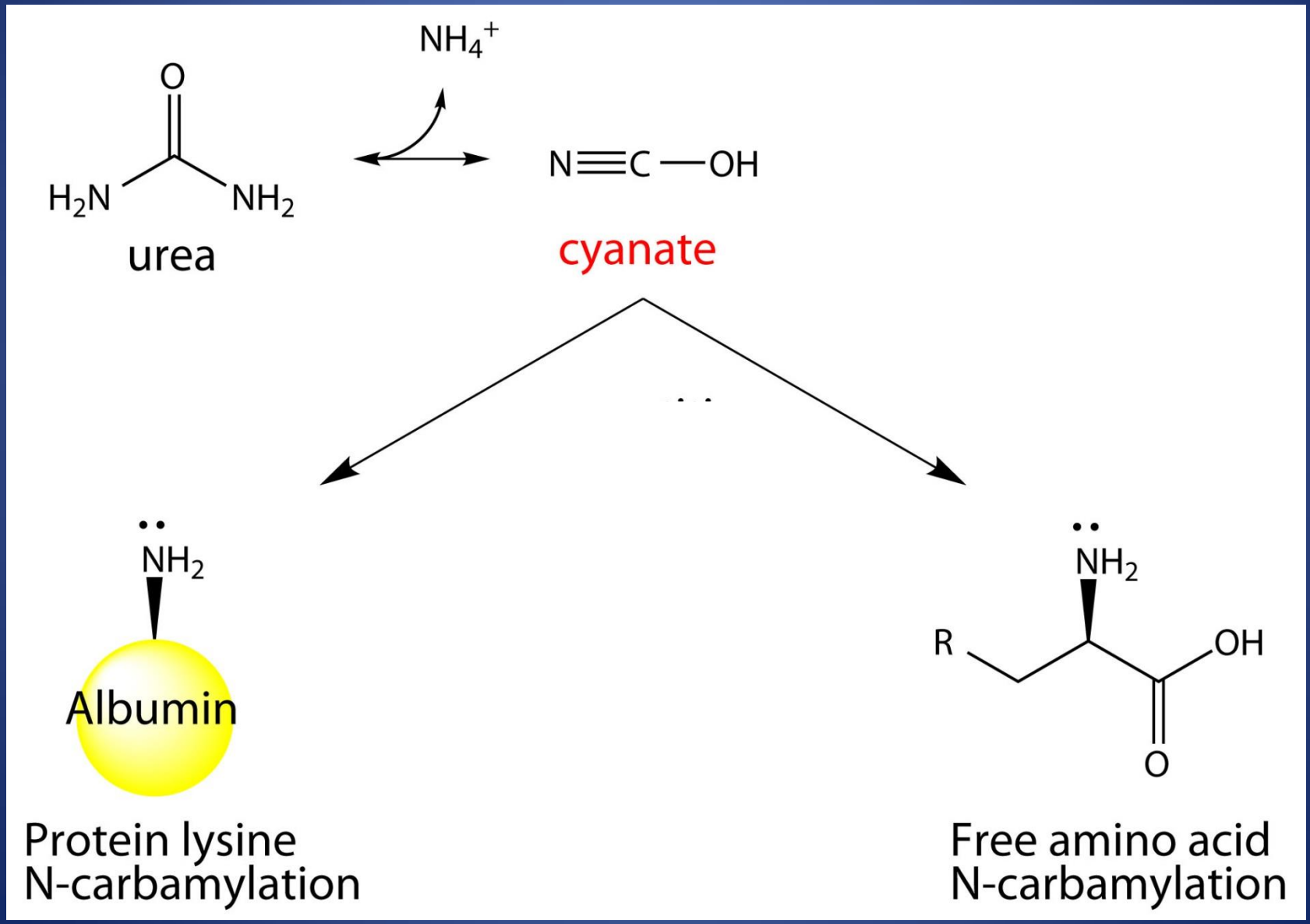


UK Prospective Diabetes Study Group. UKPDS 35:

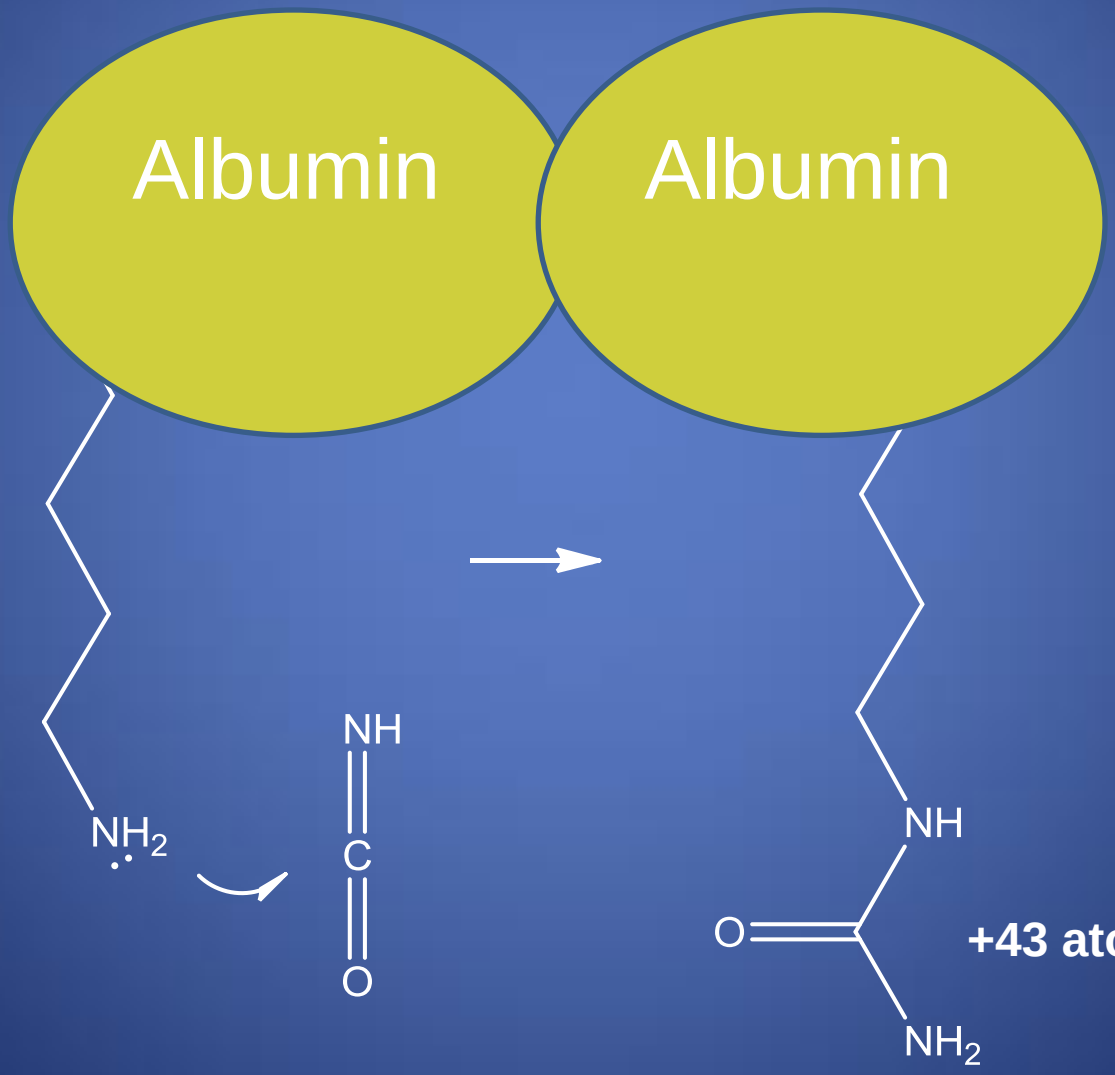
BMJ 2000; 321:405–412).

“Hemoglobin A_{1c} for uremia”

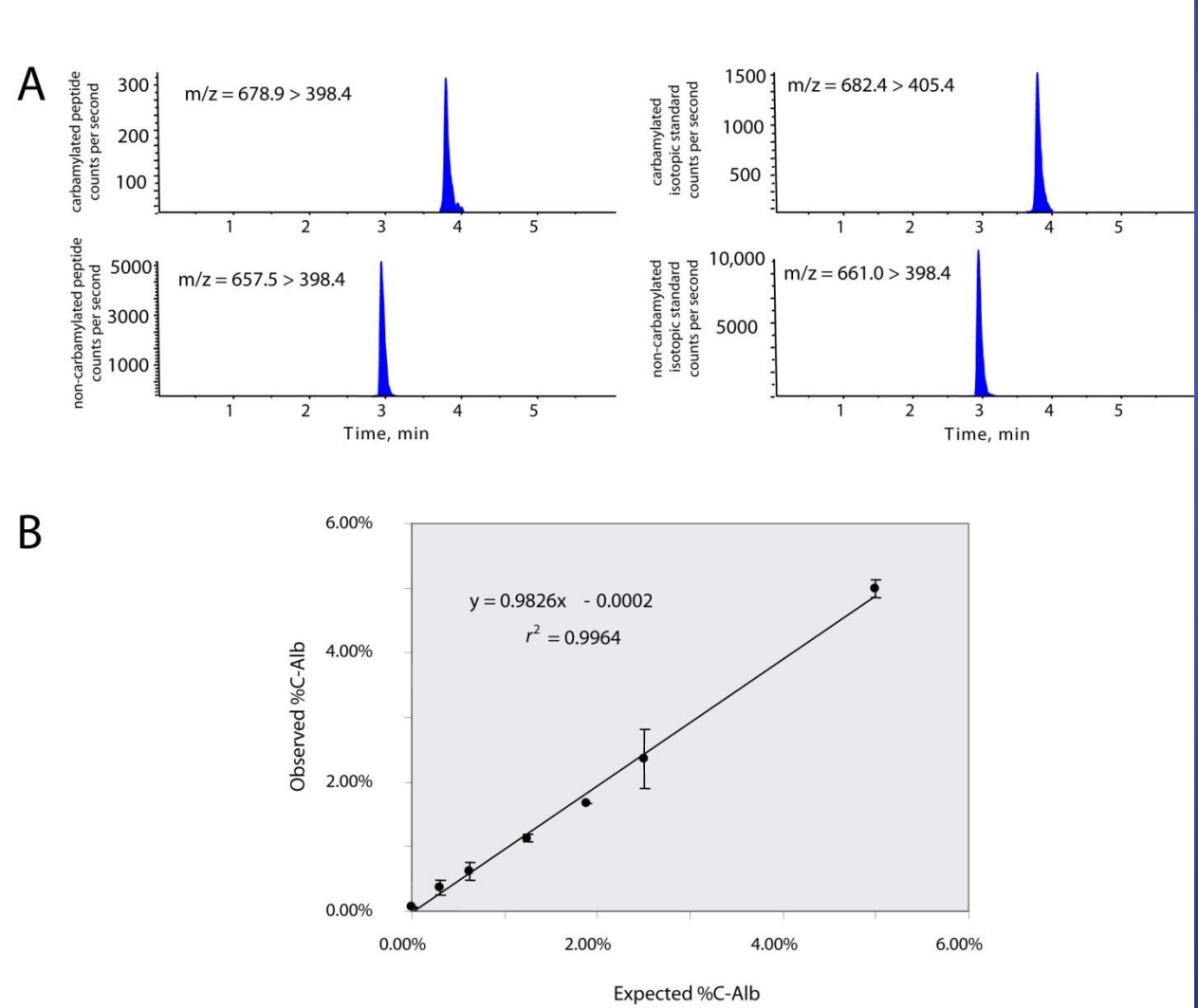
Urea modifies proteins and amino acids via carbamylation



Mass spectrometric assay for carbamylated albumin

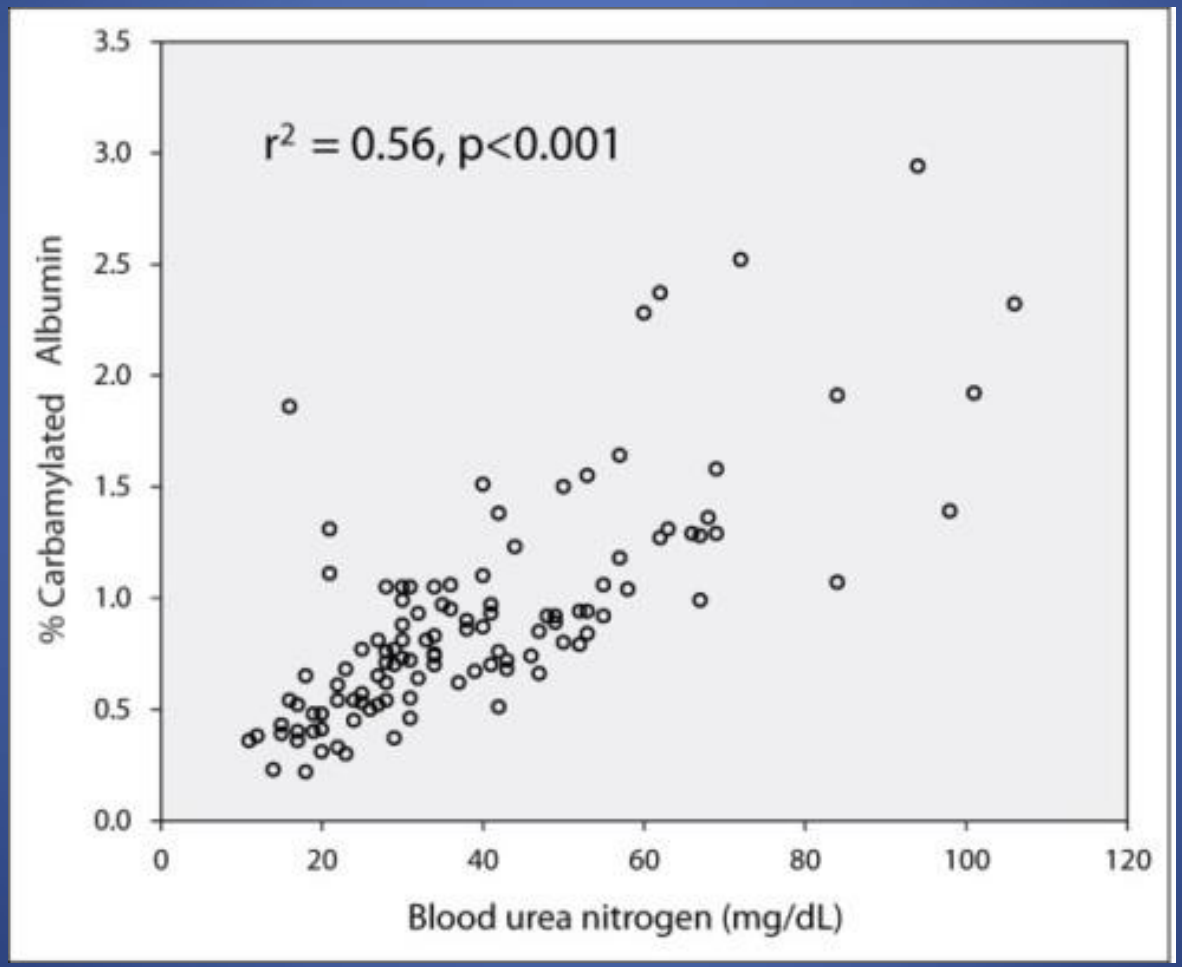


LC-MS/MS assay for Carbamylated albumin (C-Alb)



Berg et. al., Sci Trans. Med. PMCID: PMC3697767

C-Alb correlates with time-averaged blood urea *(analogous to Hgb A_{1c} and average glucose)*



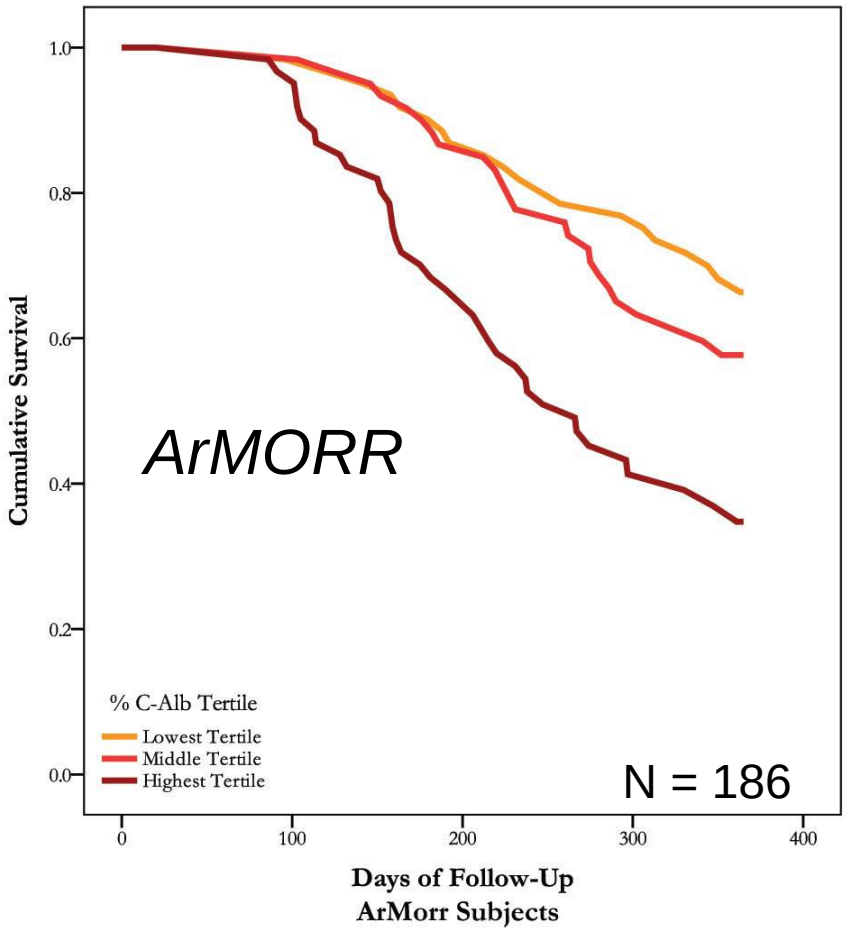
Berg et. al., Sci Trans. Med. PMCID: PMC3697767

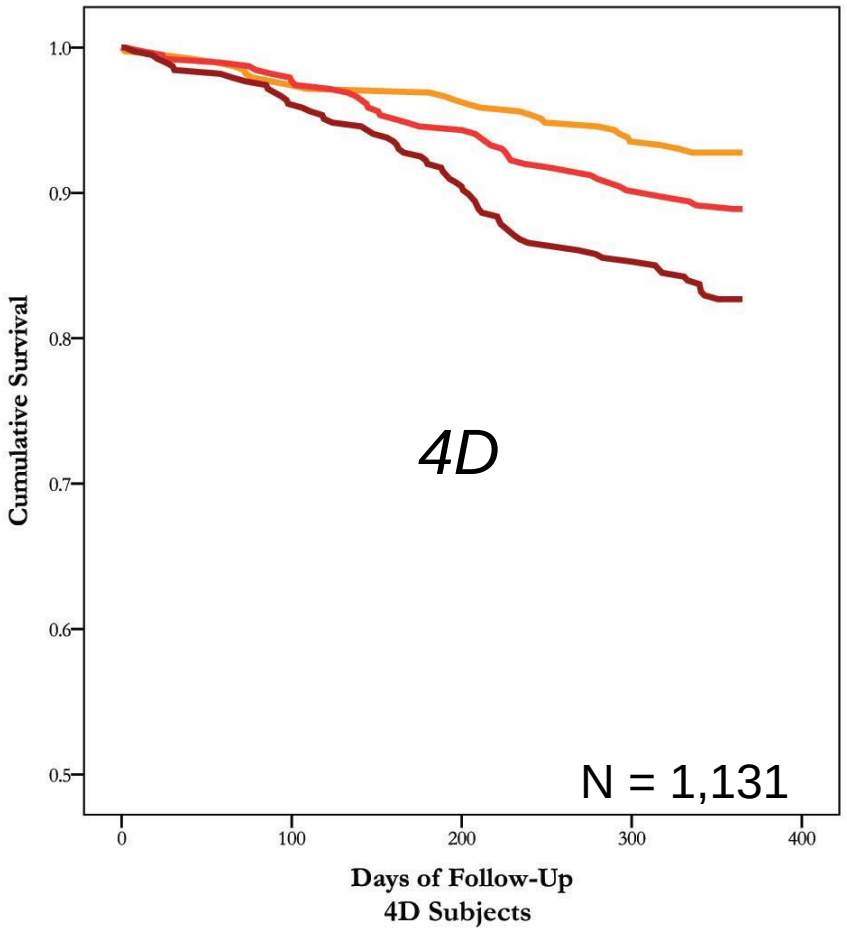
High C-Alb also correlates with low amino acids → *amino acid deficiencies associated with CKD may contribute to carbamylation*

Amino Acids	r_s	P-value
BUN	**0.431**	**<0.0001***
Arginine	-0.357	**0.0004***
Lysine	-0.310	**0.0022***
Histidine	-0.270	**0.0082***
Alanine	-0.341	**0.0007***
Glycine	**-0.216**	**0.0354***

Berg et. al., Sci Trans. Med. PMCID: PMC3697767

Increased %C-Alb is strongly associated with 1-year mortality in ESRD patients

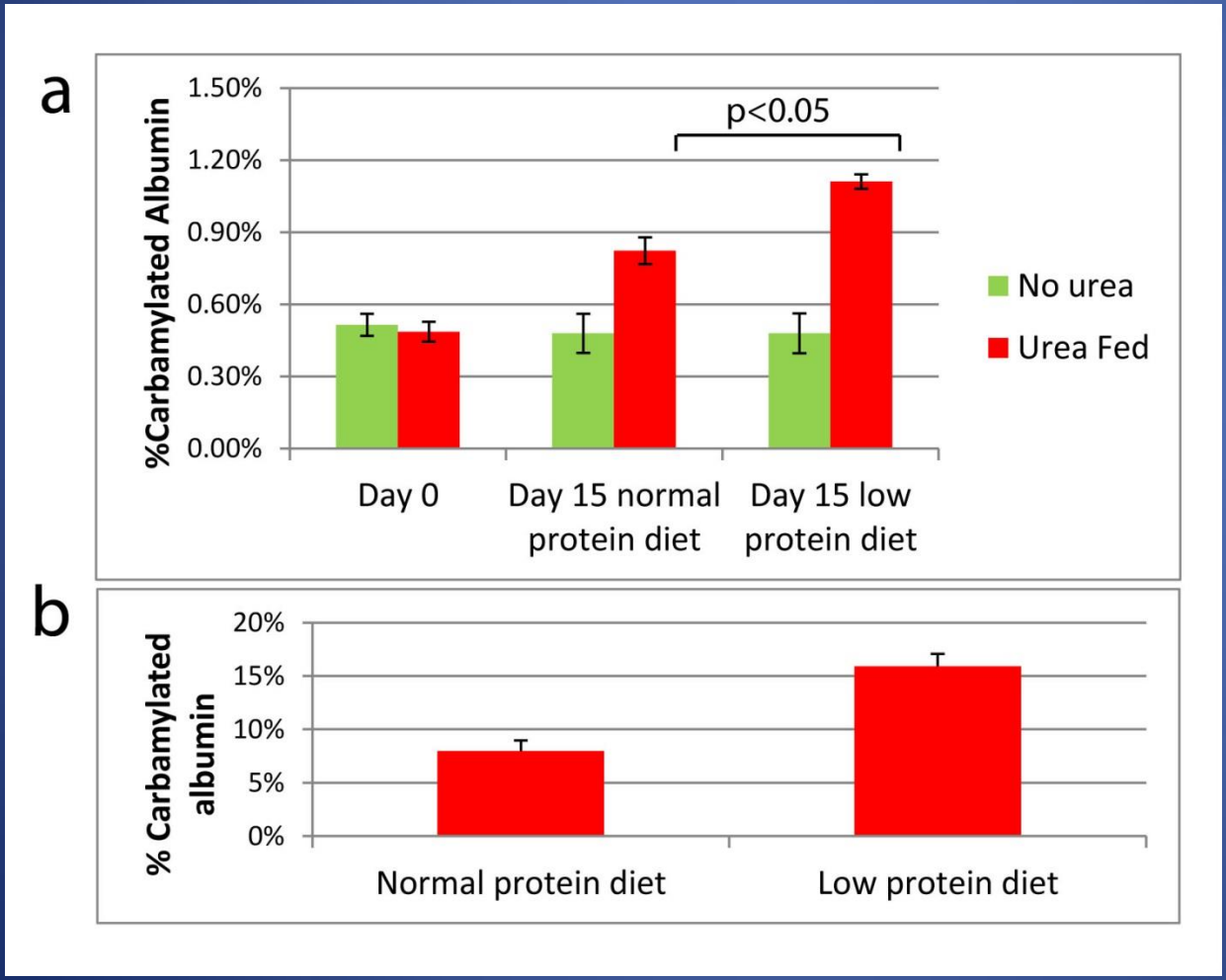




Berg et. al., Sci Trans. Med. PMCID: PMC3697767

Testing toxicity of carbamylation through animal model studies

Urea combined with amino acid deficiencies increase protein carbamylation in mice

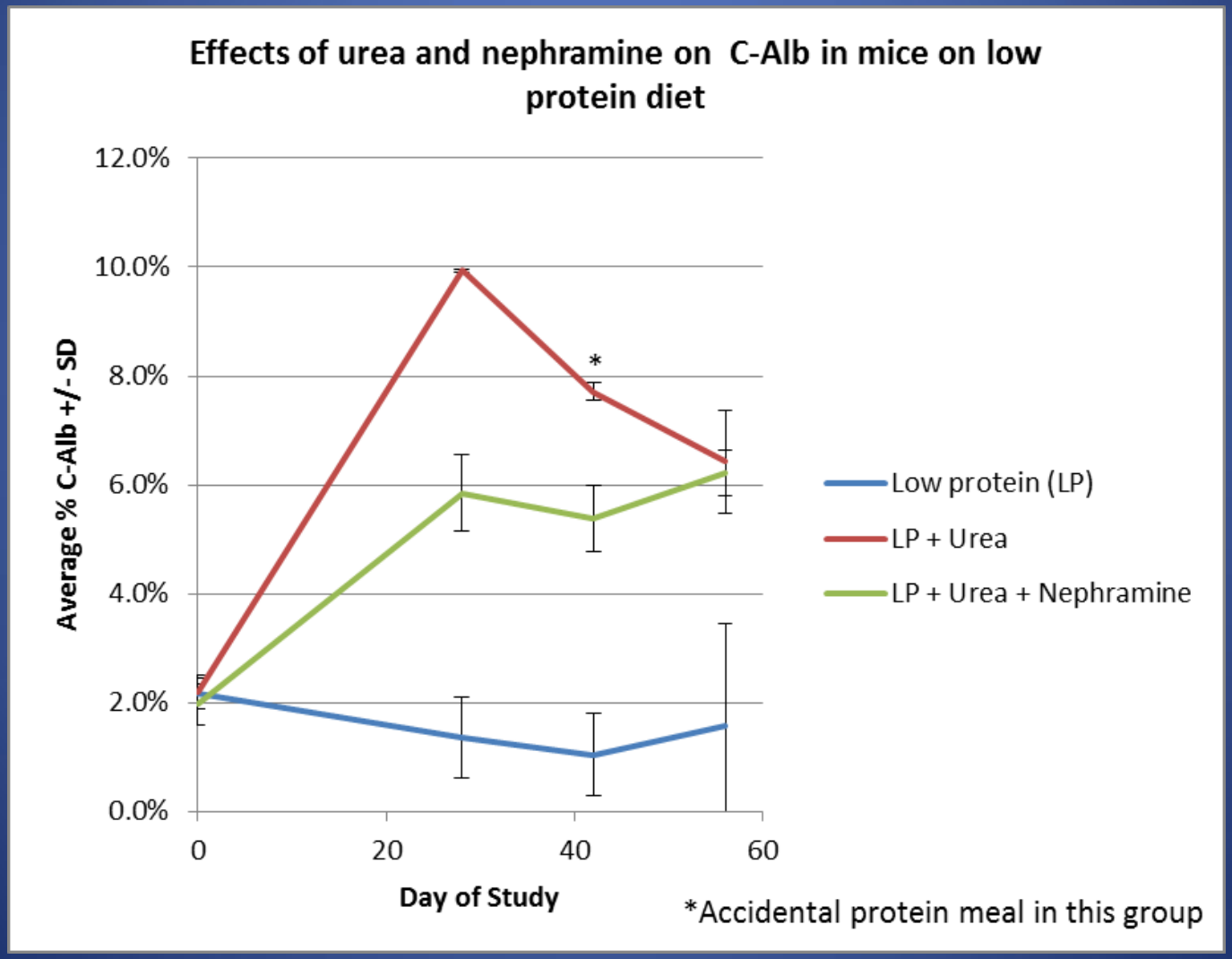


Urea-fed mice

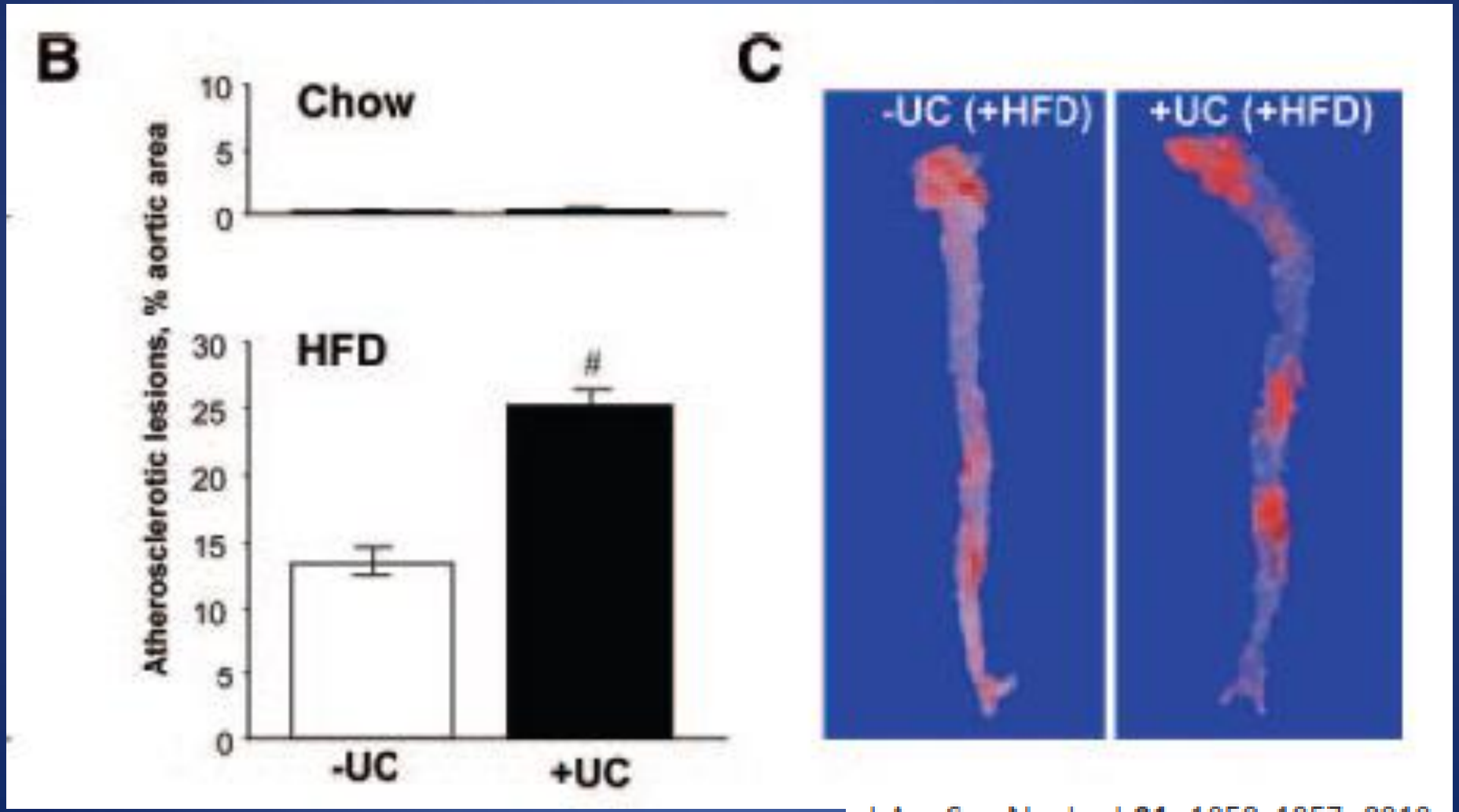
Cyanate-injected mice

Berg et. al., Sci Trans. Med. PMCID: PMC3697767

Amino acid therapy reduces urea-induced carbamylation



ApoE-null mice fed urea have accelerated atherosclerosis



J Am Soc Nephrol 21: 1852–1857, 2010

Apostolov, et. al.

E.O. Apostolov, et. al. J Am Soc Nephrol. Vol. 21. 2010.

Urea may contribute to uremic heart disease…
how do we interrupt this pathophysiology and change patient outcomes?

Reduction of carbamylation with amino acid scavenger therapy and C-Alb targeted dialysis optimization

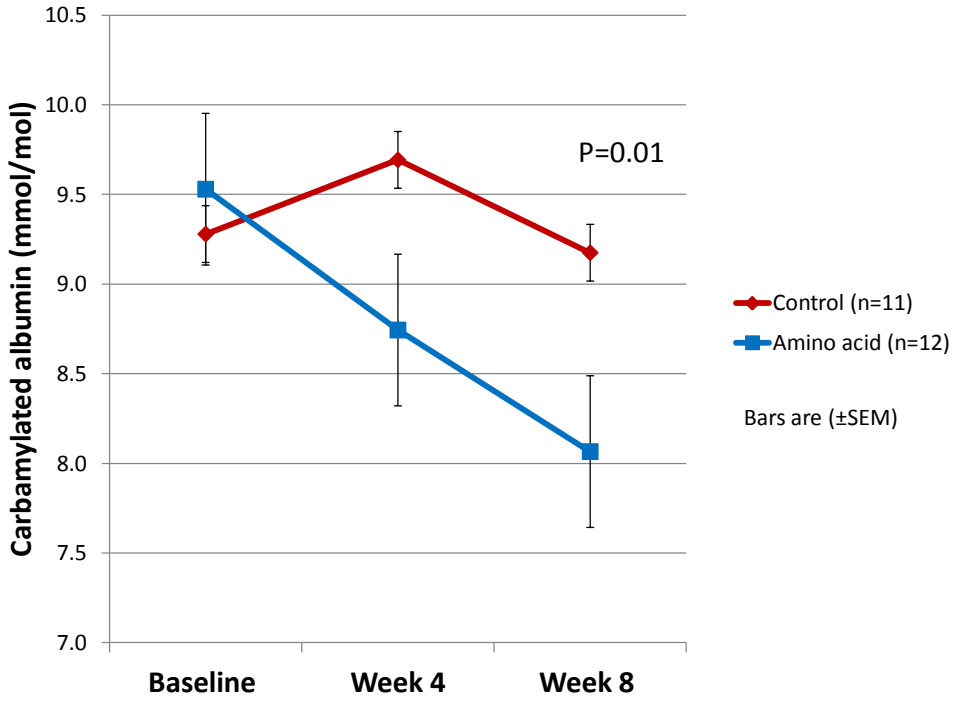
Hypothesis:

Protein carbamylation is promoted by imbalance between high blood urea and low amino acid carbamylation scavengers

Solution:

Amino acid Scavenger Supplementation Therapy

Carbamylation in Renal Disease-modulation With Amino Acid Therapy (CarRAAT)



Ravi Thadhani (MGH)

Sahir Kalim (MGH)

ClinicalTrials.gov ID: NCT01612429

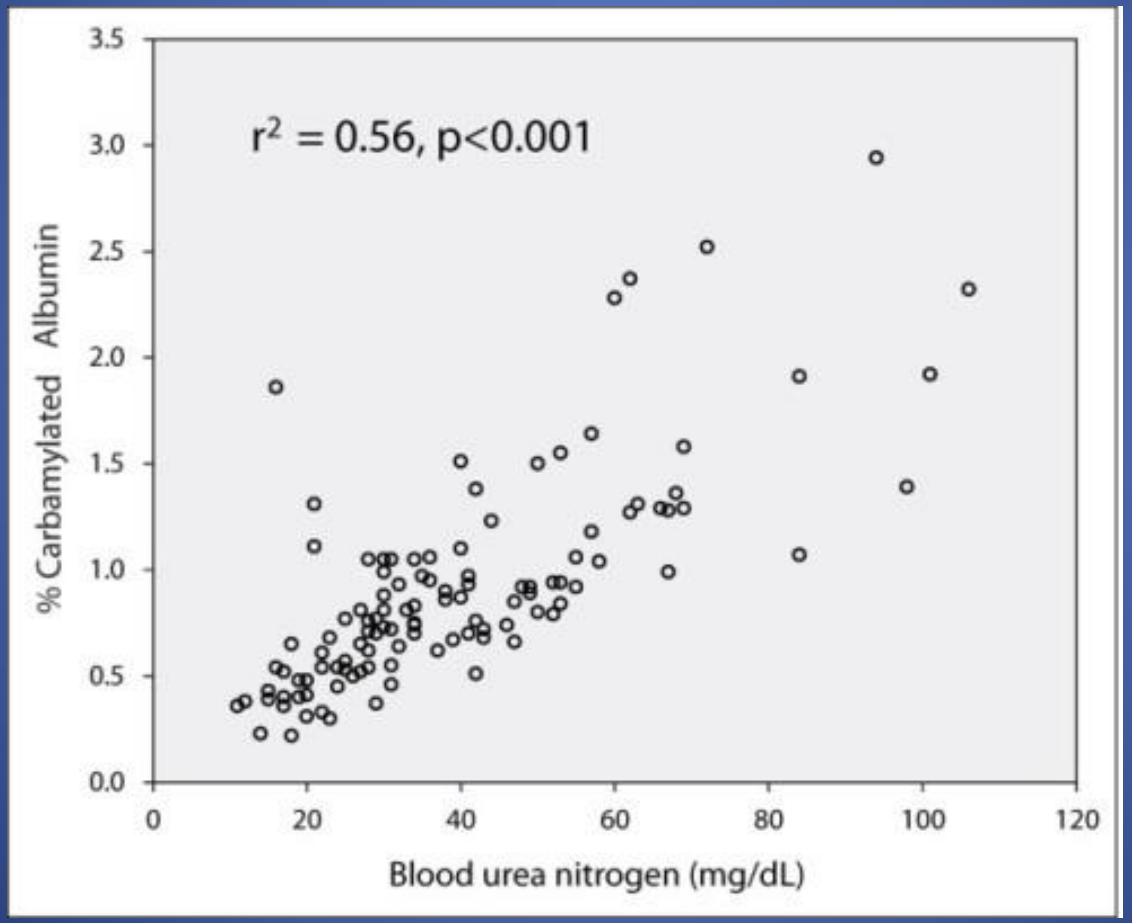
Kalim et. al., J Ren. Nutr. PMCID: PMC4469570

Specific amino acids differentially inhibit protein carbamylation→ *Pathway for more optimized therapies*

AA	%C-Alb	% Inhibition
Taurine	22 ± 1.7 %	46%
Cysteine	23 ± 1.9 %	42%
Histidine	26 ± 1.0 %	35%
Arginine	27 ± 1.6 %	34%
Lysine	31 ± 0.9 %	23%
No AA	41 ± 2.5 %	

Berg et. al., Sci Trans. Med. PMCID: PMC3697767

Is %C-Alb a better indicator of dialysis adequacy, and can it be used to optimize dialysis dose?



Study samples provided by J. Danziger, D. Friedman, n=122

Effects of intensive dialysis on carbamylation albumin

Table 1. Effects of increasing dialysis dose on C-Alb values

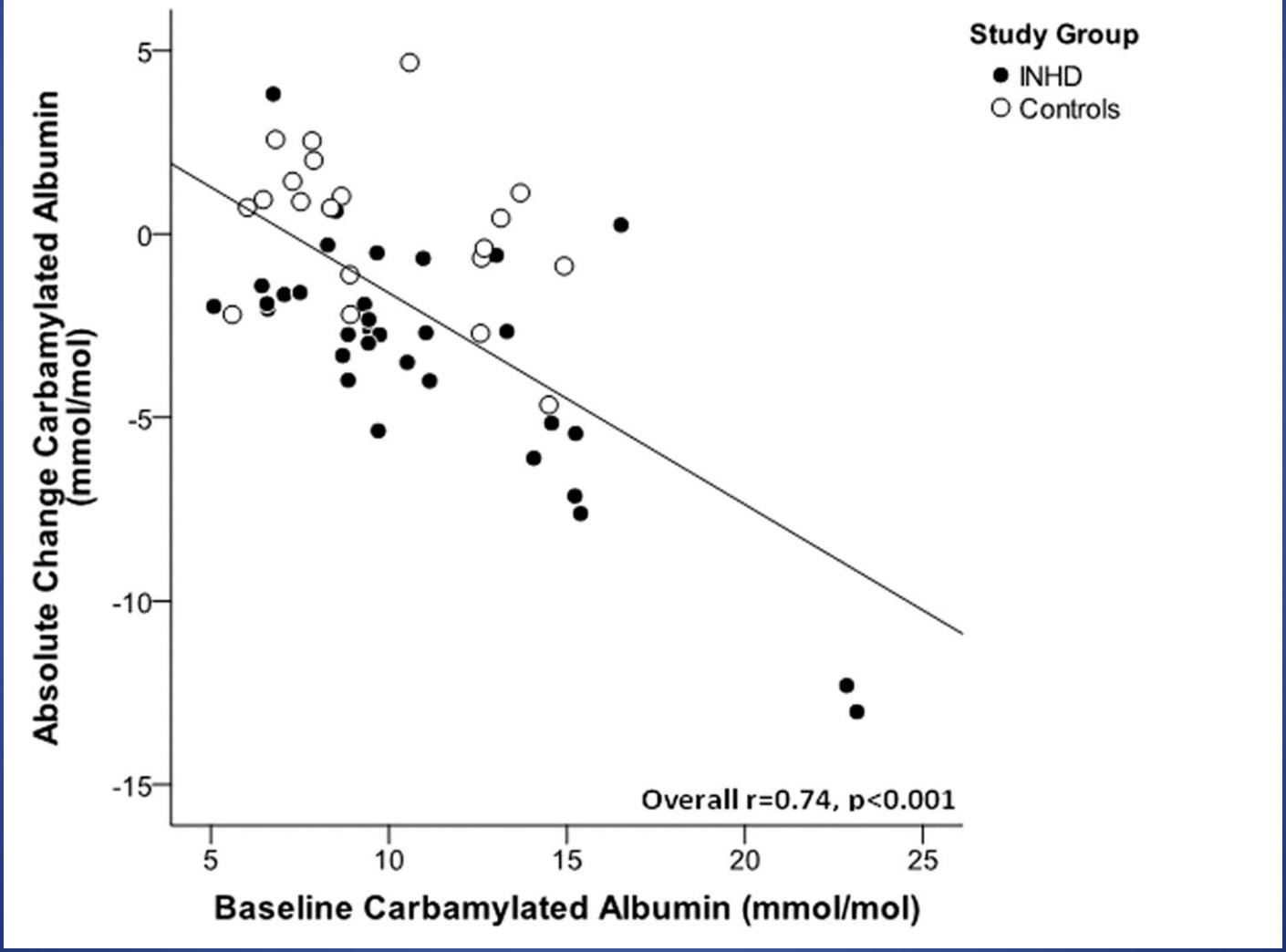
	Baseline	End of study	% change	P-value[1]
CHD control group (n=20)	9.7 ± 3.0	10.0 ± 3.0	+3%	0.36
INHD 3x per week (n=33)	11.0 ± 4.3	7.8 ± 2.7[2]	-29%	<0.0001
INHD 6 x per week (n=19)	9.5 ± 4.4	4.9 ± 1.7[3]	-48%	<0.0001

Table shows mean ± standard deviation, [1]Paired t-test for significance of difference between baseline and end of study values.[2]P = 0.009 for end of study C-Alb values in CHD vs. INHD 3x per week treatment groups.[3]P = 0.001 for end of study C-Alb values in INHD 3x per week vs. 6x per week treatment groups.

Jeffrey Perl, U Toronto

Christopher Chan, U Toronto

High baseline C-Alb correlates with greater decrease by nocturnal HD



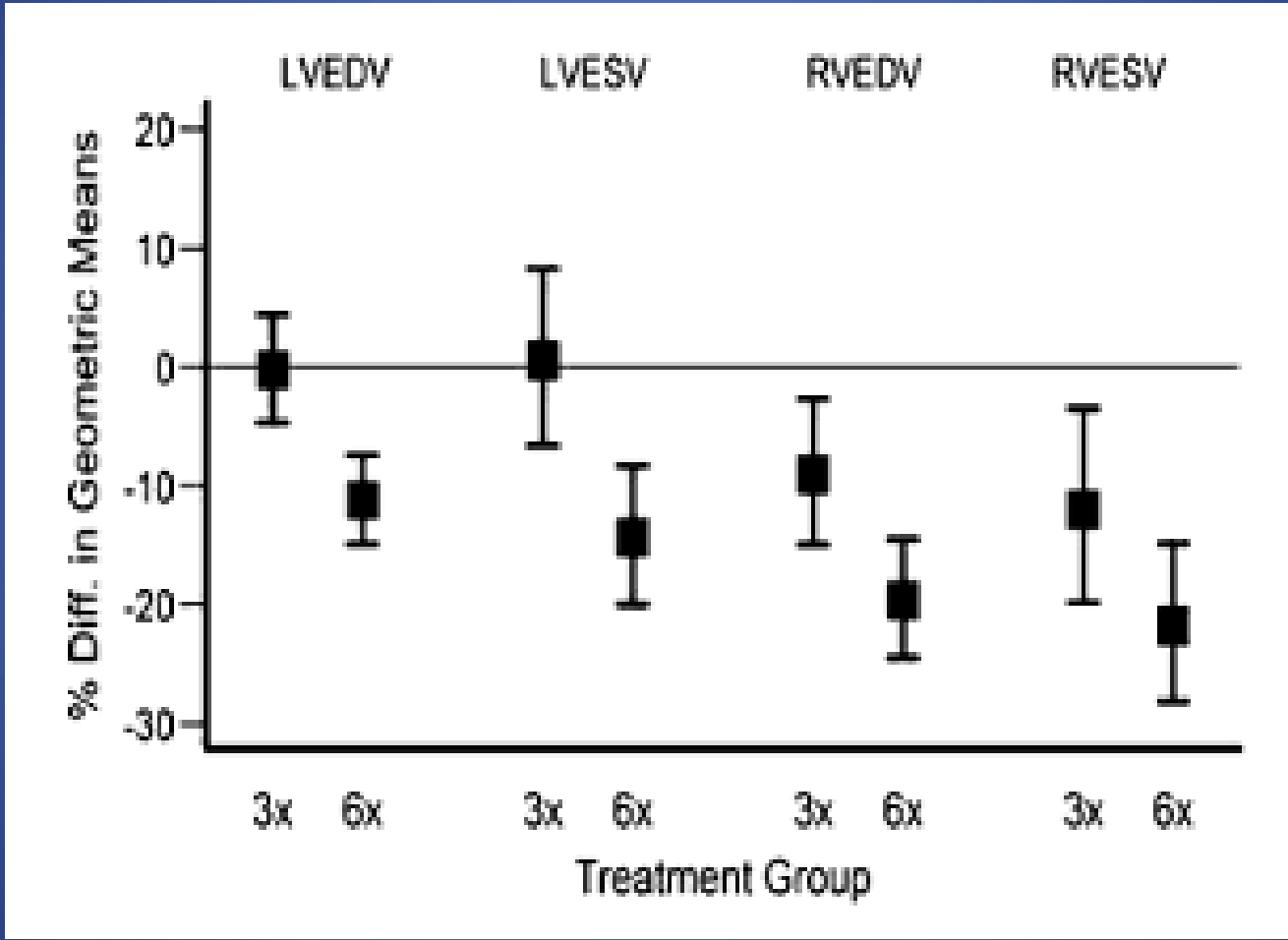
Nocturnal HD reduces urea, but increases amino acids

Table 3. Average changes in clinical and biochemical indicators by INHD treatment group

	INHD (n=33)	**Controls (n=20)**	**P-value***
Left ventricular mass (g)	-8.2 (-18.5, 2.1)	+13.6 (0.3, 27.0)	0.01*
Pre-HD Urea (mmol/L)	-2.76 (-5.13, -0.39)	+2.08 (-0.94, 5.10)	0.01*
Urea reduction ratio (%)	+11.7 (0.4, 23.9)	+0.9 (-3.1, 6.6)	<0.001*
Albumin (g/L)	+0.55 (-0.84, 1.94)	-0.40 (-2.18, 1.38)	0.40
Amino acids			
Valine (µmol/L)	+28.6 (10.2, 47.0)	-2.9 (-26.5, 20.8)	0.04*
Isoleucine (µmol/L)	+24.2 (9.4, 39.0)	+0.1 (-19.0, 19.1)	0.049*
Methionine (µmol/L)	+4.6 (-0.01, 9.3)	-2.0 (-7.9, 4.0)	0.09
Threonine (µmol/L)	+29.6 (-4.9, 64.2)	-26.7 (-71.1, 17.7)	0.049*
Lysine (µmol/L)	-12.5 (-49.7, 24.6)	-26.1 (-73.8, 21.6)	0.65
Leucine (µmol/L)	+14.7 (2.6, 26.8)	+0.2 (-15.3, 15.7)	0.15
Histidine (µmol/L)	+8.2 (-2.5, 18.9)	+1.6 (-12.2, 15.3)	0.45
Phenylalanine (µmol/L)	+7.1 (-1.8, 15.9)	-4.5 (-15.8, 6.8)	0.11
Glutathione (µmol/L)	+0.103 (-0.014, 0.220)	-0.145 (-0.295, 0.005)	0.01*
Average Essential AAs†	+0.38 (0.08, 0.68)	-0.12 (-0.50, 0.27)	0.047*
Average Non-Essential AAs†	+0.31 (0.04, 0.67)	-0.20 (-0.54, 0.14)	0.02*

BP = blood pressure, HD = hemodialysis, table shows average 12 month change in values (95% confidence intervals)

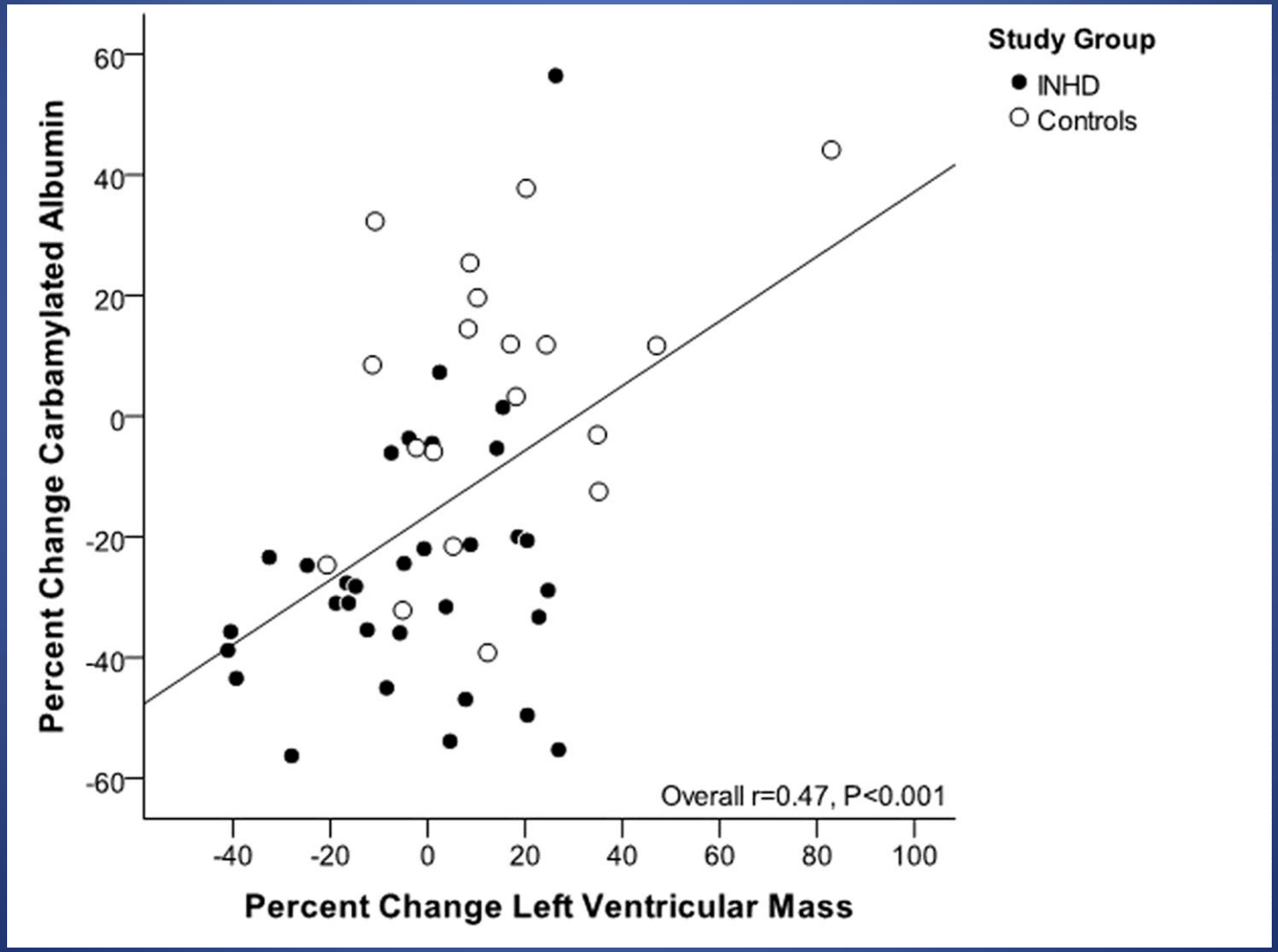
Intensive dialysis reduces ventricular hypertrophy



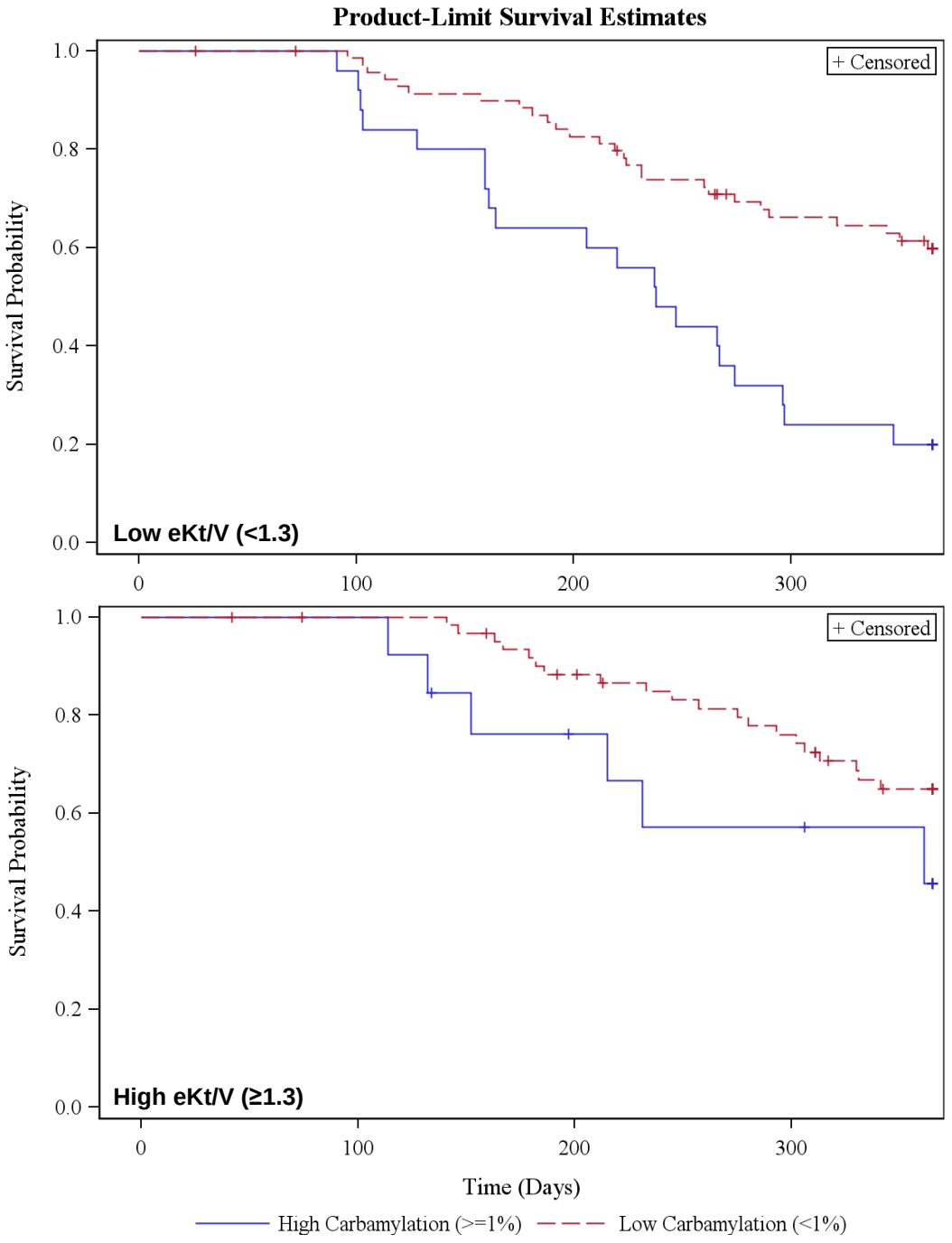
Clin J Am Soc Nephrol 8: 2106–2116, December, 2013

Frequent Hemodialysis and Ventricular Volumes, Chan et al.

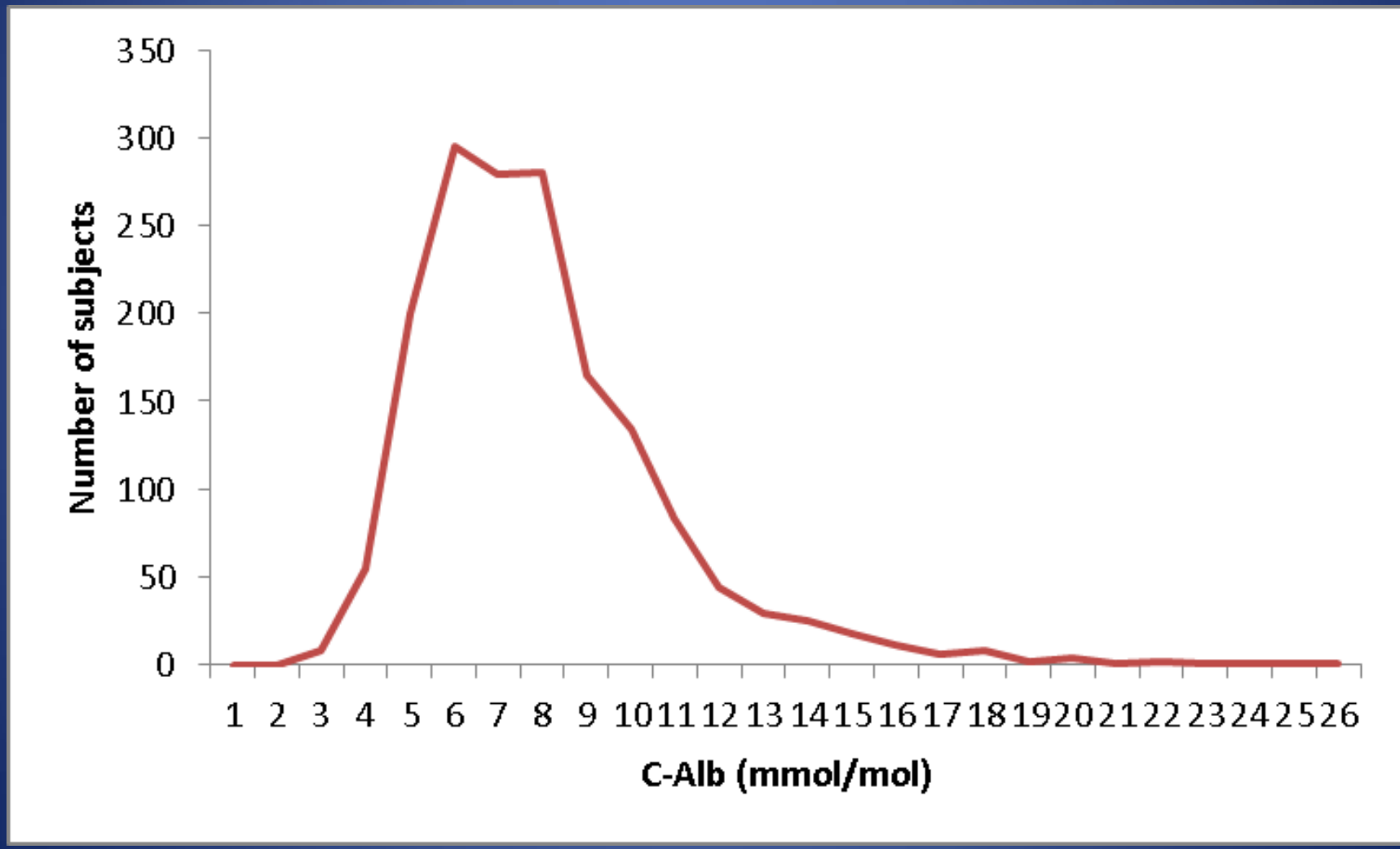
Reduction of C-Alb correlates with reduction of ventricular mass



High C-Alb *combined* with eKt/V < 1.3 was associated with especially high mortality



Analysis of carbamylated albumin in the GCKD cohort – preliminary results



New alternatives to glycated hemoglobin (HbA1c) for monitoring glycemic control in kidney patients

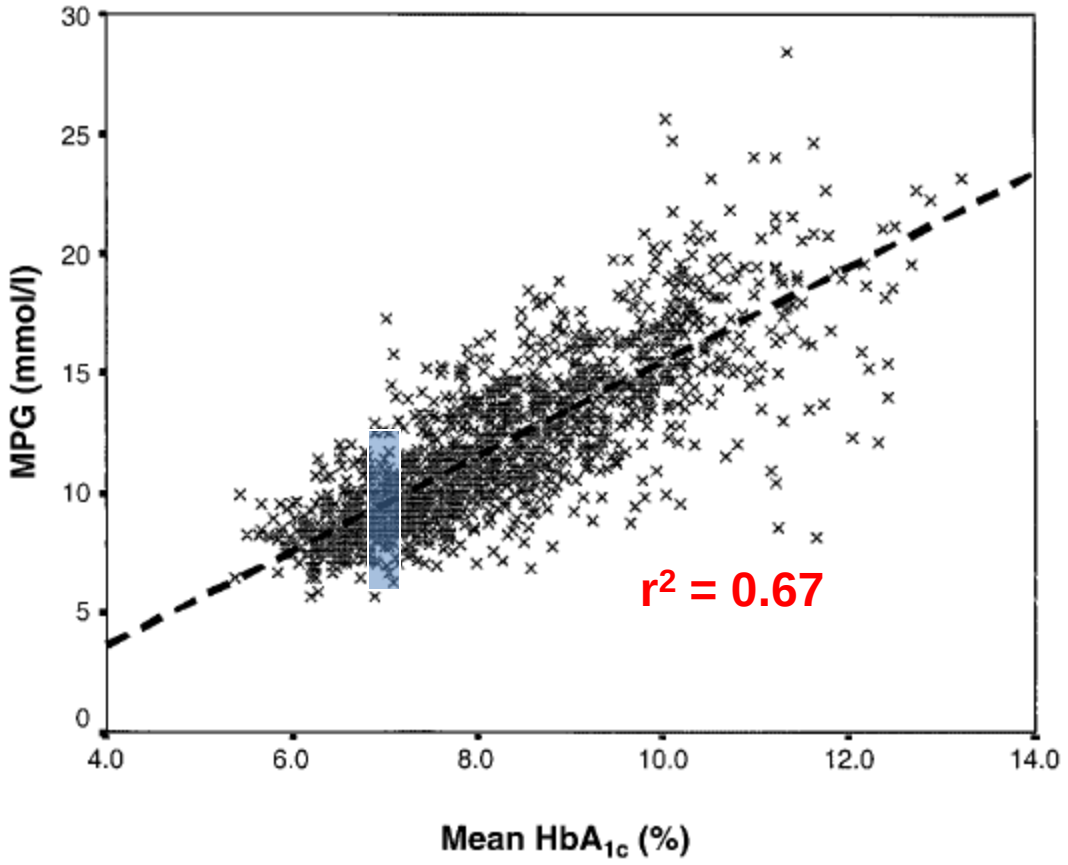
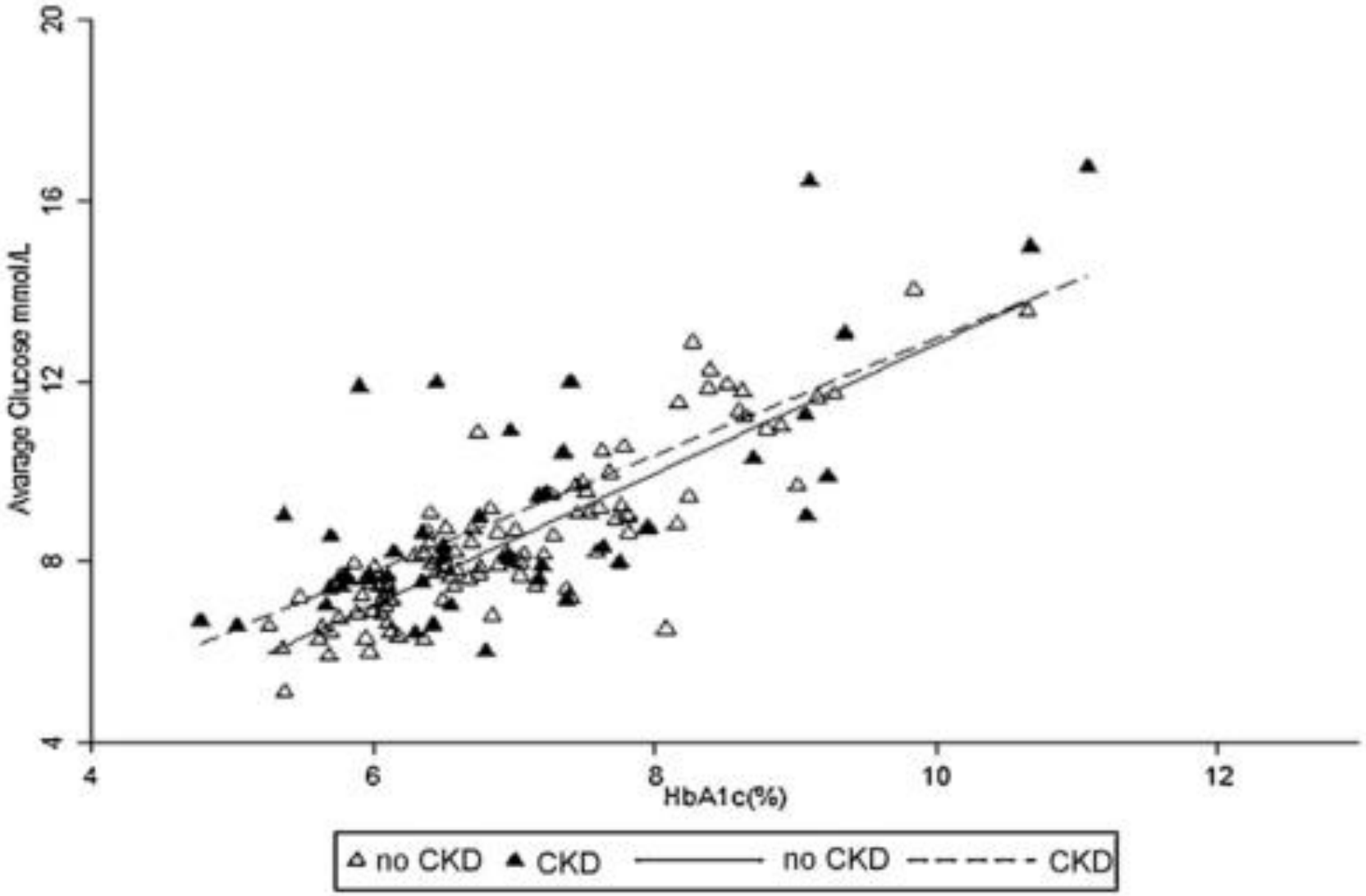


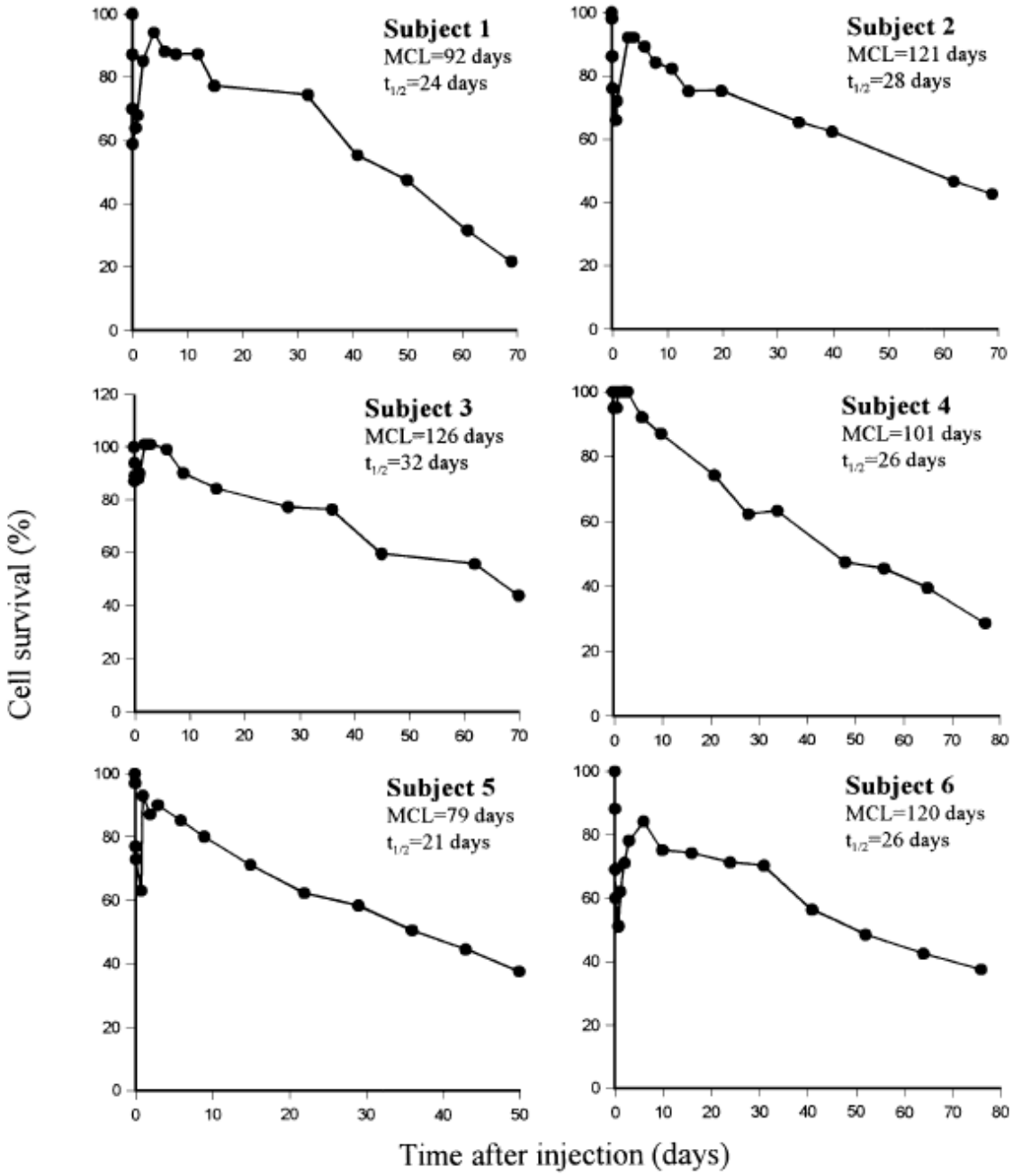
Figure 1—*MPG versus HbA_{1c}*: n = *1,439*; r = *0.82*; *PG (mmol/l) = (1.98 · HbA_{1c}) – 4.29. The dashed line indicates the regression line.*

a) Chronic kidney disease (CKD) and non-chronic kidney disease (non-CKD) cohorts.

$AG_{mmol/L} = 1.38 \times HbA1c + 0.57 \times CKD\ status - 1.16$, $R^2 = 0.66$, $p < 0.0001$ (CKD $R^2 = 0.53$ $p < 0.001$, Non CKD $R^2 = 0.75$ $p < 0.001$)



RBC life span variability



Bax, et.al., Clin. Sci., 1999

Effects of iron supplements on A1c

	Before iron mean (95% CI)	After iron mean (95% CI)	P*
A1C (%)	7.40 (6.60–8.19)	6.96 (6.27–7.25)	<0.001
Hb (g/dl)	9.71 (9.32–10.05)	10.46 (9.97–10.75)	0.001
Hct	0.302 (0.285–0.316)	0.334 (0.314–0.354)	0.007
Ferritin (μg/l)	122 (67–176)	307 (211–403)	<0.001
MBG (mmol/l)	9.55 (8.20–10.90)	9.71 (8.29–11.13)	0.071

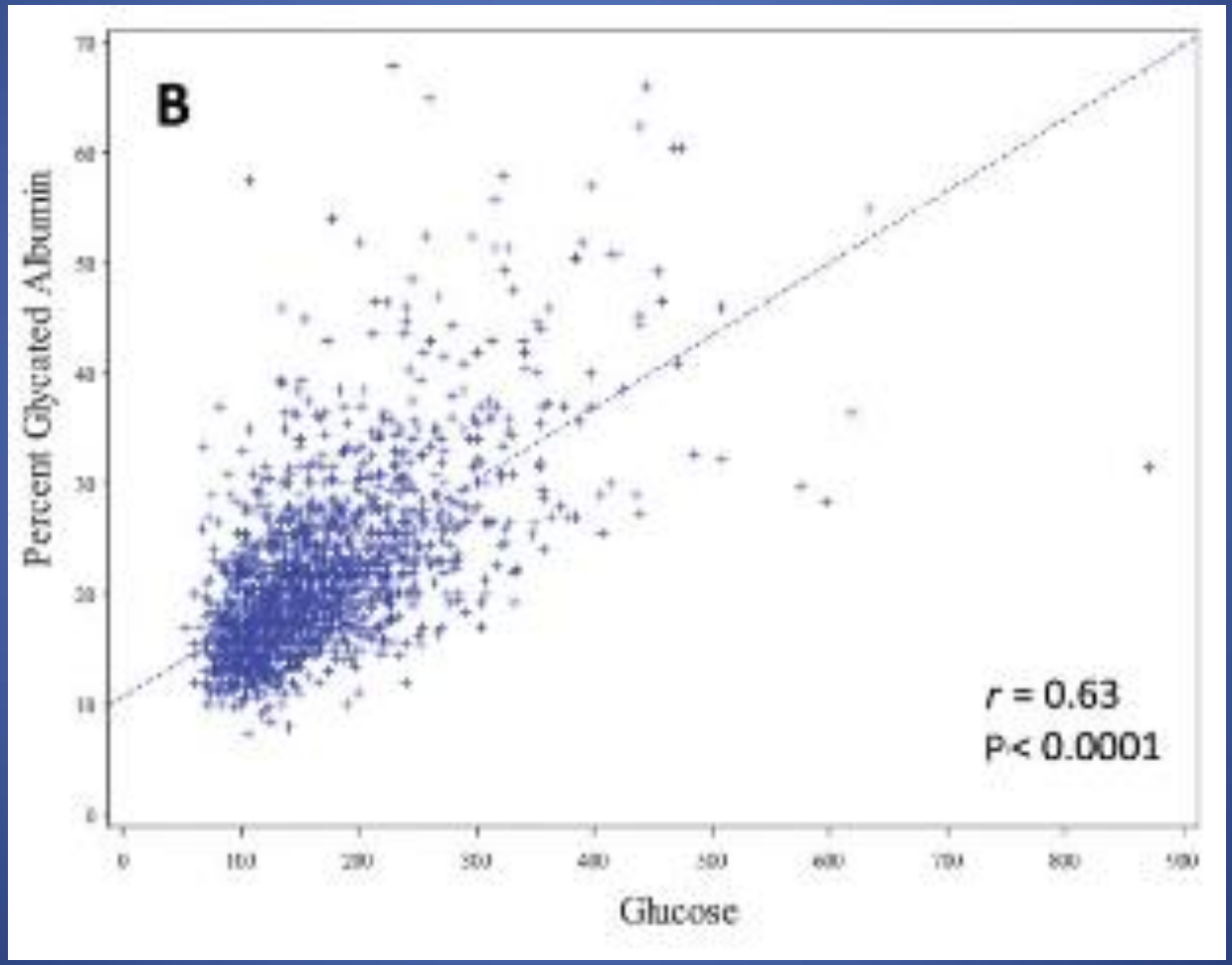
Effects of erythropoietin on A1c

	Before ESA mean (95% CI)	After ESA mean (95% CI)	P*
A1C (%)	7.31 (6.42–8.54)	6.63 (6.03–7.36)	0.013
Hb (g/dl)	9.52 (9.18–9.86)	11.51 (11.15–11.85)	<0.001
Hct	0.324 (0.296–0.350)	0.378 (0.341–0.398)	<0.001
Ferritin (μg/l)	344 (241–447)	332 (211–354)	0.37
MBG (mmol/l)	8.72 (7.31–10.12)	8.78 (7.47–9.99)	0.893

Ng, et. al.

Diabetes Care, volume 33, number 11, November 2010

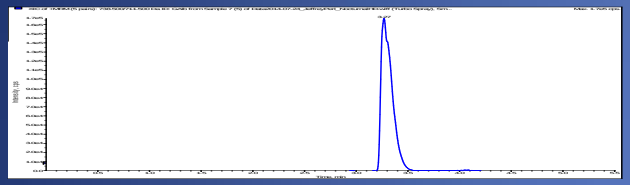
Glycated albumin as an alternative to HbA1c in CKD



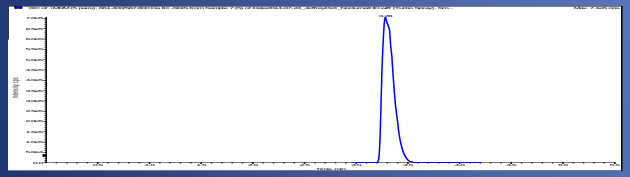
Williams, et. al.

Hemodialysis International 2015; **19**:562–571

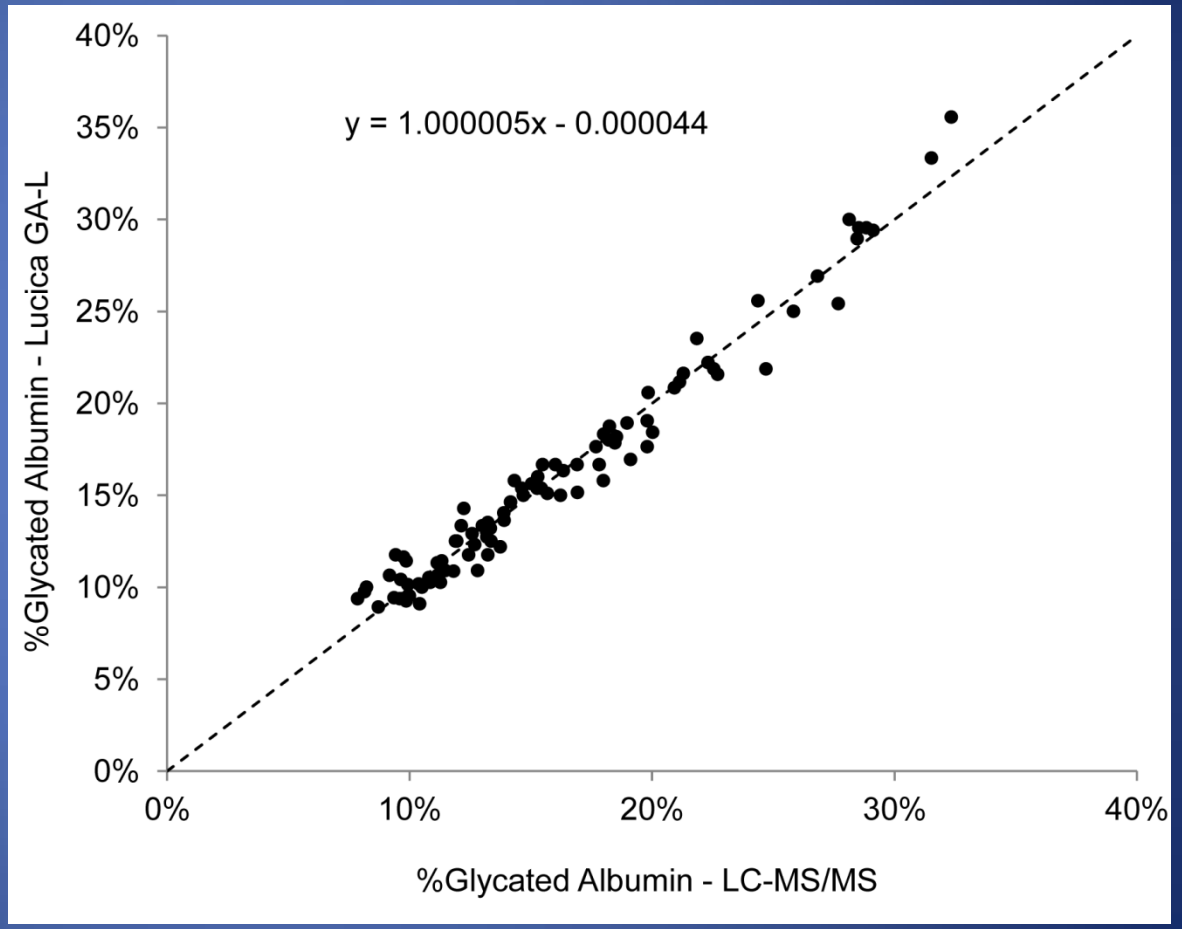
Combined LC-MS/MS carbamylated/glycated albumin assay development and validation



Glycated albumin

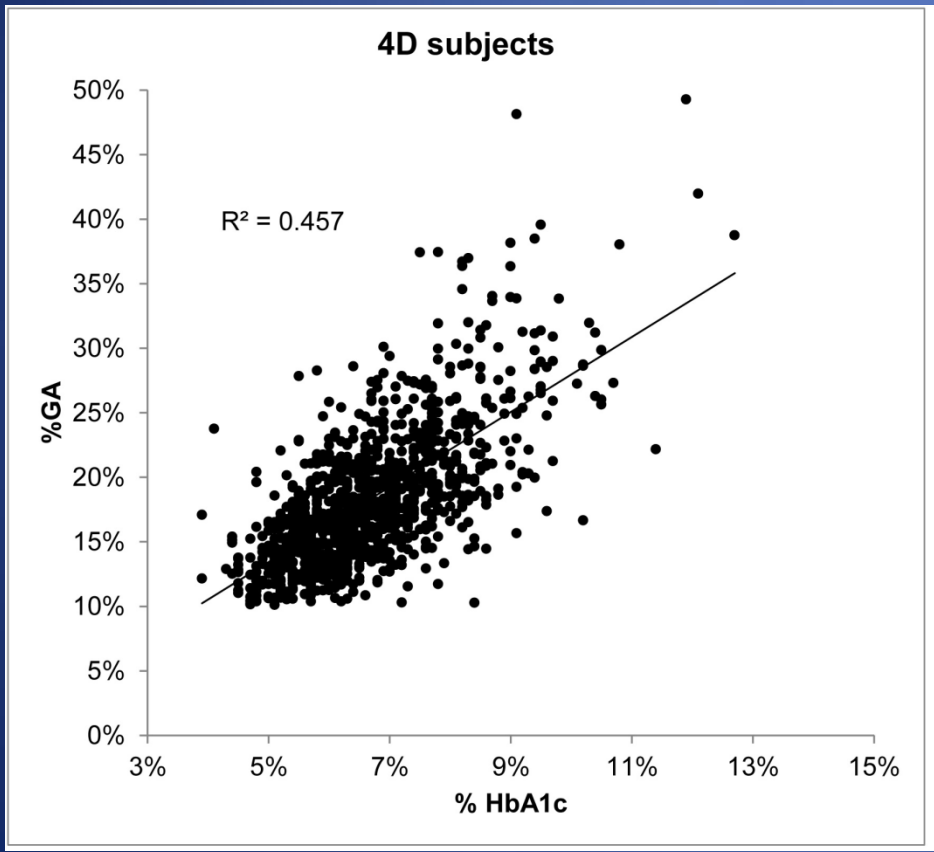


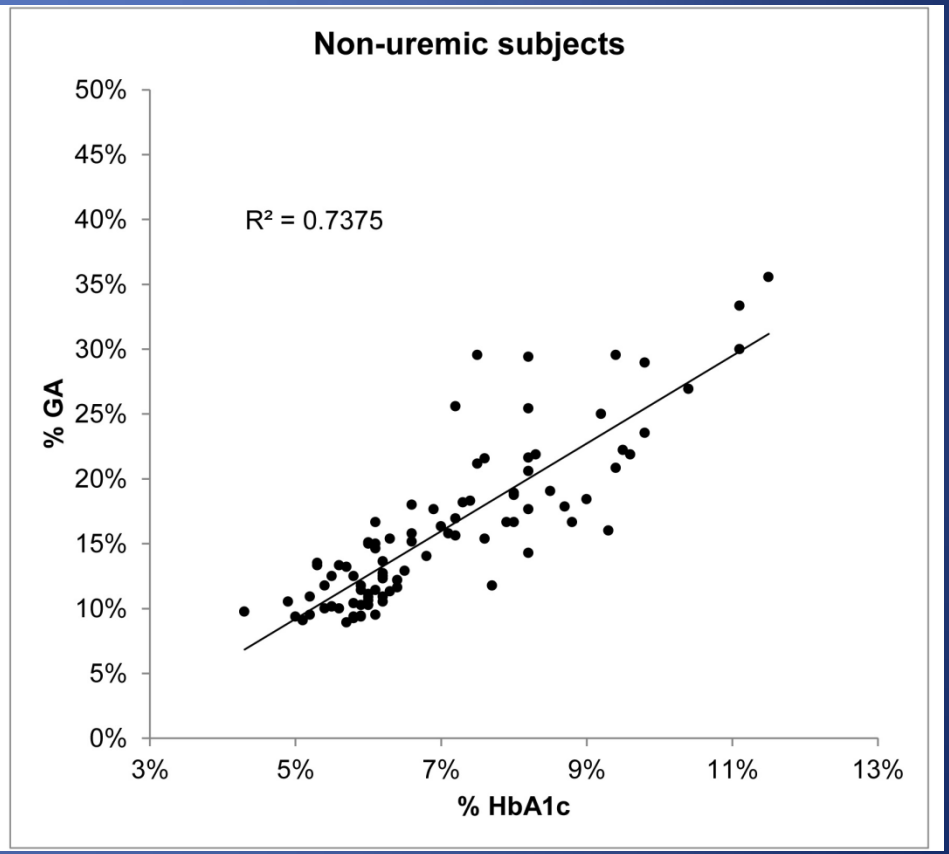
Non-glycated albumin



Intra-assay CV = 2.1%
Inter-assay CV = 6.2%

Correlation between glycated albumin and hemoglobin A1c in 4D subjects vs. non-uremic controls





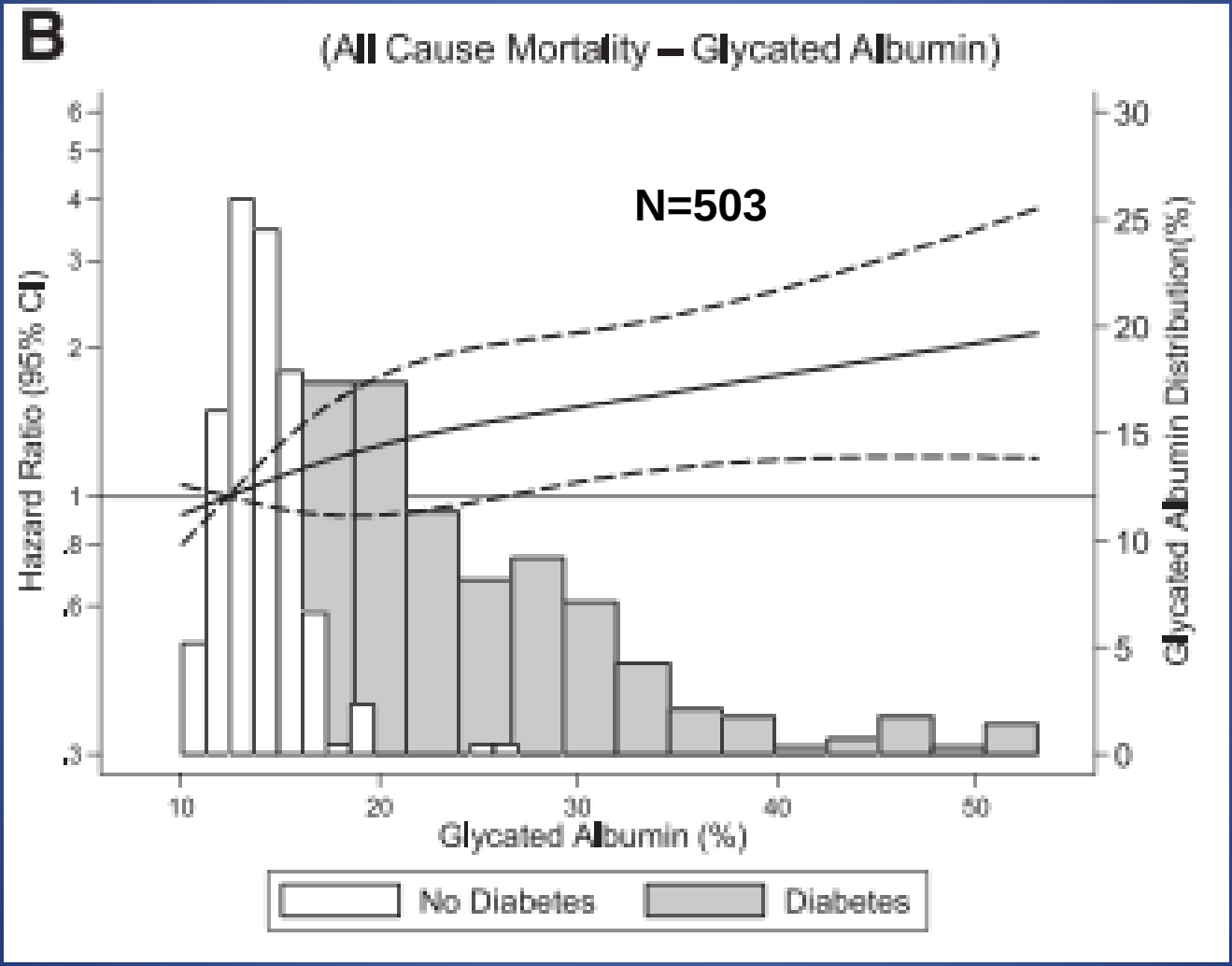
Cox-proportional hazards of baseline %Glycated albumin with 4-yr survival

	HR (95% CI)	P-value*	MV-adjusted HR (95% CI) [1]	P-value
1st quartile	(ref)		(ref)	
2nd quartile	1.23 (0.93-1.61)	0.049	1.15 (0.87 – 1.51)	0.33
3rd quartile	1.10 (0.84-1.45)	0.48	1.03 (0.78 – 1.37)	0.83
4th quartile	**1.42 (1.09-1.85)**	**0.009**	**1.32 (1.01 – 1.73)**	**0.04**

Significant at $p<0.05$

[1]Adjusted for effects of age, gender, body mass index, diabetes duration, diabetes as cause of kidney failure, history of coronary artery disease, history of congestive heart failure, systolic blood pressure, and blood concentrations of calcium, phosphate, hemoglobin, low density lipoprotein cholesterol, triglycerides, and C-reactive protein.

Mortality associated with high glycated albumin is corroborated by smaller studies



Shafi and Associates

Diabetes Care, volume 36, June 2013

Glycated Albumin - Future Directions

- Glycated albumin correlates well with blood glucose in dialysis patients.
- HbA_{1c} is unpredictably biased by CKD anemia and epo therapy.
- We have new evidence that high glycated albumin is associated with mortality.
- A larger longitudinal study is needed to compare glycated albumin vs. HbA_{1c} as indicators of ***time-averaged*** glucose in dialysis patients on and off ESA therapy.

Carbamylated Albumin Summary

- High %C-Alb in ESRD is strongly associated with heart failure and mortality
- Carbamylation of proteins may contribute to oxidative stress, atherogenesis, and uremic cardiomyopathy in patients with kidney disease.
- “Hypercarbamylation” is associated with amino acid deficiencies and may be reduced by amino acid therapy.
- Carbamylated albumin monitoring may be a new method of optimizing dialysis and nutritional therapy for patients with chronic and end-stage renal disease.

Future Directions

- Testing efficacy of different amino acid mixtures and additional nutriceutical scavengers of carbamylation in mouse models of uremia and human studies.
- Testing whether C-Alb predicts benefit from dialysis intensification.
- Testing significance of carbamylation and amino acid deficiencies in earlier stages of kidney disease (GCKD study).

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