ABSTRACT
Chronic kidney disease (CKD) is characterized by the progressive reduction of glomerular filtration rate and subsequent retention of organic waste compounds called uremic toxins. While patients with CKD are at a higher risk of premature death due to cardiovascular complications, this increased risk cannot be completely explained by classical cardiovascular risk factors such as hypertension, diabetes mellitus, and obesity. Instead, recent research suggests that uremic toxins may play a key role in explaining this marked increase in cardiovascular mortality in patients with CKD. While spermine, a tetra-amine, has previously been hypothesized to act as an uremic toxin, the following review presents a summary of recent literature that casts doubt on this assertion. Instead, acrolein, an oxidative product of spermine and the triamine spermidine, is likely responsible for the toxic effects previously attributed to spermine.

Introduction
Chronic kidney disease (CKD), and its subsequent progression to end-stage renal disease (ESRD), has grown at an exponential rate in recent years, doubling from 1991 to 2001. Although this growth has started to slow, it has been estimated that over 700,000 patients are receiving treatment for ESRD in 2015. As patient rolls have expanded, the costs of treating the disease have followed suit. While the total costs of treating ESRD in the USA totaled $22.8 billion in 2001, by 2010, the costs of treating ESRD to Medicare alone were estimated to reach almost $40 billion. Comorbidities such as diabetes mellitus, hypertension, glomerulonephritis, and other factors such as diet, lifestyle, and uremic toxins influence the progression of CKD and its response to treatment. Recognition of these factors can potentially lead to more proactive preventive and early treatment strategies for CKD.

The uremic syndrome
In patients with CKD, a progressive reduction of the glomerular filtration rate and/or the appearance of proteinuria leads to the retention of uremic toxins. This progressive retention of uremic toxins in these patients (even in those treated with standard dialysis) is termed the uremic syndrome. These compounds, also known as uremic retention solutes, are known to exert various cytotoxic activities in the serum, resulting in the deterioration of several biochemical and physiological functions and, over time, to the progression of renal failure. Over the last few decades, a large number of such compounds have been identified and their effects on various organs and tissues, especially the cardiovascular system, have been demonstrated.

Uremic toxins
Uremic toxins include low molecular weight organic substances and peptides, middle molecular weight uremic toxins, and protein-bound uremic toxins. As a result of differing hydrophobicity, low molecular weight uremic toxins may either exist as free water-soluble forms or bind reversibly to serum proteins, thereby altering protein function. Currently available dialysis modalities can adequately remove small molecular weight uremic toxins and partially remove middle molecular weight uremic toxins, but they do not adequately remove protein-bound toxins or peptides. In 2003, the European Uremic Toxin Work Group (EUTox) listed the 90 different uremic toxins known at that time. By 2008, an additional 25 toxins had been identified.

Before a retention solute can be accepted as a true uremic toxin, EUTox recommended that it should comply with a number of conditions: (i) such a compound...
should be chemically identified and an accurate quantitative analysis in biological fluids should be possible; (ii) total body and plasma levels should be higher in uremic patients as compared to nonuremic subjects; (iii) high concentrations should be related to specific uremic dysfunction and/or symptoms that decrease or disappear when the concentration is reduced; (iv) biological activity, conforming to clinical changes observed in conjunction with the uremic syndrome, should be proven in \textit{in vivo}, \textit{ex vivo}, or \textit{in vitro} studies; and (v) concentrations in these studies should conform to those found in the body fluids or tissues of uremic patients. At present there are 152 solutes listed as uremic toxins in the EUTox database. The present manuscript questions whether the tetra-amine, spermine, which belongs to a class of cations called polyamines, should be classified as a uremic toxin.

**Polyamines**

Polyamines, which include spermidine, spermine, and the related diamine putrescine, are organic cations. These molecules are present at millimolar concentrations in both prokaryotic and eukaryotic cells and are required for optimum cell growth. Cells in which polyamine production has been disrupted by mutations, or blocked by inhibitors, require the exogenous provision of at least one polyamine for continued survival.

Since the early 1970s, it has been suggested that polyamines play a role in the pathogenesis of renal anemia. Earlier studies indicated that polyamine levels were elevated in uremia. However, the methodology used to measure polyamine levels in these studies was flawed and more recent studies using high-performance liquid chromatography have shown that while the level of putrescine was increased, the level of spermine was actually decreased in the plasma of patients with chronic renal failure. This finding directly conflicts with the assertions of Sinha-Hikim et al., who have recently claimed that spermine is a reputed uremic toxin that triggers the apoptosis of vascular smooth muscle cells. The apoptotic process noted by these authors to have been caused by “the uremic toxin spermine” may in fact have been caused by acrolein, an oxidative byproduct of spermine. These authors did not investigate the role of acrolein in these processes.

While it is well known that the addition of spermidine or spermine to culture medium containing serum inhibits cellular proliferation, this effect has been shown to be caused by the oxidation products of polyamines that are generated by serum amine oxidase, which catalyzes the oxidative deamination of spermidine and spermine to produce an aminoaldehyde [N-(aminobutyryl)-aminopropionaldehyde] or an aminoaldehyde \( [\text{CH}_2\text{CHO} \text{CH}_2\text{NH}] \) is then spontaneously formed from these two amino aldehydes. Spermine oxidase can also produce 3-aminopropanal, \( \text{H}_2\text{O}_2 \), and \( \text{NH}_3 \) from spermine, which forms acrolein spontaneously.

**Acrolein**

Acrolein is an electrophilic aldehyde that, in addition to being produced from polyamines, is generated in inflammatory processes and is found in tobacco smoke and food. It is also a byproduct of the metabolism of the chemotherapeutic drugs cyclophosphamide and ifosfamide and is responsible for their prominent side effects of hemorrhagic cystitis and bladder cancer. Acrolein has been shown to react with a wide variety of proteins throughout the body via the Maillard reaction, leading to the induction of both necrosis and apoptosis in nucleated cells. Additionally, in animal models of CKD, acrolein has been shown to trigger erythropoiesis. Additionally, in animals, acrolein induces dyslipidemia and cardiac damage that can be partially prevented with the use of an oral charcoal absorbent. In cell culture media, acrolein concentrations as low as 5 \( \mu \text{M/L} \) inhibit cell growth by 50%.

As previously mentioned, plasma levels of spermine are decreased and levels of putrescine, polyamine oxidase, and acrolein are increased in patients with CKD as compared to healthy subjects. In these patients, the excess acrolein was mainly produced through the oxidation of spermine by spermine oxidase. In fact, acrolein levels in the plasma of uremic patients were 6-fold higher as compared to normal subjects and the levels of protein-conjugated acrolein were found to be correlated well with the severity of renal failure. Other work has echoed these results. These findings, when combined with previous work demonstrating the toxicity of acrolein and the fact that polyamines are necessary for cellular growth, suggest that it is in fact acrolein, and not spermine, that functions as a uremic toxin in patients with CKD.

**Conclusion**

As mentioned earlier, for a retention solute to be accepted as an uremic toxin, it should comply with five conditions. Based on these conditions, the very fact that plasma spermine levels are lower in patients with chronic renal failure than in normal subjects should disqualify this tetra-amine from being classified as an
uremic toxin. Furthermore, the 6-fold increase in acrolein levels in the plasma of uremic patients and the fact that acrolein is formed from spermine is an interesting observation that raises the important question of whether it is actually acrolein, and not spermine, that is an uremic toxin. It is also of interest to note that polyamines are an absolute requirement for growth, further raising doubts on their status as uremic toxins. These observations most clearly illuminate the need for future investigation in order to properly elucidate the proper relationship between spermine, acrolein, and kidney failure.

Disclosure statement

The author reports no conflicts of interest. The author alone is responsible for the content and writing of this article.

References

1. Norris KC, Agoda Y. Unraveling the racial disparities associated with kidney disease. Kidney Int. 2005;68:914–924.
2. Gilbertson DT, Liu J, Yue JL, et al. Projecting the number of patients with end-stage renal disease in the United States to the year 2015. J Am Soc Nephrol. 2005;16:3736–3741.
3. USRD: Annual Data Report: Atlas of end-stage renal disease in the United States. Bethesda, MD: National Institute of Health. National Institute of Diabetes and Digestive and Kidney Diseases; 2003.
4. Trivedi HS, Pang HM, Campbell A, Saab P. Slowing the progression of chronic renal failure: Economic benefits and patients’ perspectives. Am J Kidney Dis. 2002;39:721–729.
5. Vanholder R, Argiles A, Baumeister U, et al. Uremic toxicity: Present state of the art. Int J Artif Organs. 2001;24:695–725.
6. Sallée M, Dou L, Cerini C, Poitevin S, Brunet P, Burtey S. Thearyl hydrocarbon receptor-activating effect of uremic toxins from tryptophan metabolism: A new concept to understand cardiovascular complications of chronic kidney disease. Toxins (Basel). 2014;6:934–949.
7. Chao CT, Chiang CK. Uremic toxins, oxidative stress, and renal fibrosis: An interwined complex. J Ren Nutr. 2015;25:155–159.
8. Martinez AW, Recht NS, Hostetter TH, Meyer TW. Removal of P-cresol sulfate by hemodialysis. J Am Soc Nephrol. 2005;16:3430–3436.
9. Vanholder R, van Laecke S, Glorieux G. The middle-molecule hypothesis 30 years after: Lost and rediscovered in the universe of uremic toxicity? J Nephrol. 2008;21:146–160.
10. Vanholder R, van Laecke S, Glorieux G. What is new in uremic toxicity? Pediatr Nephrol. 2008;23:1211–1221.
11. http://eutoxdb.odeessoftware.com/index.php
12. Cohen SS, A Guide to Polyamines. New York: Oxford University Press; 1998.
13. Tabor CW, Tabor H. It all started on a streetcar in Boston. Annu Rev Biochem. 1999;68:1.
14. Campbell R, Talwalker Y, Bartos D. Polyamines, uremia and hemodialysis. In: Campbell RA, ed. Advances in Polyamine Research. Vol. 2. New York: Raven Press; 1978:319–324.
15. Saito A, Takagi T, Chung TG, Ohta K. Serum levels of polyamines in patients with chronic renal failure. Kidney Int. 1983;24:234–237.
16. Swendseid M, Panaquq M, Kopple JD. Polyamine concentrations in red cells and urine of patients with chronic renal failure. Life Sci. 1980;26:533–539.
17. Igarashi K, Ueda S, Yoshida K, Kashiwagi K. Polyamines in renal failure. Amino Acids. 2006;31:477–483.
18. Sinha-Hikim I, Shen R, Kovacheva E, Crum A, Vaziri ND, Norris KC. Inhibition of apoptotic signaling in sperm-treateed vascular muscle cells by a novel glutathione precursor. Cell Biol Int. 2010;34:503–511.
19. Sinha-Hikim I, Shen R, Lee W-NP, et al. Effects of a novel cysteine-based glutathione precursor on oxidative stress in vascular smooth muscle cells. Am J Physiol Cell Physiol. 2010;299:C638–C642.
20. Igarashi K, Kashiwagi K. Modulation of cellular function by polyamines. Int J Biochem Cell Biol. 2010;42:39–51.
21. Higgins ML, Tillman MC, Rupp JP, Leach FR. The effect of polyamines on cell culture cells. J Cell Physiol. 1969;74:149–154.
22. Gaugas JM, Dewey DL. Evidence for serum binding of oxidized spermine and its potent G1-phase inhibition of cell proliferation. Br J Cancer. 1979:39:548–557.
23. Bachrach U. Oxidized polyamines. Ann N Y Acad Sci. 1970;171:939–956.
24. Tabor CW, Tabor H, Bachrach U. Identification of the aminoaidehydes produced by the oxidation of spermine and spermidine with purified plasma amine oxidase. J Biol Chem. 1964;239:2194–2203.
25. Kimes BW, Morris DR. Preparation and stability of oxidized polyamines. Biochim Biophys Acta. 1971;228:223–234.
26. Morgan DM, Bachrach U, Assaraf YG, Harari E, Golenser J. The effect of purified aminoaidehydes produced by polyamine oxidation on the development in vitro of Plasmodium falciparum in normal and glucose-6-phosphate-dehydrogenase-deficient erythrocytes. Biochem J. 1986;236:97–101.
27. Gugliucci A, Lunceford N, Kinugasa E, Ogata H, Schulze J, Kimura S. Acrolein inactivates paraoxonase 1: Changes in free acrolein levels after hemodialysis correlate with increases in paraoxonase 1 activity in chronic renal failure patients. Clin Chim Acta. 2007;384:105–112.
28. Cox PJ. Cyclophosphamide cystitis-identification of acrolein as the causative agent. Biochem Pharmacol. 1979;28:2045–2049.
29. Fujii H, Nishijima F, Goto S. Oral charcoal adsorbent (AST-120) prevents progression of cardiac damage in chronic kidney disease through suppression of oxidative stress. Nephrol Dial Transplant. 2009;24:2089–2095.
30. Liu-Snyder P, McNally H, Shi R, Borgens RB. Acrolein-mediated mechanisms of neuronal death. J Neurosci Res. 2006;84:209–218.
31. Wang L, Sun Y, Asahi M, Otsu K. Acrolein, an environmental toxin, induces cardiomyocyte apoptosis via elevated intracellular calcium and free radicals. *Cell Biochem Biophys*. 2011;61:131–136.

32. Tanel A, Averill-Bates DA. The aldehyde acrolein induces apoptosis via activation of the mitochondrial pathway. *Biochim Biophys Acta*. 2005;1743:255–267.

33. Ahmed MSE, Langer H, Abed M, Voelkl J, Lang F. The uremic toxin acrolein promotes suicidal erythrocyte death. *Kidney Blood Pres Res*. 2013;37:158.

34. Conklin DJ, Barski OA, Lesgards J. Acrolein consumption induces systemic dyslipidemia and lipoprotein modification. *Toxicol Appl Pharmacol*. 2010;243:1–12.

35. Sakata K, Kashiwagi K, Sharmin S. Acrolein produced from polyamines as one of the uraemic toxins. *Biochem Soc Trans*. 2003;31:371–374.

36. Sakata K, Kashiwagi K, Sharmin S, et al. Increase in putrescine, amine oxidase, and acrolein in plasma of renal failure patients. *Biochem Biophys Res Commun*. 2003;305:143–149.