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Theoretical Analysis of D-Serine and D-Asparagine as Biomarkers for Glomerular Filtration Rate

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ABSTRACT

Chronic kidney disease (CKD) is a major global health issue, requiring accurate and noninvasive renal function assessment. Glomerular filtration rate (GFR) is the standard measure, but current methods, including inulin clearance and creatinine-based estimation, have limitations in accuracy, invasiveness, and practicality. We investigated D-serine and D-asparagine as novel endogenous biomarkers for GFR evaluation. Their clearance strongly correlates with inulin clearance, demonstrating high reliability and reduced muscle mass dependency compared to creatinine-based methods. Our findings suggest that D-amino acids offer a promising, less invasive alternative for precise renal function assessment, aiding early CKD detection and management. In addition, D-amino acids have recently been reported to be useful as biomarkers not only in the renal field but also in various other fields such as malignancy and infectious diseases. This article highlights the potential of D-amino acids in enhancing CKD diagnosis and management.

1 | Introduction

Chronic kidney disease (CKD) is a serious condition characterized by the gradual decline of kidney function, eventually requiring dialysis or kidney transplantation to sustain life. The number of patients is increasing worldwide, and according to data from the World Health Organization (WHO), CKD is estimated to affect more than 800 million people globally, making it a significant public health issue [1]. In Japan, the number of CKD patients is estimated to be approximately 13 million, with the number of patients undergoing dialysis therapy due to CKD progression expected to reach around 350 000 by 2023 [2]. Once CKD reaches an advanced stage, it becomes essentially irreversible, making early diagnosis and appropriate therapeutic interventions critical to preventing the progression of chronic kidney failure. Accurate assessment of individual renal function is essential for early diagnosis and effective therapeutic interventions. However, current renal function assessment methods used in clinical practice face challenges in terms of accuracy and simplicity. Meanwhile, our recent studies have increasingly demonstrated that the properties of D-amino acids may serve as ideal biomarkers for assessing renal function. Therefore, we aimed to develop a novel method for evaluating renal function using D-amino acids.

2 | What Is Renal Function?

Glomerular filtration rate (GFR) is a representative index used to evaluate renal function. The kidneys perform various functions, including regulating fluid volume, maintaining electrolyte balance, regulating acid–base equilibrium, controlling blood

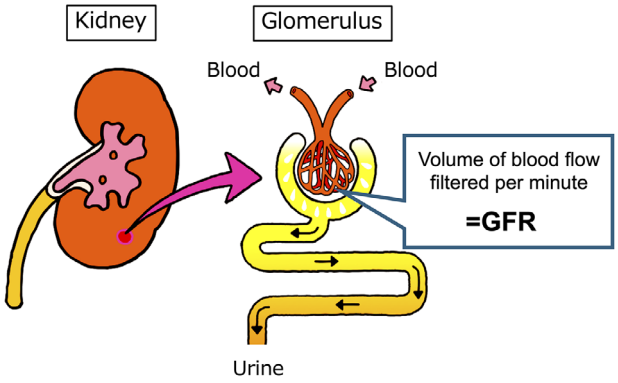

FIGURE 1 | Renal filtration capacity is defined by glomerular filtration rate (GFR). *Source:* This figure is partially revised and adapted from [3].

pressure, and promoting red blood cell production. Among these, the most critical function is filtering blood and eliminating waste products from the body as urine.

The kidneys contain hundreds of thousands to millions of functional units called nephrons. Nephrons are broadly divided into three main components: the glomeruli, Bowman's sacs, and tubules, each playing a specific role. The glomerulus filters blood, collecting waste products and excess fluid as "primary urine" in Bowman's sac. This primary urine is subsequently reabsorbed and secreted within the renal tubules, ultimately being discharged as urine.

Renal function, or the filtration capacity of the kidneys, is expressed as the volume of blood filtered by the glomeruli per minute, which is defined as GFR. GFR reflects the efficiency of blood filtration in the glomeruli and serves as an important indicator of kidney health [3] (Figure 1).

In healthy adults, GFR is generally considered normal within the range of 90–120 mL/min/1.73 m^2. A decrease in GFR below this range suggests a potential decline in renal function [4].

3 | Necessity of Renal Function Assessment

As mentioned, the measurement of GFR is used to diagnose CKD and monitor its progression. This enables the early detection of declining renal function and the evaluation of disease progression.

In addition, the adjustment of dosages for renally excreted drugs and drugs with nephrotoxic potential is also based on GFR. Furthermore, GFR is a critical factor in the selection of donors in renal transplantation. Accurate assessment of renal function is therefore essential for the safe and effective treatment of patients.

4 | Methods and Issues in Renal Function Assessment

As the direct measurement of GFR is highly challenging, clinical practice typically relies on the estimation of GFR using biomarkers of renal function. An ideal renal function biomarker should meet the following criteria: (i) free filtration in the glomerulus, (ii) no reabsorption or secretion in the tubules, and (iii) stable dynamics independent of GFR [5, 6]. Furthermore, from the perspective of minimizing patient invasiveness, the use of endogenous substances for estimation is preferred.

In routine clinical practice, GFR is estimated and evaluated using the following methods.

4.1 | Inulin Clearance (Cin)

Inulin clearance is considered the gold standard for accurate GFR measurement worldwide. Inulin is a naturally occurring polysaccharide. Because inulin is not metabolized by the body, is entirely filtered by the glomeruli of the kidney, and is neither reabsorbed nor secreted by the tubules, its clearance provides a highly accurate reflection of GFR. The measurement method involves three urine collection tests and blood tests conducted over a 90-min period, during which inulin, an exogenous substance, is continuously infused intravenously. The clearance is calculated as the average of these three clearances. However, this test requires the administration of an exogenous substance, multiple urine collections, and blood tests, making it complex and invasive for patients. As a result, it is performed at only a limited number of facilities.

4.2 | 24-h Creatinine Clearance (24 h CCr)

As creatinine is a metabolite of muscle and is almost completely filtered by the kidneys, creatinine clearance is used as a method for assessing renal function with endogenous substances. GFR is estimated using 24-h urine collection along with blood and urine creatinine levels. Although this method utilizes endogenous substances, it requires 24-h urine collection, and creatinine levels can be influenced by muscle mass, diet, age, and gender, potentially leading to inaccuracies. Additionally, errors may be particularly pronounced in patients with impaired renal function, as creatinine is partially secreted by the renal tubules.

4.3 | Estimation From Blood Creatinine Level (eGFRcreat)

The estimated GFR (eGFR) is calculated using blood creatinine levels and a formula that accounts for age and gender. In Japan and other countries, region- and race-specific formulas are applied. In Japan, the prediction formula published by the Japanese Society of Nephrology is widely used [7]. Although eGFR is estimated from blood creatinine levels and is simple and widely adopted, it is considered less accurate than the aforementioned clearance formula. Moreover, as with creatinine clearance, the use of creatinine makes it susceptible to influences from muscle mass and diet.

4.4 | Estimation From Blood Cystatin C Levels (eGFRcys)

Cystatin C is filtered by the glomeruli of the kidney but is rarely excreted in the urine because it is believed to be almost entirely

reabsorbed and completely metabolized in the renal tubules. Due to this property, blood cystatin C levels effectively reflect the kidney's GFR and are considered useful as an indicator for evaluating renal function. Compared to creatinine, cystatin C has the advantages of being largely unaffected by muscle mass, having high sensitivity, and detecting early declines in renal function. However, cystatin C levels may be influenced by drugs such as steroids and cyclosporine, as well as thyroid function, and they are not highly predictive in cases of advanced renal failure. Additionally, in many cases, insurance coverage and limited prevalence restrict rapid in-hospital measurement. Therefore, cystatin C is currently used as an adjunct to eGFR based on blood creatinine levels.

Thus, conventional methods of estimating GFR face challenges related to accuracy, invasiveness, and cumbersome measurement processes. We believe that it is essential to develop a biomarker that addresses these limitations.

5 | D-Amino Acids as a Biomarker for Kidney Disease

Proteins, which play a major role in biological functions, are composed of 20 amino acids, their smallest units. Although Krebs et al. discovered the presence of D-amino acid oxidase (DAO) in the kidney in 1935 [8], the physiological role of D-amino acids in the human body remains unclear. However, recent advancements in measurement technology have revealed that D-amino acids are present in trace amounts in mammalian bodies and exhibit a variety of physiological functions.

D-Amino acids, especially D-serine and D-asparagine, were reported to be elevated in kidney and plasma in patients with worse kidney function [9]. D-Serine and D-aspartate delivered to the kidneys are excreted almost 100% in urine. However, when kidney blood flow decreases with the decline of kidney function, the amount of D-amino acids reaching the kidneys also decreases, resulting in the accumulation of D-amino acids in the blood. This reflects kidney function [10]. On the other hand, in *Dao*-deficient mice, the metabolism of D-serine is impaired, leading to an increase in its concentration in the blood [11, 12]. *Dao* is primarily expressed in the kidneys [13], but as D-serine is almost entirely excreted in urine, the amount of D-serine metabolized by the kidneys may not be very high. The effects of Dao are based on studies conducted with completely gene-deficient animals. However, the impact of partial DAO deficiency, similar to what has been studied in relation to kidney function in humans, remains unclear.

Regarding the excretion of D-amino acids, the decrease in kidney blood flow is likely an upstream mechanism, making it the primary reason for the increase in blood concentration in worse kidney function.

Given the close relationship between the kidney and D-amino acids, we have been investigating the potential of D-amino acids as biomarkers for kidney disease. Our findings indicate that patients with CKD who exhibit high levels of D-amino acids, particularly D-serine and D-asparagine, in their blood tend to experience earlier onset of end-stage renal failure and dialysis compared to those with intermediate or low levels of D-amino acids [9, 14, 15]. Furthermore, differences in D-amino acid dynamics among the underlying diseases causing CKD suggest that these dynamics may aid in diagnosing specific diseases [16, 17].

The primary diseases of CKD vary, and each disease has different treatments and prognoses. The dynamics of D-amino acids in each disease are expected to reflect differences in tubular function, and these differences can be used to diagnose the primary disease of CKD. On the other hand, the dynamics of D-amino acids change depending on the primary disease of CKD, making strict evaluation of GFR difficult. The usefulness of D-amino acid clearance as a method of evaluating renal function we have reported has been studied in renal transplant patients, whose kidneys are considered to be less damaged by the primary disease, and therefore, it may be necessary to investigate this method in CKD patients with other primary diseases. Additionally, principal component analysis of various L- and D-amino acid concentrations in the blood of patients with CKD demonstrated that D-serine and D-asparagine form a cluster group similar to conventional renal function markers, such as creatinine [18, 19]. The results suggest that D-amino acids, but not L-amino acids, are useful as biomarkers of kidney disease.

6 | Evaluation of Renal Function by D-Serine and D-Asparagine

On the basis of these studies, we explored the potential of D-amino acids as markers for renal function evaluation, replacing creatinine and inulin. To assess the GFR, it is necessary to evaluate its clearance into urine. We measured the clearance of D-serine and D-asparagine in renal transplant donors and found that their clearance correlated strongly with inulin clearance. Additionally, these D-amino acids exhibited very similar dynamics to inulin [19, 20] (Figure 2a–c).

This strong correlation suggests that the clearance of D-serine and D-asparagine can be directly used to measure the GFR. Moreover, unlike creatinine clearance, the slope of D-serine and D-asparagine clearance closely matches that of measured inulin clearance. In other words, D-serine and D-asparagine clearance can mitigate slope bias in GFR measurement, which cannot be avoided when using creatinine clearance.

7 | Why D-Amino Acids Are Useful in Assessing Renal Function

The theoretical basis for why D-amino acids are useful for renal function assessment has been discussed. The three conditions mentioned above are essential for evaluating renal function.

7.1 | Condition 1: Glomerular Free Filtration

The molecular weights of D-serine and D-asparagine are 105 and 150, respectively, which are comparable to those of inulin and creatinine, conventionally used for measurement (inulin: 504, creatinine: 113). Therefore, it is inferred that almost all of these molecules pass freely through the glomerulus.

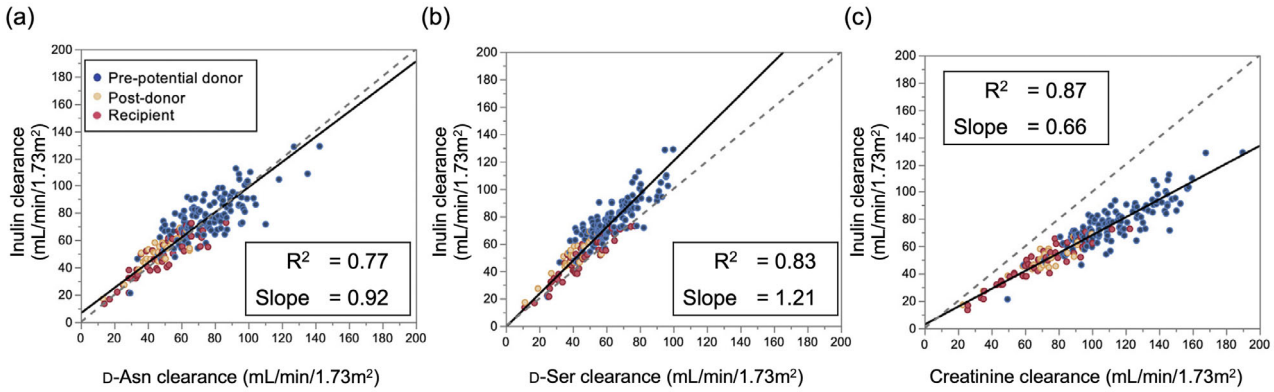

FIGURE 2 | GFR can be measured using D-amino acid clearance: (a) D-asparagine clearance, (b) D-serine clearance, and (c) creatinine clearance. *Source:* Adapted from *Kidney International Reports*, Copyright 2023, with permission from Elsevier Taniguchi et al. [19].

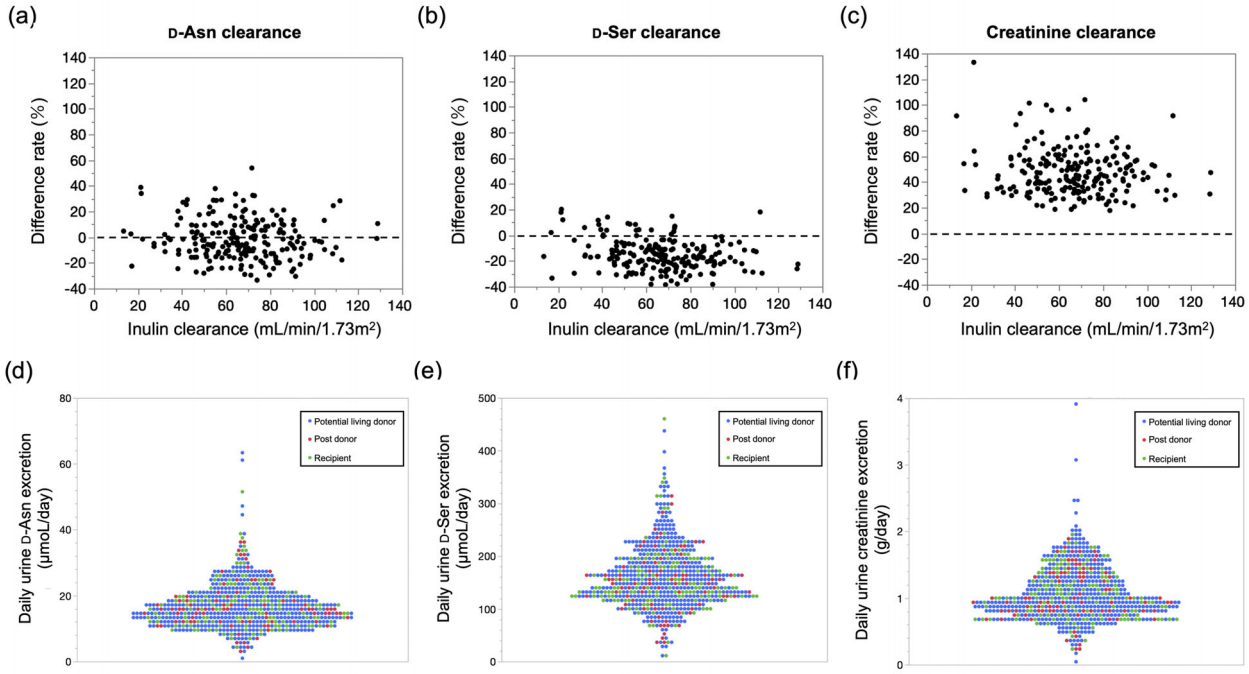

FIGURE 3 | Investigation of the in vivo dynamics of D-amino acid clearance. (a–c) Relationship between measurement errors of D-amino acids/creatinine clearance and inulin clearance, and renal function. difference rate: (clearance of each biomarker − inulin clearance)/inulin clearance: (a) D-asparagine clearance, (b) D-serine clearance, and (c) creatinine clearance. (d–f) Daily urinary excretion distribution of D-amino acids and creatinine: (d) D-asparagine, (e) D-serine, and (f) creatinine. *Source:* Adopted the image from *Nephrol Dial Transplant* Tanaka et al. [10], as the authors original version based on the Oxford University Press policy.

7.2 | Condition 2: No Tubular Reabsorption or Secretion

As mentioned above, the renal dynamics of D-serine and D-asparagine are expected to be very similar to those of inulin. In other words, D-serine and D-asparagine, like inulin, may undergo little tubular reabsorption or secretion, or reabsorption and secretion may be balanced. The similarity of the dynamics is demonstrated by calculating fractional excretion (FE), which is the ratio of the clearance of a substance to the clearance of a standard molecule, such as inulin, used to monitor the excretion ratio of the substance. Analysis of FE with inulin as a control showed that the dynamics of D-serine and D-asparagine were significantly closer to those of inulin than those of creatinine [19].

7.3 | Condition 3: Stable Dynamics Regardless of GFR

The difference between the clearance of D-serine and D-asparagine and that of inulin clearance does not vary with GFR (Figure 3a–c). A comparison of urinary excretion per unit time also revealed that interindividual differences are as small as those observed with creatinine (Figure 3d–f) [10]. In other words, their clearance remains consistent even when renal function deteriorates.

These characteristics indicate that D-serine and D-asparagine satisfy all three criteria and are valuable for renal function evaluation. Furthermore, D-serine and D-asparagine are endogenous factors and serve as useful molecules for assessing renal function.

8 | Summary and Outlook

We demonstrated the potential usefulness of D-amino acids as biomarkers of renal function. D-Serine and D-asparagine, as endogenous molecules, fulfill the requirements for biomarkers of renal function and GFR and are anticipated to see broader application in the future. Currently, our studies have primarily focused on renal transplant patients. However, we believe their utility as biomarkers of renal function across diverse patient profiles warrants further evaluation.

In particular, unlike creatinine, D-amino acids are less influenced by muscle mass, making them promising candidates for use as renal function biomarkers in pediatric patients, where this issue is especially challenging [18, 21].

Recently, D-alanine, a D-amino acid, has been identified as a biomarker for predicting the severity of influenza and COVID-19 infections [22, 23]. In the field of malignancy, D-asparagine, D-alanine, and D-proline have been reported as biomarkers for the development of bladder cancer [24].

Additionally, studies have shown that the ratio of D-amino acids in feces is lower in patients with ulcerative colitis. Moreover, in vivo D-amino acid supplementation has been demonstrated to improve colitis, hepatitis, and cholangitis in mice [25], suggesting its potential use as both a biomarker and a therapeutic target. Functionally, D-alanine can correct the circadian rhythm and its associated physiological processes, suggesting the potential to improve life style-related diseases [26]. Recent findings suggest that D-alanine plays a role in glucose metabolism and viral infection [27].

We believe that further elucidation of the dynamics of D-amino acids in the body, alongside research in the field of nephrology, will contribute to the accumulation of new knowledge and the development of treatments for a wide range of diseases currently affecting humanity.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

References

1. A. Francis, M. N. Harhay, A. C. M. Ong, et al., "Chronic Kidney Disease and the Global Public Health Agenda: An International Consensus," *Nature Reviews Nephrology* 20, no. 7 (2024): 473–485, https://doi.org/10.1038/s41581-024-00820-6.

2. M. Abe, "Latest Trends in Clinical Practice Guidelines Series: CKD Clinical Practice Guidelines 2023," *Commentary Nihon University Journal of Medicine* 82, no. 6 (2023): 319–324.

3. Japanese Society of Nephrology (Nihon Jinzo Gakkai). Japanese Society for Dialysis Therapy (Nihon Toseki Igakkai); Japan Society for Transplantation (Nihon Ishoku Gakkai); Japanese Society of Clinical Renal Transplantation (Nihon Rinsho Jin Ishoku Gakkai); Japanese Society for Peritoneal Dialysis (Nihon Fukumaku Toseki Igakkai). Strategies and Actual Conditions of Renal Failure Treatment. (2024).

4. T. K. Chen, D. H. Knicely, and M. E. Grams, "Chronic Kidney Disease Diagnosis and Management," *JAMA* 322, no. 13 (2019): 1294–1304, https://doi.org/10.1001/jama.2019.14745.

5. S. K. Swan, "The Search Continues—An Ideal Marker of GFR," *Clinical Chemistry* 43, no. 6 pt. 1 (1997): 913–914.

6. C. Maioli, M. Mangano, F. Conte, et al., "The Ideal Marker for Measuring GFR: What Are We Looking for?" *Acta Biomedica: Atenei Parmensis* 91, no. 4 (2020): e2020132, https://doi.org/10.23750/abm.v91i4.9304.

7. S. Matsuo, E. Imai, M. Horio, et al., "Revised Equations for Estimated GFR From Serum Creatinine in Japan," *American Journal of Kidney Diseases* 53, no. 6 (2009): 982–992, https://doi.org/10.1053/j.ajkd.2008.12.034.

8. H. A. Krebs, "Metabolism of Amino-Acids," *Biochemical Journal* 29, no. 7 (1935): 1620–1644.

9. T. Kimura, K. Hamase, Y. Miyoshi, et al., "Chiral Amino Acid Metabolomics for Novel Biomarker Screening in the Prognosis of Chronic Kidney Disease," *Scientific Reports* 6 (2016): 26137, https://doi.org/10.1038/srep26137.

10. R. Tanaka, S. Sakai, A. Taniguchi, et al., "Determination of Urine Volume and Glomerular Filtration Rate Using D-Serine and D-Asparagine," *Nephrology, Dialysis, Transplantation* (2024): gfae279, https://doi.org/10.1093/ndt/gfae279.

11. Y. Gonda, A. Matsuda, K. Adachi, et al., "Mammals Sustain Amino Acid Homochirality Against Chiral Conversion by Symbiotic Microbes," *Proceedings of the National Academy of Sciences of the United States of America* 120, no. 15 (2023): e2300817120, https://doi.org/10.1073/pnas.2300817120.

12. Y. Miyoshi, K. Hamase, Y. Tojo, M. Mita, R. Konno, and K. Zaitsu, "Determination of D-Serine and D-Alanine in the Tissues and Physiological Fluids of Mice With Various D-Amino-Acid Oxidase Activities Using Two-Dimensional High-Performance Liquid Chromatography With Fluorescence Detection," *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences* 877, no. 24 (2009): 2506–2512, https://doi.org/10.1016/j.jchromb.2009.06.028.

13. R. Koga, Y. Miyoshi, H. Sakaue, K. Hamase, and R. Konno, "Mouse D-Amino-Acid Oxidase: Distribution and Physiological Substrates," *Frontiers in Molecular Biosciences* 4 (2017): 82, https://doi.org/10.3389/fmolb.2017.00082.

14. T. Kimura, A. Hesaka, and Y. Isaka, "D-Amino Acids and Kidney Diseases," *Clinical and Experimental Nephrology* 24, no. 5 (2020): 404–410, https://doi.org/10.1007/s10157-020-01862-3.

15. Y. Hirakawa, T. Kimura, S. Sakai, et al., "Detection of Fast Decliner of Diabetic Kidney Disease Using Chiral Amino Acid Profiling: A Pilot Study," *Chemistry and Biodiversity* (2025): e202403332, https://doi.org/10.1002/cbdv.202403332.

16. Y. Iwata, H. Okushima, A. Hesaka, et al., "Identification of Diabetic Nephropathy in Patients Undergoing Kidney Biopsy Through Blood and Urinary Profiles of D-Serine," *Kidney360* 2, no. 11 (2021): 1734–1742, https://doi.org/10.34067/KID.0004282021.

17. H. Okushima, Y. Iwata, A. Hesaka, et al., "Intra-Body Dynamics of D-Serine Reflects the Origin of Kidney Diseases," *Clinical and Experimental Nephrology* 25, no. 8 (2021): 893–901, https://doi.org/10.1007/s10157-021-02052-5.

18. A. Hesaka, S. Sakai, K. Hamase, et al., "D-Serine Reflects Kidney Function and Diseases," *Scientific Reports* 9, no. 1 (2019): 5104, https://doi.org/10.1038/s41598-019-41608-0.

19. A. Taniguchi, M. Kawamura, S. Sakai, et al., "D-Asparagine Is an Ideal Endogenous Molecule for Measuring the Glomerular Filtration Rate," *Kidney International Reports* 8, no. 6 (2023): 1192–1200, https://doi.org/10.1016/j.ekir.2023.03.009.

20. M. Kawamura, A. Hesaka, A. Taniguchi, et al., "Measurement of Glomerular Filtration Rate Using Endogenous D-Serine Clearance in Living Kidney Transplant Donors and Recipients," *EClinicalMedicine* 43 (2022): 101223, https://doi.org/10.1016/j.eclinm.2021.101223.

21. T. Morishita, N. Nishizaki, S. Taniguchi, et al., "Plasma D-Asparagine and the D/L-Serine Ratio Reflect Chronic Kidney Diseases in Children Regardless of Physique," *Amino Acids* 56, no. 1 (2024): 38, https://doi.org/10.1007/s00726-024-03400-x.

22. S. Kimura-Ohba, M. N. Asaka, D. Utsumi, et al., "D-Alanine as a Biomarker and a Therapeutic Option for Severe Influenza Virus Infection and COVID-19," *Biochimica et Biophysica Acta: Molecular Basis of Disease* 1869, no. 1 (2023): 166584, https://doi.org/10.1016/j.bbadis.2022.166584.

23. S. Kimura-Ohba, Y. Takabatake, A. Takahashi, et al., "Blood Levels of D-Amino Acids Reflect the Clinical Course of COVID-19," *Biochemistry and Biophysics Reports* 34 (2023): 101452, https://doi.org/10.1016/j.bbrep.2023.101452.

24. A. Yamamoto, A. Kawashima, S. Sakai, et al., "Serum D-asparagine Concentration Adjusted for eGFR Could Serve as a Novel Screening Tool for Urothelial Carcinoma," *Biochemical and Biophysical Research Communications* 733 (2024): 150701, https://doi.org/10.1016/j.bbrc.2024.150701.

25. S. Umeda, T. Sujino, K. Miyamoto, et al., "D-Amino Acids Ameliorate Experimental Colitis and Cholangitis by Inhibiting Growth of Proteobacteria: Potential Therapeutic Role in Inflammatory Bowel Disease," *Cellular and Molecular Gastroenterology and Hepatology* 16 (2023): 1011–1031, https://doi.org/10.1016/j.jcmgh.2023.08.002.

26. S. Sakai, Y. Tanaka, Y. Tsukamoto, et al., "D-Alanine Affects the Circadian Clock to Regulate Glucose Metabolism in the Kidney," *Kidney360* 5, no. 2 (2024): 237–251, https://doi.org/10.34067/KID.0000000000000345.

27. T. Kimura, S. Sakai, and Y. Isaka, "D-Alanine, a Circadian Metabolite That Regulates Glucose Metabolism and Viral Infection," *ChemBioChem* 26: e202500018.