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Nutrition and metabolism in kidney diseases

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Nutrition and metabolism in kidney diseases

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Editorial: Nutrition and metabolism in kidney diseases

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Editorial on the Research Topic
Nutrition and metabolism in kidney diseases

Introduction

The impairment of kidney function, which occurs in chronic kidney disease (CKD) and acute kidney injury (AKI), promotes specific alterations in nutrient metabolism (1) and induces a pro-inflammatory state (2). These alterations affect the nutritional status of the patients and increase morbidity and mortality risk. Among the many factors that are associated with poor outcomes in this population, protein-energy wasting, malnutrition, and sarcopenia play a significant role.

Nutritional management in individuals with impaired kidney function varies depending on the disease severity, nutritional status, cause of disease, comorbidities, medications, and treatment methods. Therefore, understanding the available methods for assessing nutritional status, establishing dietary requirements, and strategies for preventing or treating potential nutritional derangements is essential for optimal care of patients with kidney diseases. This Research Topic focuses on recent studies exploring nutrition and metabolism in CKD.

Energy requirements and CKD

In individuals with CKD, energy requirements have traditionally been considered to be higher based on early nitrogen balance studies. However, more recent studies have questioned these higher recommendations (3–5). Indirect calorimetry is the gold standard for measuring resting energy expenditure (REE); however, its availability in clinical settings is limited. Ramos-Acevedo et al., performed a study in individuals with CKD stages 3–5 without kidney replacement therapy, incorporating measures of nutritional status and other clinical variables. They found good concordance between their models and others validated in CKD. The authors concluded that clinicians should consider using formulas that include nutritional status and other variables, such as weight, fat-free mass, comorbidities, sex, and age, to estimate energy requirements.

Similarly, Abi et al. utilized indirect calorimetry to measure REE in patients with stage 3–5 CKD and assess the relationship with the adipokines leptin, IL-6 and adiponectin in serum. In an initial analysis, the authors found that REE was positively correlated with leptin in males and females and negatively correlated with adiponectin in males only. However, when fat mass

was accounted for using a multivariate linear regression model, the only significant relationship observed was between REE and leptin in males. This study highlights the importance of considering the degree of adiposity when studying adipokines, as well as the possibility of a sex-specific relationship between adipokines and energy expenditure in CKD patients.

Plasma biomarkers in CKD

Cardiovascular mortality is increased in populations with CKD, and there is considerable interest in how commonly assessed lipid risk factors can be utilized to understand and modulate cardiovascular risk (6). In a large retrospective study of Chinese peritoneal dialysis (PD) patients, Wu et al. observed that there was a U-shaped relationship between LDL cholesterol levels and cardiovascular mortality. When a subgroup analysis was performed, this *U*-shaped relationship only remained significant in those with serum albumin of <36 g/L, used as a biomarker of malnutrition. The authors concluded that nutritional status modifies the relationship between LDL cholesterol and cardiovascular mortality in PD.

Lecamwasam et al. compared clinical and metabolomic data between patients with diabetes and early or late-stage CKD. In this study, the authors found no difference in LDL cholesterol between groups, although low-density lipoprotein triglyceride (LDL-TG) was increased in the late CKD group. The ratio between Apolipoprotein B1 and Apolipoprotein A1 (ApoB/ApoA1), a well-established risk factor for cardiovascular disease (7), was increased in the late CKD group, driven by a reduction in ApoA1. These findings suggest that apolipoproteins may be useful for assessing cardiovascular risk in high risk populations such as CKD patients.

Trimethylamine *N*-oxide (TMAO) is a uremic toxin which has been implicated in cardiovascular disease (8). Murray et al. fed pigs a highly heat-treated diet, high in resistant protein and found a profound alteration in the composition of the gut microbiota and changes in the plasma metabolome. They found that the resistant protein diet increased plasma TMAO and reduced plasma acetate, a beneficial short-chain fatty acid that is produced predominantly by the gut microbiota. This provides emerging evidence for the role of dietary resistant protein modulating the gut microbiota in the pathogenesis of cardiovascular disease in CKD.

Adverse body composition in CKD and interventions to improve it

Sarcopenia is highly prevalent in patients living with CKD (9), yet a reliable and simple means to assess it is lacking. The product of serum creatinine and the estimated glomerular filtration rate based on cystatin C was recently proposed as a sarcopenia index. Lin et al. evaluated the sarcopenia index in 297 patients with non-dialysis stage 3b-5 CKD. They found the sarcopenia index had acceptable discriminative ability to detect sarcopenia. As such, sarcopenia index could be used as a surrogate marker for sarcopenia and may be helpful for screening in advanced CKD.

Aside from measuring body composition, bioelectrical impedance analysis also provides the phase angle, a composite marker influenced by hydration and integrity of the body cell membrane (10). Reis et al. evaluated whether the phase angle could be used as a nutritional marker and predictor of mortality in PD patients. Their findings revealed an inverse correlation between the phase angle and coronary artery calcium score, a predictor of the incidence of acute myocardial infarction and death from cardiovascular disease.

Oral nutritional supplementation (ONS) is one of the interventions recommended to manage muscle wasting in patients on maintenance hemodialysis if diet alone does not provide sufficient energy and protein intake (11). In a randomized controlled trial of 56 patients with protein energy wasting, Sahathevan et al. showed improvement in muscle mass parameters assessed by ultrasound after a 6-month ONS compared to nutritional counseling. In a small open randomized pilot trial, González-Ortiz et al. showed both intradialytic or at home ONS improved not only nutritional parameters, such as body mass index and normalized protein nitrogen appearance, as well as sleep quality in 23 patients on maintenance hemodialysis.

Obesity is also a critical issue in the CKD population. Post-transplant increases in fat mass are usually associated with insulin resistance and cardiovascular risk factors in kidney transplant recipients (12). Another intervention approached in this Research Topic was a personalized digital health intervention, with the aim to prevent weight gain after kidney transplantation. Castle et al. showed this intervention was feasible and acceptable for recent kidney transplant recipients.

Malnutrition and diet management

Malnutrition may also be associated with kidney-related outcomes, such as increased susceptibility to AKI (13, 14). A study by Liang et al. observed that elderly patients with malnutrition, assessed by the Controlling Nutritional Status score (CONUT score), who underwent percutaneous coronary intervention, had a 2-fold increased adjusted risk of contrast-associated AKI, compared to those with no malnutrition.

Zinc is an essential micronutrient involved in numerous metabolic processes. AKI is associated with low plasma zinc (15), but outcomes with zinc supplementation in critically ill patients with AKI remain limited. Xia et al. investigated the effectiveness of zinc supplementation in 9811 patients with AKI. They found zinc supplementation was associated with improved survival in critically ill patients with AKI. Whilst further study is needed, this study highlights potential benefits of zinc supplementation in critically ill patients with AKI.

Diet management plays a crucial role in treating potential or ongoing nutritional deficiencies and derangements in patients undergoing hemodialysis (11). However, current dietary recommendations may not be culturally appropriate. In a qualitative study, Song et al. conducted semi-structured interviews with 23 patients in China on hemodialysis to explore their perceptions and attitudes toward diet. Findings showed diet behavior in Chinese patients undergoing hemodialysis is strongly influenced by culture. Culturally sensitive interventions regarding the improvement of diet intake are urgently needed.

Healthy lifestyle behaviors, including a healthy diet, weight control and physical activity promote the kidney health of children, reducing long-term kidney damage in adulthood (16). Physical activity may have an impact on hydration status and kidney health,

but the interaction of hydration status and physical activity level on kidney function is not well-studied in children. Li et al. explored associations of kidney damage with the interaction of hydration status and physical activity level in 1,914 primary school children from China. They found longitudinal interactions of hydration status and physical activity level on early kidney damage and found increased dehydration among the children over time. These results support the importance of adequate water intake, and suggest that children can be protected from early kidney damage by euhydration, either with sufficient or insufficient physical activity.

Perspectives

In summary, the studies included in the Research Topic on "*Nutrition and metabolism in kidney diseases*" enrich our understanding on a wide variety of topics, from nutritional assessment aspects, such as energy expenditure, body composition, bioelectrical impedance analysis parameters, and new and traditional biomarkers evaluation, to nutritional management of the main nutritional disorders in CKD and AKI, including malnutrition, sarcopenia, macro And micronutrients deficiency, and obesity. The articles included in this collection highlight future research directions, assist in the development of novel therapeutic approaches, and contribute to improvements in clinical practice.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Muscle Status Response to Oral Nutritional Supplementation in Hemodialysis Patients With Protein Energy Wasting: A Multi-Center Randomized, Open Label-Controlled Trial

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Background: Muscle wasting, observed in patients with end-stage kidney disease and protein energy wasting (PEW), is associated with increased mortality for those on hemodialysis (HD). Oral nutritional supplementation (ONS) and nutrition counseling (NC) are treatment options for PEW but research targeting muscle status, as an outcome metric, is limited.

Aim: We compared the effects of combined treatment (ONS + NC) vs. NC alone on muscle status and nutritional parameters in HD patients with PEW.

Methods: This multi-center randomized, open label-controlled trial, registered under ClinicalTrials.gov (Identifier no. NCT04789031), recruited 56 HD patients identified with PEW using the International Society of Renal Nutrition and Metabolism criteria. Patients were randomly allocated to intervention (ONS + NC, $n = 29$) and control (NC, $n = 27$) groups. The ONS + NC received commercial renal-specific ONS providing 475 kcal and 21.7 g of protein daily for 6 months. Both groups also received standard NC during the study period. Differences in quadriceps muscle status assessed using ultrasound (US) imaging, arm muscle area and circumference, bio-impedance spectroscopy (BIS), and handgrip strength (HGS) methods were analyzed using the generalized linear model for repeated measures.

Results: Muscle indices as per US metrics indicated significance ($p < 0.001$) for group × time interaction only in the ONS + NC group, with increases by 8.3 and 7.7% for quadriceps muscle thickness and 4.5% for cross-sectional area (all $p < 0.05$). This effect was not observed for arm muscle area and circumference, BIS metrics and HGS in both the groups. ONS + NC compared to NC demonstrated increased dry weight ($p = 0.039$), mid-thigh girth ($p = 0.004$), serum prealbumin ($p = 0.005$), normalized protein catabolic rate ($p = 0.025$), and dietary intakes ($p < 0.001$), along with lower malnutrition–inflammation score (MIS) ($p = 0.041$). At the end of the study, lesser patients in the ONS + NC group were diagnosed with PEW (24.1%, $p = 0.008$) as they had achieved dietary adequacy with ONS provision.

Conclusion: Combination of ONS with NC was effective in treating PEW and contributed to a gain in the muscle status as assessed by the US, suggesting that the treatment for PEW requires nutritional optimization *via* ONS.

Keywords: oral nutritional supplementation, nutrition counseling, hemodialysis, protein energy wasting, quadriceps muscle, ultrasound imaging

INTRODUCTION

Treating underlying muscle wasting in malnourished patients with chronic kidney disease (CKD) is challenging. The onset of muscle wasting establishes at the early stages of CKD, and the commencement of the dialysis treatment at end-stage kidney disease is an iatrogenic factor for malnutrition as it also promotes muscle proteolysis (1). The issue of muscle wasting is associated with protein energy wasting (PEW) in patients who undergo dialysis, a syndrome affecting 28–54% of patients worldwide (2). Increased risk for muscle wasting occurs in the presence of uremia, metabolic acidosis, inflammation, and insulin resistance, since each condition promotes muscle proteolysis (1). In patients who undergo dialysis, low muscle mass is associated with frailty, depression, malnutrition (3), and poor quality of life (4–6); and is a strong predictor of hospitalization and mortality (7, 8). The patients who undergo dialysis and having greater muscle mass achieve better physical ability, quality of life and survival (7, 9), indicating a priority to target treatment to mitigate muscle wasting.

Nutritional supplementation and exercise training are recommended to treat muscle wasting in patients with hemodialysis (HD) (6). However, the evidence of benefit for muscle status is inconclusive depending on treatment duration, feeding frequency, nutrient composition of supplementation, the severity of malnutrition, as well as assessment parameters (10, 11). Anomalies arise from malnutrition diagnosis that adopts body mass index (BMI) and serum albumin cut-offs, as muscle and fat compartments of the body are not differentiated, and albumin values are influenced by the presence of inflammation (12, 13). In contrast, although diagnosis with composite nutritional indices, such as subjective global assessment and malnutrition-inflammation score (MIS) (14–16) indicate that oral nutritional supplementation (ONS) improved the overall nutritional status, but muscle mass improvement could not be ascertained. In addition, anomalies in muscle mass assessment arise from sensitivity to detect change as per muscle indices (11) and site of measurement (17) when using skinfold measurements (15), handgrip strength (HGS) (18), and bio-impedance spectroscopy (BIS) (19).

Till now, ONS investigations addressing muscle wasting in patients with HD reflect either ONS use alone (15, 19, 20) or in combination with exercise (11, 18, 21). Studies on ONS intervention alone found no changes in lean body mass (LBM) (15, 19, 20). Exercise therapy alone did not find any improvement in LBM but was associated with improvements in the physical functioning and quality of life (18). In studies evaluating ONS combined with resistance exercise, no significant gains in LBM were apparent (18, 21). These studies investigating exercise alone or in combination with ONS, report no improvement in muscle mass with both treatments. Additionally, these studies recruited patients with mild to severe malnutrition defined by BMI, arm circumference, serum albumin, dietary intake, and subjective global assessment scores (15–17, 20), thus highlighting recruitment without PEW diagnostic criteria.

Assessment of ONS as a treatment strategy in patients with established PEW lacks in terms of the limitation of the methods to indicate improvement in muscle mass. These studies have not adopted direct measurement of muscle mass quantification of the lower limb muscle, which is sensitive to degradation from inflammation-related malnutrition (22). Additionally, there has been no standardization in diagnosing PEW. We addressed these gaps by purposively selecting only HD patients with PEW as the study population and provided them intervention in the form of ONS combined with NC or NC alone. The outcome measure resulted in the change in the muscle status as assessed using ultrasound (US) imaging as per the thickness of the quadriceps muscle and its cross-sectional area (23). PEW was identified in a HD population using the diagnostic criteria of the International Society of Renal Nutrtition and Metabolism (ISRNM) (14). The aims of this study therefore were (i) to assess muscle status changes in response to treatments using the US method and

(ii) to determine PEW prevalence post-intervention between the treatment groups.

MATERIALS AND METHODS

Study Design and Patient Recruitment

This multi-center, randomized open-label controlled trial was conducted between June 2016 and July 2019 with recruitment from 16 outpatient HD facilities representing government, private, and non-governmental organization sectors in the Klang Valley. Since serum prealbumin is a stable biomarker for protein synthesis in line with anabolism (24), the sample size calculation was therefore based on the study by Malgorzewicz et al. (16) who used serum prealbumin as an endpoint to ONS provision. In our calculation, using the mean difference in serum prealbumin at 0.57 ± 8.1 g/L, the effect size calculated at 0.70 (moderate effect) with power at 80% and the level of significance set at 5%, the minimum required sample size was 25 patients per treatment arm. Assuming a 20% dropout, the final sample size was inflated to 30 patients per arm.

Eligibility criteria included HD patients receiving standard dialysis treatment (3 sessions per week, 4 h per session) for $\geq$3 months, aged between 18 and 70 years old, and diagnosed with PEW using the ISRNM criteria (13). The PEW was identified when any 3 out of 4 ISRNM diagnostic criteria were met: BMI $<$23 kg/m^2, reduction $>$10% in MAMC related to the 50th percentile of the reference population, serum albumin $<$38 g/L, and dietary energy intake (DEI) $<$25 kcal/kg ideal body weight (IBW).

Patients with a history of poor adherence to HD treatment, prolonged hospitalization, or surgery in the past 3 months prior to recruitment, diagnosed with inflammatory diseases or malignancy, vegetarian, or on regular ONS were excluded.

The study was approved by the Medical Research and Ethics Committee, Ministry of Health, Malaysia (NMRR-16-2525-32068) and the Research Ethics Committee of National University of Malaysia (NN-081-2016). This trial was also registered on www.clinicaltrials.gov (NCT04789031).

Intervention and Control Groups

The treatments provided were ONS and nutrition counseling (NC). The selected ONS was a renal-specific product (Novasource^{TM} Renal; Nestle Health Science, Malaysia) providing 475 kcal and 21.7 g of protein per serving given on a daily basis. This was a ready-to-drink formula available as a 237 ml tetrabrik pack. The selected product fulfilled the criteria of been calorie and protein dense within a limited volume of supplement, which enables in achieving the necessary nutrient adequacy to improve the nutritional status as well as avoid overhydration in patients with HD. Patients were advised to consume the beverage 30 min after commencing their dialysis session on dialysis days and at home on non-dialysis days. The nutritional information of the product is provided in the **Supplementary Table S1**.

Nutrition counseling was provided to both the treatment groups by dietitians who counseled on achieving nutritional adequacies for energy and protein whilst limiting sodium, phosphate, potassium, and fluid intakes as per the Kidney Disease Outcomes Quality Initiative Guidelines (25). NC sessions were organized at baseline, third, and sixth months of the study as per standard healthcare protocol.

Recruited patients were randomized in a ratio of 1:1 to receive either ONS + NC or NC only. The NC only group served as the control. Block randomization was carried out at each study site using a computerized randomization calculator (Random Allocation Software Version 1.0) after the baseline data was collected. Randomization was performed by the study statistician (KC), who was not clinically involved in the trial. Both the groups were matched for age, gender, serum prealbumin, and BMI. Both the groups received treatment for 6 months.

Outcome Measures

Evaluation of nutritional outcomes was performed at baseline, third, and sixth months of the study and related to the following parameters:

Muscle Indices

Quadriceps muscles: The thickness of the mid-length (MID) of quadriceps muscle, *rectus femoris* (RF_{MID}) and *vastus intermedius* (VI_{MID}) muscles, and cross-sectional area (CSA) of the RF (RF_{CSA}) at the mid-thigh were assessed using a portable US imaging device (GE Logiq e Digital Portable Color Doppler, GE Healthcare, Wauwatosa, USA). Only one leg was consistently measured for all timed events, with selection for each patient dependent on either the dominant leg or leg without the vascular access. Standardized anatomical landmarking was performed at the MID site as per the International Society for Advancement of Kinanthropometry (ISAK) protocol (26) by an ISAK-trained anthropometrist (TK) as detailed previously (27). Two US scan readings were obtained for each measured site, and the mean value was used for data analysis. Researchers (SS and BHK) performed the US scan 2 h after the commencement of dialysis, with dialysis chairs adjusted for the supine position and both knees extended but relaxed. The same assessor performed all measurements for the same patient throughout the study. The intra- and inter-observer reliability for US measurements has been reported elsewhere (27).

Arm muscles: In order to determine the mid-arm muscle circumference (MAMC) and mid-arm muscle area (MAMA) (28), the skinfold thickness and the MAMC of triceps were measured according to the ISAK protocol on the dominant or non-fistula arm (26) using the Harpenden skinfold caliper (HSK-BI; British Indicators, West Sussex, UK) and a no-stretchable tape (Lufkin®, Apex Tool Group, LLC, NC, USA). All measurements were collected before the commencement of the dialysis by the same dietitian (SS) to minimize inter-observer variation.

Bio-impedance spectroscopy (BIS) analysis: Body composition was assessed using a portable whole-body BIS device (Body Composition Monitor, Fresenius Medical Care, Bad Homburg, Germany) before the dialysis session on a mid-week day, with the patient resting in the supine position.

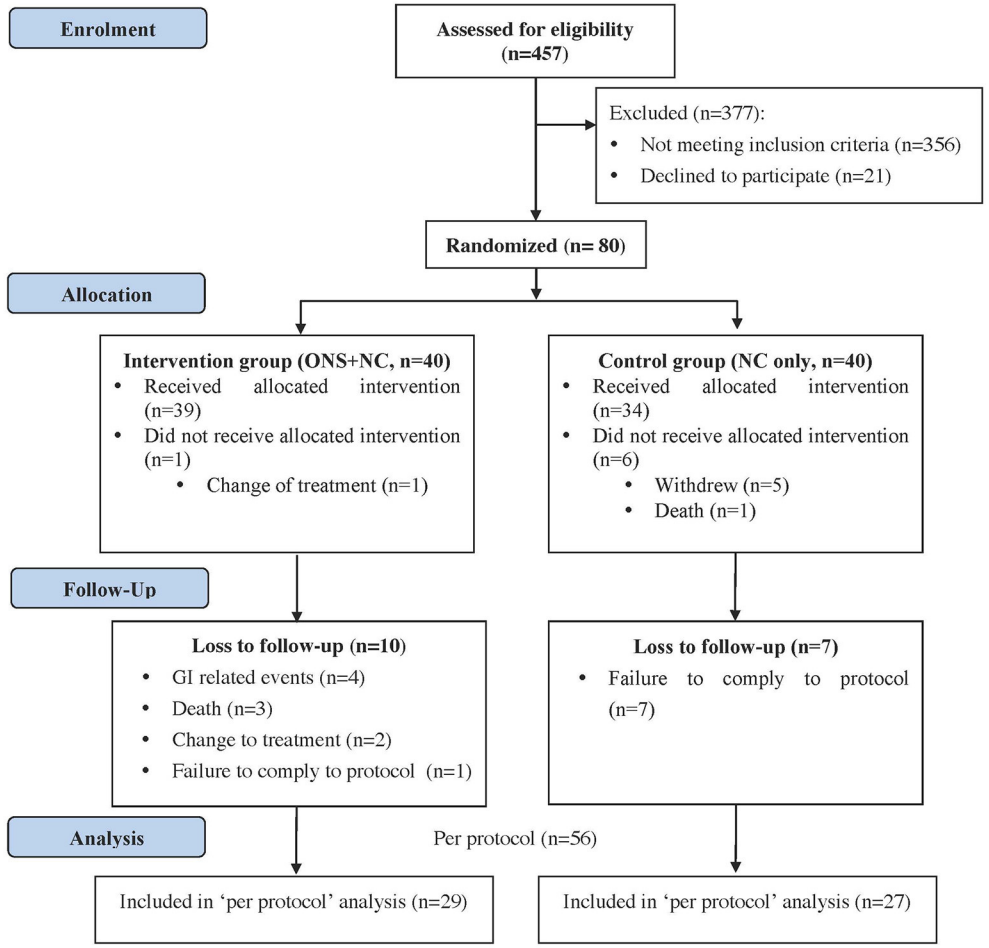

FIGURE 1 | Study flow diagram.

Hydration status, lean tissue mass (LTM), LTM corrected for height (lean tissue index, LTI), and body cell mass (BCM) data generated were based on the physiological tissue model (29).

Handgrip strength (HGS) test: HGS was assessed using a digital hand dynamometer (Jamar® Plus +, Sammons Preston, Illinois, USA) on the dominant or non-fistula hand, in a standing position with the arm held straight, at 90° to the trunk of the body (30). The median of the three readings was taken. All measurements were collected before the commencement of dialysis.

Diagnosis of Malnutrition

Malnutrition-inflammation score (MIS) evaluation: The MIS form was used to assess the severity of the malnutrition-inflammation complex syndrome (31). This fully quantitative nutrition screening tool assessed the domains of weight changes, dietary intake, gastrointestinal system, functional capacity, presence of comorbidities, presence of muscle, and fat depletion as well as BMI, serum albumin, and total iron-binding capacity. The cumulative score for MIS ranges between 0 (normal) and 30 (severely malnourished).

Other Nutritional Indicators

- Postdialysis weight was measured using a digital scale (SECA, Model 220, SECA, Germany). This weight was used to calculate the BMI based on the Quetelet's Index (32).
- The mid-thigh girth measurement was taken at the mid-point of the same leg as assessed for the quadriceps, following the ISAK protocol (26).
- Laboratory measures for serum albumin (bromocresol green method), serum prealbumin (immunoturbidimetric method), and high-sensitivity C-reactive protein (hsCRP) (particle-enhanced immunoturbidimetric assay) were analyzed by an accredited external laboratory (Clinipath Sdn Bhd). Interleukin-6 (IL-6) was measured by the sandwich enzyme-linked immunosorbent assay method in our laboratory (SSN and SS) using a commercial kit, IL-6 High Sensitivity Human ELISA kit (Abcam, Cambridge, MA, USA). All biochemistry analyses were based on the mid-week collection of fasting blood samples.
- The appetite of the patient was assessed using the first question from the original 44-item Appetite and Dietary Assessment Tool used in the Hemodialysis Study Group study (33). It

TABLE 1 | Baseline characteristics of study patients.

Patient characteristics	ONS + NC ($n = 29$)	NC ($n = 27$)	p-value
Age (years)[a]	50.90 ± 11.41	48.85 ± 15.97	0.582
Gender (male/female)	17/12	18/9	0.534
Dialysis vintage (months)	91 ± 85	61 ± 53	0.130
Charlson comorbidity index	4.17 ± 1.49	4.52 ± 2.01	0.465
Co-morbidities (n, %)[b]			
Diabetes	4 (13.8)	11 (40.7)	0.023
Hypertension	20 (69.0)	21 (77.8)	0.457
Hepatitis B or C	5 (17.2)	5 (18.5)	0.587
Cardiovascular disease	2 (6.9%)	3 (11.1%)	0.465
Kt/V	1.73 ± 0.46	1.76 ± 0.32	0.790
Vascular access (n, %)			
Fistula	24 (82.8%)	21 (77.8%)	0.636
Catheter	5 (17.2%)	6 (22.2%)	
Nutritional parameters			
BMI (kg/m^2)	19.85 ± 2.00	19.83 ± 2.49	0.984
MAMC (cm^2)	21.05 ± 2.32	21.14 ± 2.33	0.895
Serum albumin (g/L)	41.83 ± 3.71	41.85 ± 3.12	0.984
Serum prealbumin (g/L)	0.28 ± 0.09	0.26 ± 0.07	0.386
DEI (kcal/kg IBW)	25.21 ± 7.03	24.14 ± 6.01	0.546
MIS score	7.41 ± 2.77	6.19 ± 2.95	0.114
PAL (MET-minutes/week)	198 (0–487)	198 (0–396)	0.980
Hydration status (kg)	2.46	2.62	0.737

[a]*Continuous data were analyzed using Students t-test and presented as mean ± SD or median (interquartile).*

[b]*Categorical data were analyzed using Chi-square test and presented as frequency (percentage).*

BMI, body mass index; DEI, dietary energy intake; IBW, ideal body weight; MAMC, mid-arm muscle circumference; MIS, malnutrition-inflammation score; NC, nutrition counseling; PAL, physical activity level; Kt/V, dialysis adequacy; ONS, oral nutritional supplementation.

was a single, self-administered question with multiple-choice responses: *During the past week (7 days), how would you rate your appetite?* Patients were required to indicate their responses using a scale of 1–5: (1) very good, (2) good, (3) fair, (4) poor, or (5) very poor. Obtained ratings were further classified as "good" (*very good* and *good*) or "diminished" (*fair*, *poor*, and *very poor*) appetite.

- Physical activity level (PAL) was assessed using an interviewer-administered International Physical Activity Questionnaire (34), which included activities, such as walking, moderate- and vigorous-intensity activities. Scores were expressed in MET-min/week, whereby a minimum of 600 MET-min was identified as moderate-active.

Monitoring Parameters

Dietary assessment: Twenty-four-hour dietary recalls were collected for 3 days, inclusive of a dialysis day, a non-dialysis day, and a weekend (25). This was an interviewer-administered questionnaire with familiarization of household measurements to assist patients in quantifying their dietary intake. Energy and protein intakes were analyzed using Nutritionist Pro^{TM} 2.2.16 software (First DataBank Inc., 2004).

Clinical parameters: Normalized protein catabolic rate (nPCR), an indirect measure of dietary protein intake (35), was calculated by the participating dialysis centers using an online urea kinetic modeling calculator (36). Dialysis adequacy (Kt/V) was also calculated using the same approach (36). Levels of routine biochemistry parameters, analyzed in-house, such as serum urea, creatinine, and phosphate were obtained from the medical record of the patients.

Compliance and Product Acceptance

Dialysis nurses at respective study sites ensured that the patients ingested the supplement in their presence on dialysis days. Patients receiving ONS were replenished with a biweekly supply of supplements for home consumption on non-dialysis days. Empty tetrabrik packs or unused supplements were collected biweekly by the researcher to record and monitor the actual intake of ONS. Patients with consecutive ONS intakes <50% during the first 3 months of supplementation were classified as non-compliant.

Patients also fulfilled a product acceptance form (32, 37) on a three-monthly basis. Using a 5-point *Likert* scale, patients evaluated the ONS product based on taste, odor, and portion provided, and rated their overall liking toward the product.

Charlson Comorbidity Index

The Charlson Comorbidity Index was computed using 19 comorbid conditions, which were weighted and summed to an index on a 0–33 scale (38).

Statistical Analysis

"Per protocol" analysis was used to exclude patients who either withdrew their participation from the study or those who were non-compliant. Variables were presented as mean ± SD, median (interquartile) or frequency (percentage). The normal distribution of continuous variables was assessed using Kolmogorov–Smirnov test. Continuous variables were analyzed using Student's t-test, whereas categorical variables were evaluated using Chi-square test. The group × time interaction on all outcome measures were analyzed using a generalized linear model for repeated measures, with Bonferroni *post-hoc* test. Univariate analysis was used to compare percentage change between groups. All analyses were computed using the IBM Statistical Package for Social Sciences version 26.0 (IBM SPSS Statistics Inc. Chicago IL. USA). Statistical significance was set at $p < 0.05$ for all evaluated parameters.

RESULTS

Study Stock Flow

Out of 101 eligible HD patients identified with PEW, only 80 patients consented to participate in this study. Upon randomization, 40 patients were allocated to each treatment arm. The stock flow of patients according to the Consort diagram is presented in **Figure 1**.

Study withdrawals after randomization and consent giving occurred at baseline from patients withdrawing consent

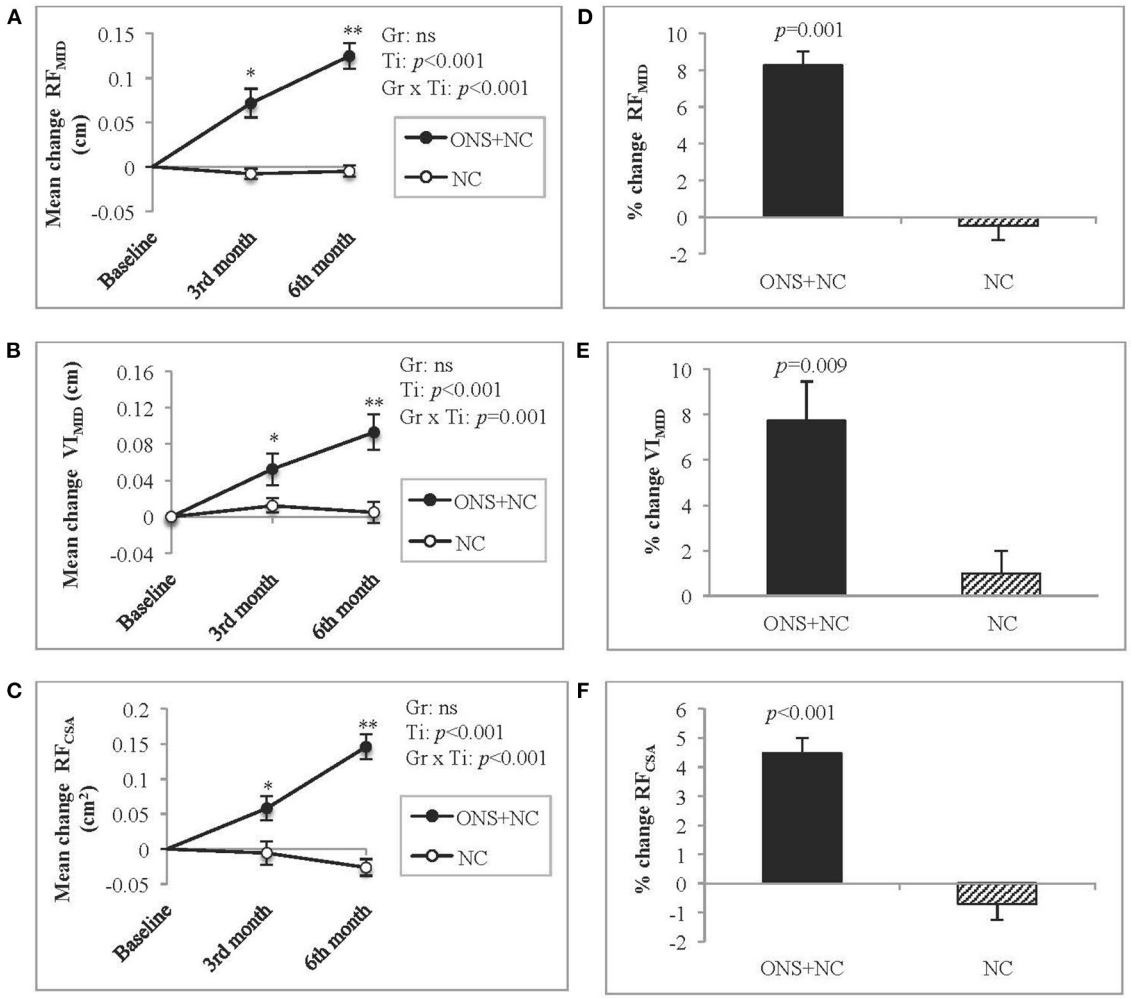

FIGURE 2 | Changes in US-derived muscle metrics. **(A–C)** represent mean changes according to US metrics for **(A)** RF_{MID}, **(B)** VI_{MID}, and **(C)** RF_{CSA}, whereas **(D–F)** represent the percentage change for **(D)** RF_{MID}, **(E)** VI_{MID}, and **(F)** RF_{CSA}. Gr, main effect of Group; Ti, main effect of Time, Gr × Ti, Group × Time interaction; $^{*}p < 0.05$; $^{**}p < 0.001$; Data were adjusted for age, gender, dialysis vintage, and presence of diabetes mellitus. CSA, cross-sectional area; MID, mid-point; NC, nutrition counseling; ns, not significant; ONS, oral nutritional supplementation; RF; *rectus femoris*; US; ultrasound; VI, *vastus intermedius*.

(NC, $n = 5$) or who became eligible for transplant (ONS + NC, $n = 1$). One death from a cardiac event (NC) occurred before the initiation of the study. The dropout rate at the end of the intervention was 30%.

Baseline Characteristics of Patients

Baseline characteristics of patients completing the protocol are shown in **Table 1**. Age, gender, dialysis vintage, the Charlson comorbidity index, comorbidities, dialysis adequacy, and type of vascular access were not significantly different between groups except for the presence of diabetes mellitus ($p = 0.023$). Similarly, no difference in nutritional status and PAL was observed between both groups.

Muscle Status

Ultrasound-Derived Muscle Metrics

Mean changes according to treatment response (group × time interactions) for US-derived muscle metrics are provided in the **Supplementary Table S2**. The group × time interactions were significant for RF_{MID}, VI_{MID}, and RF_{CSA} (**Figures 2A–C**). Increasing trends in all US metrics observed only in the ONS + NC group, were significant for mean changes from baseline to 3rd and 6th months of the study. The NC group experienced no change to these metrics. ONS + NC patients also experienced significant increases of 8.3% for RF_{MID} ($p = 0.001$), 7.7% for VI_{MID} ($p = 0.009$), and 4.5% for RF_{CSA} ($p < 0.001$) compared to minimal changes in these metrics in the NC group (**Figures 2D–F**).

TABLE 2 | Changes in other muscle indices according to treatment groups.

	ONS + NC ($n = 29$)					NC ($n = 27$)				
	Baseline	**3rd month**	**6th month**	**Δ^{t3}**	**Δ^{t6}**	**Baseline**	**3rd month**	**6th month**	**Δ^{t3}**	**Δ^{t6}**
Arm circumference										
MAMC (cm^2)[b]	20.4 ± 0.5	20.9 ± 0.5	20.8 ± 0.5	0.5 ± 0.1**	0.4 ± 0.2	20.6 ± 0.4	20.9 ± 0.4	20.8 ± 0.3	0.3 ± 0.2	0.2 ± 0.3
MAMA (cm^2)[b]	24.9 ± 1.5	26.8 ± 1.5	26.4 ± 1.6	1.9 ± 0.4**	1.4 ± 0.6	25.9 ± 1.2	26.8 ± 1.2	26.2 ± 1.1	1.0 ± 0.6	0.3 ± 0.9
Body composition										
LTM (kg)	29.2 ± 1.3	29.5 ± 1.3	29.4 ± 1.3	0.3 ± 0.3	0.2 ± 0.4	28.6 ± 1.0	28.6 ± 0.9	28.5 ± 1.0	−0.1 ± 0.2	−0.1 ± 0.3
LTI (kg/m^2)	11.4 ± 0.5	11.7 ± 0.5	11.6 ± 0.5	0.3 ± 0.2	0.1 ± 0.2	11.7 ± 0.4	11.8 ± 0.4	11.8 ± 0.4	0.1 ± 0.1	0.2 ± 0.2
BCM (kg)	15.3 ± 1.0	15.5 ± 1.0	15.4 ± 1.0	0.3 ± 0.3	0.1 ± 0.4	15.7 ± 0.8	15.9 ± 0.7	15.9 ± 0.7	0.2 ± 0.3	0.2 ± 0.4
Muscle strength										
HGS (kg)	19.0 ± 1.3	19.1 ± 1.3	19.6 ± 1.4	0.1 ± 0.4	0.6 ± 0.4	18.3 ± 0.8	18.6 ± 0.9	19.1 ± 0.9	0.3 ± 0.4	0.8 ± 0.4

*All baseline comparisons between group were not significantly different as per Independent t test; Data adjusted for age, gender, dialysis vintage, and presence of diabetes mellitus are presented as mean ± SD; [a]Main effect of Group, [b]Main effect of Time, [c]Group × Time interaction; Δ^{t3}, Mean change at 3rd month; Δ^{t6}, Mean change at 6th month; *$p < 0.05$ compared to baseline, **$p < 0.001$ compared to baseline.*

BCM, body cell mass; HGS, handgrip strength; LTI, lean tissue index; LTM, lean tissue mass; MAMA, mid-arm muscle area; MAMC, mid-arm muscle circumference; NC, nutrition counseling; ONS, oral nutritional supplementation.

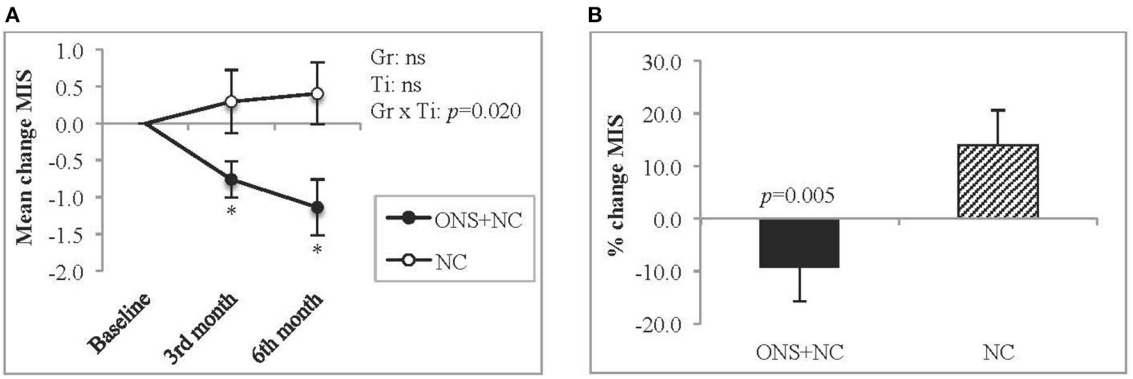

FIGURE 3 | Changes in MIS score. This figure represents **(A)** mean changes and **(B)** percentage change for MIS score. Gr, main effect of Group; Ti, main effect of Time; Gr × Ti, Group × Time interaction; *$p < 0.05$; Data were adjusted for age, gender, dialysis vintage, and presence of diabetes mellitus. MIS, malnutrition-inflammation score; NC, nutrition counseling; ns, not significant; ONS, oral nutritional supplementation.

Other Muscle Indices

Treatment responses for other muscle indices are shown in **Table 2**. Group × time interactions were not significant for MAMC, MAMA, BIS-derived muscle measures, and HGS (all $p > 0.05$). However, a significant increase over time was detected at the 3rd month for ONS + NC treatment as per MAMC ($p < 0.001$) and MAMA ($p < 0.001$), but this effect was not apparent at the 6th month. The NC treatment effected no change for these measures.

Malnutrition Diagnosis

MIS Evaluation

The group × time interactions for MIS was significant, with declining trends in ONS + NC groups at both the 3rd ($p = 0.032$) and 6th months ($p = 0.041$) of the study (**Figure 3A**). In contrast, the NC group experienced no improvement in MIS scores. The percentage change for MIS score in ONS + NC group was significant (−9.4 %, $p = 0.005$) compared to 14% increase in NC group (**Figure 3B**).

PEW Status

Eligibility criteria for PEW diagnosis met by patients were mainly reduced for the BMI (100%), MAMC (ONS + NC = 89.7% vs. NC = 88.9%), and DEI criteria (ONS + NC = 62.1% vs. NC = 77.8%) (all $p > 0.05$) (**Table 3**). Only a small percentage of patients met the low serum albumin criteria (ONS + NC = 20.7% vs. NC = 18.5%) ($p > 0.05$). By the end of the 6-month treatment, a change in PEW eligibility criteria only occurred as per the DEI criteria with patient numbers reducing in the ONS + NC group compared to the NC group (ONS + NC = 24.1% vs. NC = 70.4%, $p = 0.001$). This resulted in lower PEW prevalence with ONS + NC compared to NC treatment (24.1 vs. 59.3%, $p = 0.008$).

Other Nutritional Outcomes

Treatment response (group × time interactions) for other nutritional parameters are provided in the

TABLE 3 | Effect of treatment on PEW criteria status.

PEW criteria[a,b]	Baseline		*p*-value	6th month		*p*-value
	ONS + NC (*n* = 29)	NC (*n* = 27)		ONS + NC (*n* = 29)	NC (*n* = 27)	
BMI <23 kg/m^2	29 (100%)	27 (100%)	NA	26 (89.7%)	24 (88.9%)	0.630
MAMC >10th percentile	26 (89.7%)	24 (88.9%)	1.000	24 (82.8%)	22 (81.5%)	0.587
Serum albumin <38 g/L	6 (20.7%)	5 (18.5%)	0.838	5 (17.2%)	7 (25.9%)	0.429
DEI <25 kcal/kg IBW	18 (62.1%)	21 (77.8%)	0.201	7 (24.1%)	19 (70.4%)	0.001

[a]*Categorical data were presented as frequency (percentage).*

[b]*Data was analyzed using Chi-square test.*

BMI, body mass index; DEI, dietary energy intake; IBW, ideal body weight; MAMC, mid-arm muscle circumference; NA, not available; NC, nutrition counseling; ONS, oral nutritional supplementation; PEW, protein energy wasting.

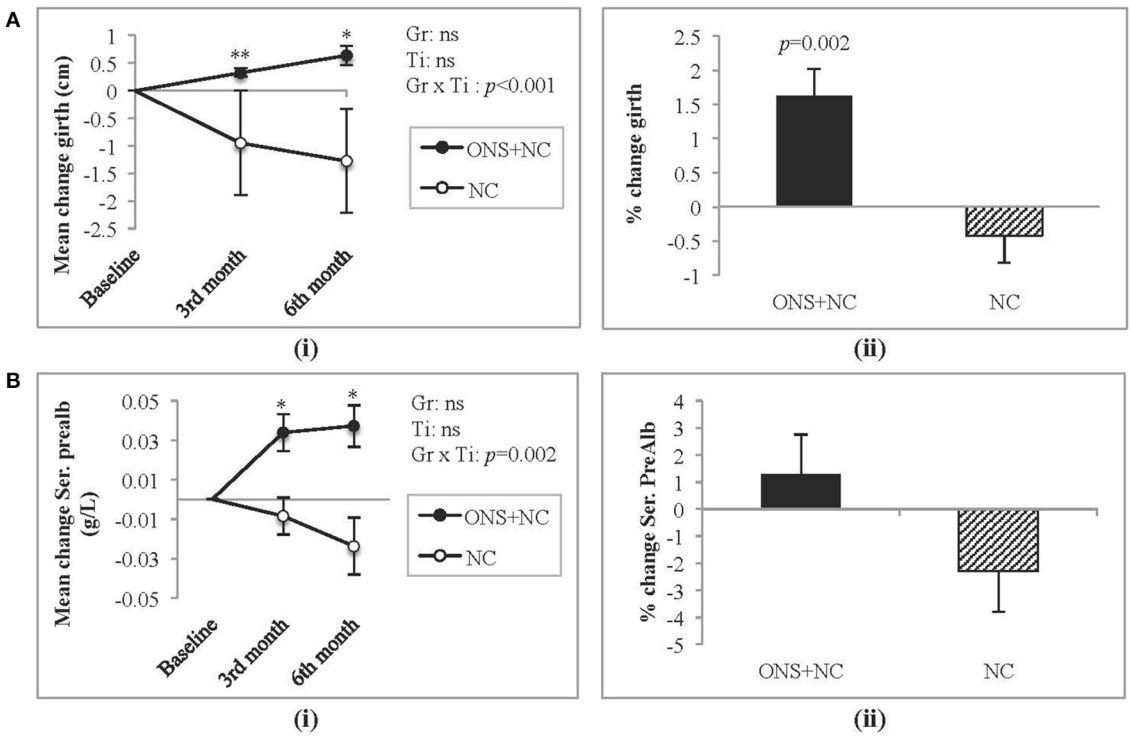

FIGURE 4 | Changes in mid-thigh girth and serum prealbumin levels. **(A)** Represents (i) mean change and (ii) percentage change in mid-thigh girth, whereas **(B)** represents (i) mean change and (ii) percentage change in serum prealbumin. Gr, main effect of Group; Ti, main effect of Time; Gr × Ti, Group × Time interaction; *$p <$ 0.05; **$p <$ 0.001; Data were adjusted for age, gender, dialysis vintage, and presence of diabetes mellitus. NC, nutrition counseling; ns, not significant; ONS, oral nutritional supplementation; Prealb, prealbumin.

Supplementary Table S3. Parameters that were not significantly different between and within treatment groups were BMI, serum albumin, creatinine, phosphate, hsCRP, IL-6, appetite ratings, and PAL. Positive improvements, however, were gained only by the ONC + NC group at 6 months for dry weight (mean change = 1.1 ± 0.4 kg, $p = 0.039$). For this group, specific significant increases in mid-thigh girth (**Figure 4A**) and prealbumin (**Figure 4B**) occurred with each time point, resulting in a significant percentage change increase of approximately 2% only for mid-thigh girth. Adequacy with ONS supplementation reflected in improved *n*PCR (mean change = 0.2 ± 0.1 g/kg, $p = 0.025$) and dietary parameters (mean change for energy intake = 366 ± 60 kcal/day, $p < 0.001$; mean change for protein intake =17.4 ± 3.2 g/day, $p < 0.001$).

Product Monitoring and Acceptance

There was no change in the hydration status or hospitalization frequency over the 6 months of intervention (data not shown). The average compliance rate achieved by patients receiving ONS was 81%. Product acceptance in relation to taste, flavor, and portion size was reported at 90% with minimal (<10%) reporting

of dislike toward the odor of the product, satiety, and adverse events for the ONS group.

DISCUSSION

Treatment strategies toward reducing the progression of muscle wasting are challenging given the complex etiology of PEW, and the current lack of evidence to support ONS or exercise or both options as beneficial in mitigating muscle wasting. The major finding from our study, which recruited only patients with PEW, was that those receiving ONS with nutrition counseling (ONS + NC) demonstrated significant improvement in quadriceps muscle indices, namely RF_{MID}, VI_{MID}, and RF_{CSA}, as measured by the US approach. Additionally, these patients demonstrated improvements in the nutritional status and lower MIS scores with concomitant gains in dry weight, mid-thigh girth, serum prealbumin and *n*PCR. These improvements were not observed in the group receiving only NC.

We detected improvements in muscle status in response to ONS treatment using the US approach. The clinical significance of our data indicates 8.3, 7.7, and 4.5% improvements in RF_{MID}, VI_{MID}, and RF_{CSA}, respectively. In contrast, only one study reported a 4.2% increase in arm muscle circumference in patients with HD supplemented with ONS for 12 weeks (21). We do note that studies reporting US measure for various clinical outcomes in different populations. The thickness of the lower quadriceps muscle predicted fall injury (39) and PEW risk (40) in HD patients. The thickness of the lower quadriceps muscle was also associated with prolonged hospitalization in critically ill patients (41), whereas lower RF_{CSA} had been associated with hospital readmission or death in patients with chronic respiratory disease (42). The advantage of US is that it directly quantifies muscle thickness and CSA (43), allowing for the detection of small changes in muscle status attributed to nutritional intervention (43). Importantly, overhydration status is not an issue for US measurements as consistency of readings for pre- and post-dialysis is reported (44). The usability and low-cost US for muscle status assessment contrasts with gold standard methods, such as dual energy X-ray absorptiometry, magnetic resonance imaging, or computed tomography, which although having high accuracy and validity are not feasible for routine clinical use as they require trained personnel, are costly, and also pose radiation risk to patients with CKD (4, 45, 46). This further justifies the use of the US as an alternative bedside measure as it has been validated against computed tomography (24) to detect muscle wasting in HD patients (40). Interestingly, the NC group did not demonstrate any significant improvement in US measures as experienced by the group receiving ONS. Of note, the absence of deterioration in their muscle status could be attributed to the provision of nutrition counseling.

No change in muscle indices was observed with BIS or HGS assessments as comparator assessments. It should be noted that BIS only provides mathematical estimates of muscle mass (19, 29) and its precision in estimating LTM is affected by hydration status (47). Ideally, the BIS assessment should be performed post-dialysis, as patients are closest to their dry weight (47, 48). This may perhaps explain the lack of significance we observed as the BIS measurement was performed pre-dialysis to suit the convenience of the patients. As regards the non-significant outcomes of HGS measurement reported in this study, weakness arising from poor physical activity, a common scenario in the HD population may contribute to "muscle disuse" (17). Indeed, in an earlier cross-sectional study of US measurement in Malaysia, we noted that there was no difference in HGS between PEW and non-PEW HD patients (40).

We additionally provided MIS evaluation in the assessment monitoring protocol, as the complex milieu of malnutrition-inflammation is implicated in muscle wasting. Malnutrition coexists with inflammation in dialysis patients (31), and inflammation is a contributive factor to malnutrition and poor appetite (2, 49). We found the patients receiving ONS + NC compared to the NC group achieved significantly lower MIS scores by the end of 6 months in tandem with an improvement in nutritional status, although inflammatory markers were not different after treatments. Ko et al. (50) have noted low levels of leptin, an appetite-suppressing hormone associated with proinflammatory properties and that high CRP levels were associated with malnourished patients with HD, who were identified using MIS scores. Comparatively, patients in the present study had lower CRP levels, which is similar to a Japanese HD cohort in the Phase 3 *Dialysis Outcomes and Practice Pattern Study* (51). There is no strong evidence supporting the improvement of inflammation status *via* nutritional intervention (52, 53). Alternatively, treatment strategies targeted at improving dialysis-induced inflammation factors or anti-cytokine therapies could be explored (52).

Prealbumin, a negative acute-phase protein is a biomarker sensitive toward rapid changes in nutritional status (14, 54) due to its shorter half-life compared to serum albumin (∼2–3 vs. 20 days). Prealbumin is commonly used in nutritional interventions to indicate response toward treatment (55). In our study, the patients on ONS did achieve a significant increase in prealbumin levels as expected from nutritional interventions (14, 35). We did note a non-significant increase in serum albumin levels in the ONS + NC group, which concurs with other ONS studies (13, 15, 56). However, the magnitude of change in albumin status depends on the duration of ONS feeding (10), severity of hypoalbuminemia (56, 57), chronic inflammation (58) and hydration status (59, 60), not withstanding the pro-inflammatory nature of dialysis treatment (61).

We used the PEW-ISRNM diagnostic criteria to identify PEW (13), as it requires objective assessments of muscle wasting. Combination treatment of ONS with NC was beneficial in patients with PEW, as indicated by a significant decline in PEW prevalence at the end of the 6th month. This effect concurs with other studies treating general malnutrition in HD patients (15, 37, 62). Interventional approaches to treat PEW diagnosed by the ISRNM criteria applied by other researchers indicated some limitations to interpretations. Enrolment of both PEW and non-PEW patients occurred with one study (21), another study failed to report the remission of PEW post-treatment (24), whereas the 3rd study was underpowered (total $n = 16$) and targeted only elderly HD patients (18). Further, whether exercise alone in

comparison to combination treatments for HD patients are valid strategies that remain inconclusive, as study design limitations, such as inclusion of young and well-nourished patients (11), suboptimal intensity and duration of exercise (63), small sample size (11, 18) and sensitivity of method in assessing muscle mass, are noted (11). Additionally, aiming for dietary energy sufficiency was not planned in these strategies (63).

In terms of the PEW remission associated with ONS intervention that we reported, more patients achieved dietary adequacy compared to NC alone as per DEI >25 kcal/kg IBW (38.0 vs. 7.4%, $p = 0.001$). This indicated that ONS treatment was able to optimize dietary adequacy in malnourished HD patients, thereby fulfilling the study objective. However, we note that the nutritional composition of ONS does differ as reported by various studies, depending on the objectives of the outcome. We ourselves found that a protein only supplementation did not correct for energy deficiency in malnourished peritoneal dialysis patients (64). Supplementation studies in HD patients do concur achieving dietary adequacy for both energy and protein intake were met *via* ONS (11, 21, 65) but not with protein only supplementation (66, 67). Other associated markers of nutritional status that improved with ONS intervention were significant gains in dry weight, mid-thigh girth, serum prealbumin, and *n*PCR levels, which align with dietary adequacy. Nutritional adequacy promotes positive nitrogen balance thus minimizing the catabolic impact of PEW *via* gluconeogenesis (2, 68). In a secondary analysis looking at protein kinetics, Gamboa et al. (69) reported that well-nourished HD patients receiving ONS achieved positive amino acid balance based on increases in their net protein balance in the forearm skeletal muscle.

Poor compliance is a common issue affecting successful ONS intervention (70, 71). Poor compliance (<70%) reported in previous studies were related to taste perceptions, presence of adverse events, and fear of overhydration (70, 71). The dropout rate of 30% in the present study was similar to 31.8% reported by Caglar et al. (70). We avoided the risk of poor compliance in our study by prestesting available renal-specific ONS products in HD patients ($n = 10$) outside the recruitment of this study. This approach was also reported by Patel et al. (56). Additionally, early satiety with ONS intake and reduced dietary intake (37) were avoided by allowing for flexible consumption of ONS between main meals and before going to bed, which allowed patient nutritional intakes to achieve adequacy (37, 71).

The novel finding from this study is improvement in muscle changes in response to ONS treatment, which was detected by the US method, answering the gap in literature for an appropriate impact measure to detect response. Other strengths were using the ISRNM-PEW diagnosis to standardize patient selection criteria, assessing the presence of malnutrition-inflammation complex syndrome as per MIS score, a longer duration of supplementation, and adequate ONS dose. These factors are known to influence the efficacy of ONS in improving the muscle status of HD patients (10, 11, 15). The study methodology adopted only a single leg to measure, so as to minimize technical error of measurement that are likely to happen in the intervention studies.

A major limitation of this study was the unequal distribution of patients with diabetes mellitus between treatment groups. Although the data were adjusted for the presence of diabetes mellitus, it should be noted that diabetes mellitus is the main cause of CKD (71) and insulin resistance is contributive to muscle wasting in HD patients (1, 72). The 6-month study duration was insufficient to measure the impact of muscle change on clinical endpoints as regards to infection rates, hospitalization, and mortality. Furthermore, taste fatigue, a common issue when patients are consuming ONS daily for a prolonged period may have hindered a greater compliance (73, 74) despite the 81% achievement in the current study.

In conclusion, gains in quadriceps muscle status detected using the US approach in patients with PEW on HD were attributed to dietary optimization *via* the ONS provision. A significant reduction in PEW prevalence occurred with the ONS intervention by patients achieving dietary adequacy. Nutritional interventions for the treatment of muscle wasting associated with malnutrition should consider the US approach to monitor the outcomes for clinical relevance.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Research and Ethics Committee, Ministry of Health, Malaysia (NMRR-16-2525-32068) and the Research Ethics Committee of National University of Malaysia (NN-081-2016). This trial was also registered on www.clinicaltrials.gov (NCT04789031). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SS and TK designed the study. SS was the main author of the manuscript, performed all the assessment of nutritional outcomes, analyzed, and interpreted data. TK supervised the project. B-HK and BKSS assisted in performing the nutritional assessments. KC assisted with the statistical analysis. SS, TK, B-HK, ZAMD, EF, AS, AHAG, SB, RY, RV, ZW, B-LG, ZM, BCB, and HSW and assisted in the interpretation of the results and writing the manuscript. All authors contributed to the article and approved the submitted manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.743324/full#supplementary-material

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Implications of Malnutrition on Contrast-Associated Acute Kidney Injury in Young and Old Patients Undergoing Percutaneous Coronary Intervention: A Multicenter Prospective Cohort

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Background: The relationship between malnutrition and the risk of contrast-associated acute kidney injury (CA-AKI) and the resulting prognosis in patients undergoing percutaneous coronary intervention (PCI) is still not well known.

Methods: Patients undergoing PCI were consecutively enrolled in a multicenter study in China (NCT01402232), categorized by nutritional status (non-malnutrition, malnutrition) based on two different cut-off values (i.e., traditional threshold and the best cut-off value based on the receiver operating characteristic (ROC) curve) for the controlling nutritional status (CONUT) score. The primary endpoint was CA-AKI, diagnosed as a rise in serum creatinine >0.3 mg/dl or >50% than the baseline level occurring within 48 h after the intervention. The secondary endpoint was all-cause mortality. The relationships of malnutrition, CA-AKI, and all-cause mortality were examined using multivariate-adjusted logistic and Cox regression analyses, respectively.

Results: Among 2,083 patients undergoing PCI (age: 62.8 ± 11.1 years; 79.0% men), 1,258 (60.4%) were malnourished. During hospitalization, 80 (3.8%) patients developed CA-AKI events. The incidence of CA-AKI in patients who did not have malnutrition (the non-malnutrition group) and those who did have malnutrition (the malnutrition group) was 1.7% and 5.25%, respectively. Patients with malnutrition had a 2-fold increased adjusted risk of CA-AKI compared to those with no malnutrition [adjusted odds ratio (aOR) (95% confidence interval CI): 2.41 (1.22 to 5.22)]. Malnutrition was associated with a 3-fold increased adjusted risk of CA-AKI in patients aged ≤75 years [*N* = 1,791, aOR (95% CI): 3.39 (1.46–9.25)]. Malnourished patients with CA-AKI had a higher risk of all-cause mortality than the others. Similar results were observed in the grouping of **Supplemental Analyses** based on the optimal cut-off value of the CONUT score identified by the ROC curve.

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Conclusions: Malnutrition is strongly associated with an increased risk of CA-AKI in both young and old patients undergoing PCI. Malnourished patients with CA-AKI had a significantly higher risk of all-cause mortality. Further studies are needed to prospectively assess the efficacy of nutritional interventions on outcomes in patients undergoing PCI.

Keywords: malnutrition, percutaneous coronary intervention, contrast-associated acute kidney injury, the controlling nutritional status score, 1-year mortality

INTRODUCTION

Contrast-associated acute kidney injury (CA-AKI) is a major complication of percutaneous coronary intervention (PCI) and is associated with poor prognosis (1, 2). The incidence of CA-AKI in patients undergoing PCI ranged from 6 to 18% (3–5). Due to limited CA-AKI treatment strategies, early screening and preventive measures for this high-risk population are essential.

Malnutrition is strongly associated with oxidative stress and the inflammatory process (6, 7). CA-AKI is highly related to neutrophils and albumin, which are well-known biomarkers of inflammation (8, 9), and is also affected by malnutrition. Protein-caloric malnutrition is related to kidney hemodynamic changes, the reduction of renal blood flow, glomerular filtration rate, and the ability of renal tubules to excrete acid (10, 11), which are involved in the physiological mechanisms that occur during CA-AKI.

Compared with other risk factors, malnutrition is easier to recognize and reverse by physicians (12). The controlling nutritional status (CONUT) score is an efficient and simple tool to detect malnutritional status, based on only three indexes (serum albumin, cholesterol, and lymphocytes), and has been widely used in the cardiovascular field (13, 14). Recent evidence has shown that malnutrition is highly prevalent in patients with cardiovascular diseases, increasing the risk of complications and adverse clinical outcomes (15–17). However, the relationship between malnutrition and the risk of CA-AKI and the resultant prognosis in patients undergoing PCI have not been adequately addressed.

Therefore, this study investigates the implications of malnutrition on CA-AKI and the resulting mortality among all patients undergoing PCI in a large multicenter cohort.

METHODS

Study Population

The REICIN study, a prospective, multicenter study (NCT01402232), enrolled a total of 4,271 patients from three different provinces of China, admitted to one of 12 hospitals between Jan 2013 and February 2016 and undergoing coronary angiography. Initially, all patients who underwent PCI with the diagnosis of coronary artery disease (CAD) were initially considered for inclusion. Patients corresponding to the following criteria were then excluded: (1) Patients who did not meet the CONUT score conditions (available serum albumin, lymphocyte, total cholesterol); (2) patients with missing follow-up data; (3) patients with a lack of preoperative serum creatinine or lack of further creatinine measurement within 48 h after PCI. Eventually, 2,083 patients were included in the final analysis (**Figure 1**). The ethics committee waived the requirement for written informed consent by participants because our study was retrospective in nature (No. GDREC2012141H). The date of approval by the ethics committee was 2011-11-19.

Data Collection

The data from the primary and secondary care records were retrieved from electronic medical records from the hospitals, including demographic characteristics, laboratory data, comorbid conditions, and medical therapy. Follow-up information was collected from follow-up appointments, re-hospitalization records of the clinical management system, and conversations between the patients and their families and the attending physicians or well-trained research assistants, over the telephone. Serum creatinine (Scr) was measured at least twice, before PCI, and 1 to 2 days following the procedure. Baseline values of laboratory examinations (e.g., serum total cholesterol, albumin, and total lymphocyte counts) were obtained from preoperative blood sampling data.

Malnutrition Screening Tools

The controlling nutritional status (CONUT) score was chosen as the malnutrition assessment method in our study because of its high applicability to cardiovascular disease (18). Based on the traditional threshold of the CONUT score, we divided the patients into two groups: non-malnutrition (scores of 0 to 1) and malnutrition (scores of 2 to 12). We added a **Supplementary analysis** to the attachment, using receiver operating characteristic (ROC) to determine the optimal cut-off value (2.50). The population was divided into two groups: CONUT $\leq$2 (lower risk of malnutrition, scores of 0 to 2) and CONUT $>$2 (higher risk of malnutrition, scores of 3 to 12).

Endpoint and Definition

The primary endpoint was CA-AKI diagnosed as a rise in serum creatinine $>$0.3 mg/dl or $>$50% than the baseline level, occurring within 48 h after the intervention. The secondary endpoint was all-cause mortality. Kidney function was assessed by the estimated glomerular filtration rate (eGFR), which was calculated using the Modification of Diet in Renal Disease(MDRD) study equation. Chronic kidney disease (CKD) was defined as eGFR $<$ 60 mL/min/1.73

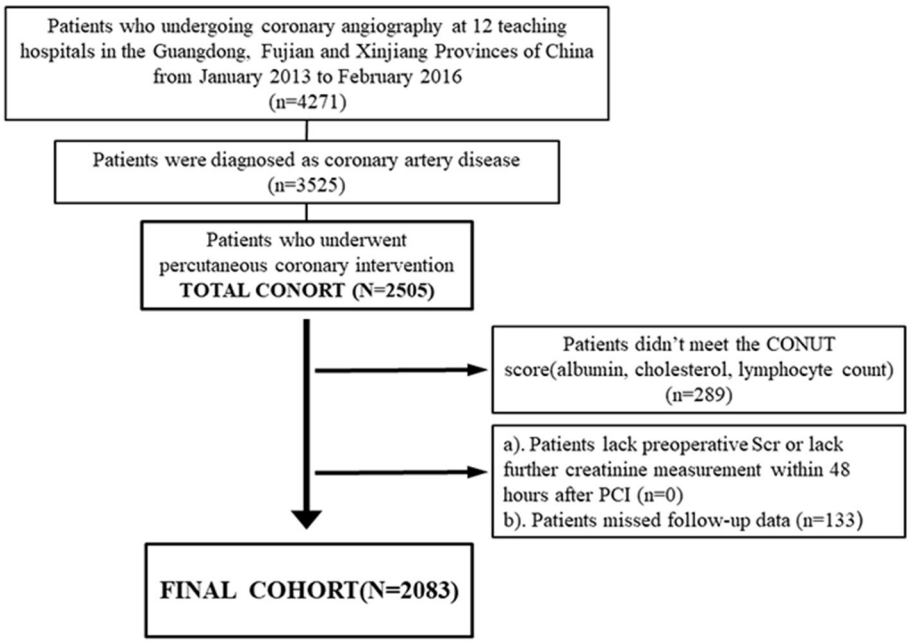

FIGURE 1 | Patient flow diagram.

m^2 at baseline (19). Congestive heart failure (CHF) was diagnosed as Killip class > 1 or New York Heart Association class > 2.

Statistical Analysis

Descriptive statistics were reported as mean ± standard deviation (SD) for continuous variables, and categorical variables were expressed as numbers (percentages) at baseline. Student *t*-tests and nonparametric tests (Mann–Whitney) were used to compare normally and not normally distributed variables, respectively. Differences in categorical variables were compared using the chi-square test. Multivariate logistic regression analysis was used to evaluate the association between malnutrition and CA-AKI, with or without adjustment for age, preoperative Scr, anemia, body mass index (BMI), contrast volume, CHF, intra-aortic balloon pump, and diuretics. Combined with the sample size, the confounding variables were associated with known risks of CA-AKI or mortality, based on previous studies and clinical plausibility, or a *p*-value of < 0.1, in univariable analysis.

The optimal cut-off value of the CONUT score was determined by the best performance based on the appropriate sensitivity and specificity ratio on the ROC curve. Time-to-event data were graphically presented using the Kaplan–Meier curves. The log-rank tests were used to compare survival between groups. Multivariable Cox regression models were used to estimate hazard ratios for all-cause mortality across combined nutritional statuses and the occurrence of CA-AKI, with adjustment for age, sex, CKD, and CHF.

All tests were two-sided and *P* values < 0.05, were considered statistically significant. All statistical analyses were performed using the R software (ver. 4.0.3).

RESULTS

Baseline Characteristics

Among the 2,083 patients undergoing PCI, the mean age was 62.8 ± 11.1 years, 79% were men, and 258 (60.4%) were malnourished. Compared with the non-malnutrition group, malnourished patients were older, but there was no significant difference in the proportion of sex, diabetes mellitus (DM), and smoking. Malnourished patients had a higher prevalence of anemia, hypertension, CKD, CHF, and the use of diuretics, as well as higher Scr, but lower BMI. There were no significant differences between the two groups in contrast agent dose, statin use, and other preoperative medications. More data on baseline demographic and clinical characteristics of the patients are shown in **Table 1**. Similar trends of baseline characteristics based on the cut-off value (2.5) of the CONUT score are detailed in **Supplemental Table 1**.

Clinical Outcomes

During the study period, 80 patients (3.8%) developed CA-AKI events. The incidence of CA-AKI in patients with and without malnutrition was 1.7 and 5.3%, respectively ($p < 0.001$), and malnutrition was observed among patients aged ≤75 years (58.2%) and those aged >75 years (73.4%) (**Figure 2**). The incidence of CA-AKI, graded by age and nutritional status, is shown in **Figure 2**. Controlling potential confounding variables, malnutrition was associated with a 2-fold increased risk of CA-AKI in patients with PCI [adjusted odds ratio (95% confidence interval), aOR (95% CI): 2.41(1.22–5.22)] (**Table 2**). It is worth noting that malnutrition was associated with a 3-fold increase in the risk of CA-AKI in patients undergoing PCI with age ≤75 years in the multivariate-adjusted logistic regression

TABLE 1 | Baseline characteristics stratified by risk of malnutrition (Cut-off value based on a traditional threshold).

Characteristics	Overall	Non-malnutrition	Malnutrition	*p*-value
	(*n* = 2,083)	(*n* = 825)	(*n* = 1,258)	
Demographic characteristics				
Age, year	62.81 (11.09)	60.0 ± 11.0	64.6±10.7	<0.001
Male, *n* (%)	1,646 (79.02)	641 (77.70)	1,005 (79.89)	0.252
Basic information				
SBP, mmHg	131.12 (20.32)	133.59 ± 19.59	129.50 ± 20.64	<0.001
DBP, mmHg	76.31 (11.97)	77.79 ± 11.67	75.33 ± 12.07	<0.001
BMI, kg/m^2	24.12 (3.27)	24.47 ± 3.30	23.88 ± 3.24	<0.001
Medical history				
Anemia, *n* (%)	653 (31.39)	145 (17.62)	508 (40.41)	<0.001
DM, *n* (%)	597 (28.66)	223 (27.03)	374 (29.73)	0.199
Hypertension, *n* (%)	1,171 (56.22)	431 (52.24)	740 (58.82)	0.004
Smoke, *n* (%)	899 (43.16)	364 (44.12)	535 (42.53)	0.501
CKD, *n* (%)	1,111 (53.34)	374 (45.33)	737 (58.59)	<0.001
CHF, *n* (%)	493 (23.67)	173 (20.97)	320 (25.44)	0.022
AMI, *n* (%)	792 (38.02)	281 (34.06)	511 (40.62)	0.003
IABP, *n* (%)	44 (2.11)	15 (1.82)	29 (2.31)	0.545
Laboratory findings				
Hemoglobin, g/L	133.90 (16.63)	138.74 ± 14.41	130.73 ± 17.23	<0.001
TC, mmol/L	4.59 (1.29)	5.18 ± 1.08	4.20 ± 1.26	<0.001
LYMPH, 10∧9/L	1.92 (0.97)	2.29 ± 1.18	1.68 ± 0.71	<0.001
ALB, g/L	36.55 (4.40)	38.96 ± 2.90	34.98 ± 4.50	<0.001
Scr, umol/L	85.00 [73.00, 104.00]	83.00 [72.00, 96.00]	88.00 [74.00, 108.00]	<0.001
eGFR, ml/mim/1.73 m^2	80.30 [63.22, 95.24]	84.81 [69.07, 98.09]	77.04 [59.39, 92.74]	<0.001
TBIL, mg/dl	14.50 (6.62)	14.31 ± 6.28	14.63 ± 6.83	0.277
Uric acid, μmol/L	374.73 [315.00, 446.00]	379.00 [325.72, 446.00]	370.56 [309.00, 444.75]	0.053
CRP, mg/L	1.98 [0.00, 7.39]	1.73 [0.00, 5.58]	2.31 [0.00, 10.10]	0.029
Urine pro, g/L	0.25 [0.10, 0.70]	0.25 [0.20, 0.30]	0.25 [0.10, 0.70]	0.977
Contrast volume, ml	110.00 [100.00, 150.00]	100.00 [100.00, 150.00]	115.00 [100.00, 150.00]	0.171
Treatment				
Pre-statin, *n* (%)	1,346 (64.62)	545 (66.06)	801 (63.67)	0.286
Pre-CCB, *n* (%)	203 (9.75)	75 (9.09)	128 (10.17)	0.459
Pre-ACEI/ARB, *n* (%)	861 (41.33)	347 (42.06)	514 (40.86)	0.617
Pre-diuretics, *n* (%)	221 (10.61)	53 (6.42)	168 (13.35)	<0.001

DM, diabetes mellitus; CKD, chronic kidney disease; CHF, congestive heart failure; AMI, acute myocardial infarction; IABP, intra-aortic ball on pump; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; TC, serum total cholesterol; ALB, albumin; Scr, serum creatinine; eGFR, estimated glomerular filtration rate; TBIL, serum total bilirubin; UA, uric acid; CRP, C-reactive protein; Urine pro, Urine protein; Pre-CCB, Pre-calcium channel blocker; Pre-ACEI/ARB, Pre-angiotensin-converting enzyme inhibitor/ angiotensin receptor blocker; CONUT score, Controlling Nutritional Status score.

model [N = 1,791, aOR (95% CI): 3.39 (1.46–9.25)] (**Table 3**). We also observed a higher risk of CA-AKI in patients with malnutrition based on the cut-off value (2.5) of the CONUT score (**Supplemental Tables 2, 3**). Kaplan–Meier curves for all-cause mortality across nutritional statuses and the occurrence of CA-AKI are shown in **Supplemental Figure 2**. In the Cox model, malnourished patients with or without CA-AKI had a significantly higher risk of all-cause mortality after adjusting for confounding factors. Patients with malnutrition and CA-AKI had the highest risk of mortality [adjusted hazard ratio (95% CI), aHR (95% CI): 4.1(2.17–7.75)]. Similar results are reported in **Supplemental Table 4**, using the optimal cut-off value determined by ROC.

DISCUSSION

This was a national prospective multicenter cohort study to demonstrate the association between malnutrition and CA-AKI in patients undergoing PCI. Compared with no malnutrition, we found that malnutrition was significantly associated with a 2-fold increased risk of developing CA-AKI. The incidence of CA-AKI was higher in elderly patients aged >75 years, while the malnutrition-associated risk of CA-AKI in younger patients aged ≤75 years appeared to be higher. Malnourished patients with CA-AKI had a higher risk of all-cause mortality.

Malnutrition is prevalent in all patients undergoing PCI. In our study, nearly 60% of the patients were defined as

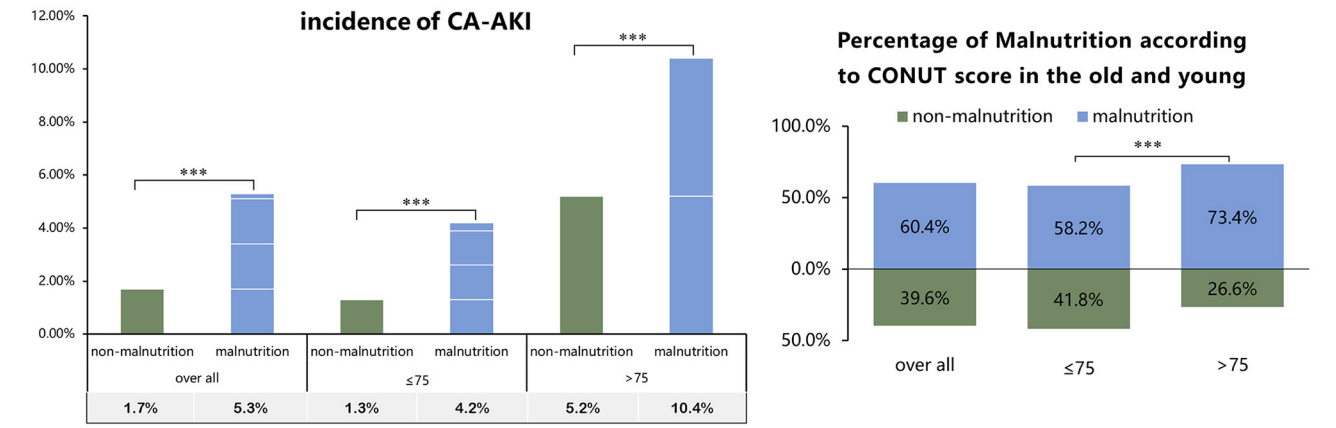

FIGURE 2 | Percentage of contrast-associated acute kidney injury (CA-AKI) incidence and malnutrition in old and young patients with percutaneous coronary intervention (PCI) (Cut-off value based on traditional threshold). ***$p < 0.001$.

TABLE 2 | Risk of contrast-associated acute kidney injury (CA-AKI)-associated nutritional state in patients undergoing percutaneous coronary intervention (PCI) (Cut-off value based on a traditional threshold).

	Univariate		Multivariate	
Risk factors	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**
Non-malnutrition	Ref		Ref	
Malnutrition	3.207 (1.846–5.984)	<0.0001	2.408 (1.218–5.215)	0.017
Age, year			1.048 1.018–1.080)	0.002
Scr, umol/L			1.006 (1.000–1.011)	0.025
Anemia			1.032 (0.565–1.846)	0.916
BMI, kg/m			1.027 (0.939–1.120)	0.555
Contrast volume, ml			1.001 (0.995–1.005)	0.764
CHF			0.728 (0.348–1.423)	0.374
IABP			8.090 (2.367–23.937)	<0.0001
Pre-diuretics			2.007 (0.964–3.941)	0.051

**Odds ratio adjusted for age, Scr, serum creatinine; Anemia, HGB, hemoglobin; BMI, body mass index; contrast volume, CHF, congestive heart failure; IABP, intra-aorticball on pump; and pre-diuretics.*

malnourished by the CONUT score. Malnutrition is prevalent in both young and elderly PCI patients and is more common in older patients. Few studies have reported the prevalence of malnutrition in younger patients undergoing PCI, and our findings are in line with the available evidence. Xiao et al. have shown that 61.1% of patients undergoing PCI were malnourished, using the CONUT score in 1,308 patients aged ≥75 years (20). Sergio et al. found malnutrition in 50 to 60% of patients, evaluated by CONUT and nutritional risk index (NRI) scores, in a cohort of 6,023 patients with the acute coronary syndrome (ACS) (18). This study showed a considerable number of malnourished patients who underwent PCI. However, malnutrition is not seriously considered as a factor by clinical cardiologists. Patients who are admitted for PCI need to be screened for nutritional status and receive timely diagnoses and treatments. The indexes in the CONUT score used in this study are simple for clinicians to obtain and calculate.

We confirmed that malnutrition was strongly associated with an increased risk of CA-AKI, as assessed by the CONUT score. Previous studies have shown that malnutrition is an important risk factor for acute kidney injury (AKI). Recently, Acarbaş et al. reported that preoperative malnutrition, assessed using the prognostic nutritional index (PNI), CONUT score, and geriatric NRI (GNRI), was a predictor for AKI in a cohort of 454 patients (21). Miyeun et al. revealed that patients with a low PNI had an independent association with CA-AKI (22). The malnutrition index analyzed in our study focused on the CONUT score. The CONUT score included only three laboratory values (serum albumin, total cholesterol levels, and total lymphocyte count), and the assessed nutritional state was positively correlated with these three values (13). These laboratory indexes have

TABLE 3 | Risk of CA-AKI-associated nutritional state in patients undergoing PCI with age ≤75 years (N = 1791) (Cut-off value based on traditional threshold).

	Univariate		Multivariate	
Risk factors	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**
Non-malnutrition	Ref		Ref	
Malnutrition	3.250 (1.694–6.881)	0.001	3.386 (1.458–9.253)	0.008
Age, year			1.021 (0.982–1.064)	0.319
Scr, umol/L			1.005 (0.998–1.011)	0.094
Anemia			0.881 (0.399–1.844)	0.743
BMI, kg/m			1.022 (0.916–1.136)	0.689
Contrast volume, ml			1.003 (0.997–1.008)	0.338
CHF			0.675 (0.256–1.567)	0.390
IABP			8.451 (1.762–30.502)	0.002
Pre-diuretics			1.701 (0.592–4.190)	0.280

**Odds ratio adjusted for age, Scr, serum creatinine; Anemia, HGB, hemoglobin; BMI, body mass index; contrast volume, CHF, congestive heart failure; IABP, intra-aortic ball on pump; and pre-diuretics.*

previously been demonstrated as risk factors for CA-AKI. Decreased albumin levels were independently associated with an increased risk of CA-AKI in a cohort of 394 patients undergoing PCI (23). Lymphocyte count is an independent risk factor for the development of contrast-induced nephropathy (24). Qin et al. showed that total cholesterol was significantly higher in patients with CA-AKI than in non-CA-AKI patients (25). The rationale behind these associations may be explained by the main mechanisms of vasoconstriction and oxidative stress in CA-AKI.

The potential mechanisms underlying the relationship between malnutrition and CA-AKI include the role of proteins, lipid profiles, and lymphocytes. Albumin not only reflects the nutritional status of the patients but also influences microvascular integrity and participates in the inflammatory pathways (26). A lower serum albumin level is an independent prognostic predictor of several cardiovascular diseases, such as ACS, coronary artery disease (CAD), and heart failure (27, 28). Low levels of albumin may be involved in the development of CA-AKI through the vascular and oxidative inflammatory pathways. Lymphocytes, markers of inflammatory response and immune status, have been shown to participate in the initiation, proliferation, and recovery stages of AKI in the previous study (29). Lipid profiles have long been regarded as risk factors for cardiovascular and kidney diseases (30, 31). Previous studies have reported that low cholesterol levels might be related to predisposing catabolic comorbidity (32, 33), thus exacerbating metabolic disorders in CA-AKI. Failure to identify and intervene in malnourished patients before surgery may lead to the unnecessary occurrence of CA-AKI. Therefore, CA-AKI can be prematurely prevented by screening for malnutrition.

It is also worth mentioning that patients aged ≤75 years have a significantly high risk of CA-AKI in malnourished nutritional status. Compared with patients without malnutrition, patients with worse nutritional status aged ≤75 years showed a 3-fold increase in the incidence of CA-AKI, while those aged >75 years showed a 2-fold increase. This suggests that younger patients are more susceptible to nutritional status. Few studies have analyzed the relationship between malnutrition and CA-AKI in patients aged ≤75 years undergoing PCI. Wei et al. showed that malnutrition was an independent risk factor for CA-AKI in a cohort of 1,308 elderly patients aged >75 years undergoing PCI, based on the CONUT score (20). In our study, patients aged ≤75 years constituted a large proportion of the overall PCI population. Nutrition screening on admission and subsequent treatments should be performed in patients with PCI regardless of age, in the elderly and young.

Malnutrition increased the risk of mortality in patients who underwent PCI with or without CA-AKI. Malnourished patients with CA-AKI had the strongest association with all-cause mortality. Recent studies have shown that malnutrition was a significant risk factor of all-cause death in patients with CAD (34). Patients with CA-AKI were more likely to have adverse outcomes than those without CA-AKI (35). Malnutrition and kidney damage due to exposure to contrast media affect physical regulation and repair, which are related to adverse outcomes (36, 37).

All our findings strongly support that malnutrition is a potentially modifiable risk, a therapeutic target, and physicians should add screening for malnutrition in their daily practice. Early diagnosis of malnutrition may be associated with CA-AKI risk stratification, provide a warning sign, and guide clinicians to adopt secondary prevention measures for patients. Preoperative malnutrition assessment is difficult in patients undergoing PCI because of the limited time in an emergency. However, the CONUT score is easy to calculate for clinicians to effectively identify poor nutritional status. Screening patients who underwent PCI for malnutrition might help to identify the risk of CA-AKI, and these patients might benefit from tailoring dehydration prevention programs and nutritional supplements to prevent CA-AKI and ameliorate the prognosis. Intravenous hydration is a common strategy used by physicians to prevent CA-AKI (38). Increased hydration volume could accelerate the excretion of the contrast agent, decrease the release of vasoconstrictors and reactive oxygen species, and reduce direct renal toxicity (39). To reduce the residual risk and improve the prognosis, cardiologists should keep pace with current evidence and follow the nutrition guidelines in

advance. Multiple strategies have been advocated to prevent and intervene in malnutrition, including dietary counseling, exercise standards, oral nutritional supplements, and educational interventions. Moreover, these nutritional interventions need to be performed during hospitalization and maintenance therapy after discharge. More rigorous research is needed to evaluate the efficiency of malnutrition intervention in patients with CA-AKI and mortality.

LIMITATIONS

First, this was an observational study with a cross-sectional nature, so our inferences did not reflect direct causality. In addition, we must always recognize the potential for residual, uncontrolled confounding, which might partly explain the associations. Second, we did not have information about the marital status, educational attainment, or socio-economic information that might help us to apply more comprehensive nutritional assessments to identify the nutritional status. The complexity of malnutrition, especially in fatter or older adults, might be explained by the wide range of determinants and the diversity in etiology. Lastly, due to the lack of other endpoints of mortality, we could have missed some other points to predict the prognosis of patients who underwent PCI. We welcome other researchers and other countries with different social systems and healthcare to confirm our findings.

CONCLUSION

In all patients undergoing PCI, malnutrition significantly increased the risk of developing CA-AKI. Malnourished patients with CA-AKI had a significantly higher risk of all-cause mortality. Clinicians must evaluate and monitor the nutritional levels of patients undergoing PCI early. Further studies are needed to prospectively assess the efficacy of nutritional interventions on outcomes in patients undergoing PCI.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

YHu, SC, JLia, and LZ provided research idea and study design. JLia, ZH, YHe, YLin, KC, MY, ML, GL, YLia, and YLiu handled the data acquisition. JLiu and SC took care of data analysis and interpretation. ZH, JLia, and JLiu handled the statistical analysis. YHu, SC, and LZ done the supervision, writing guidance, and mentorship. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions on the accuracy or integrity of any portion of the work are appropriately investigated and resolved. All authors read and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.795068/full#supplementary-material

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A Novel Application of Serum Creatinine and Cystatin C to Predict Sarcopenia in Advanced CKD

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Sarcopenia is highly prevalent in patients with advanced chronic kidney disease (CKD), yet a reliable serum index has not been established. The product of serum creatinine and the estimated glomerular filtration rate based on cystatin C (Cr×eGFRcys) was recently proposed as a sarcopenia index (SI), approximately to 24-h filtered creatinine through the glomerulus. We aimed to evaluate the diagnostic validity of the novel SI in advanced CKD. In 297 patients with non-dialysis stage 3b-5 CKD, aged 68.8 ± 12.9 years, the total skeletal muscle mass (SMM), handgrip strength (HGS), and usual gait speed were assessed. Sarcopenia was defined based on the Asian Working Group for Sarcopenia 2019 consensus update. The prevalence of sarcopenia in this cohort was 20.2%. The SI correlated moderately with SMM ($r = 0.503$, $P < 0.001$), HGS ($r = 0.508$, $P < 0.001$), and gait speed ($r = 0.381$, $P < 0.001$); the independency of the SI with three muscle metrics was confirmed after extensive adjustment. For sarcopenia prediction, the SI had acceptable discriminative powers in males [area under the receiver operating characteristic curve (AUC) 0.646, 95% confidence interval (CI) 0.569–0.718] and females (AUC 0.754, 95% CI 0.670–0.826). In males, the best cut-off was 53.9, which provided 71.1% sensitivity, 58.0% specificity, 32.9% positive predictive value (PPV), and 87.4% negative predictive value (NPV); in females, the best cut-off was 45.8, which provided 81.8% sensitivity, 62.3% specificity, 31.0% PPV, and 94.3% NPV. In conclusion, Cr×eGFRcys could be served as a surrogate marker for sarcopenia and may be helpful for sarcopenia screening in advanced CKD. Further studies are needed to expand our investigation.

Keywords: creatinine, cystatin C, sarcopenia, chronic kidney disease, skeletal muscle

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INTRODUCTION

Sarcopenia, which is characterized by a progressive decline of skeletal muscle mass, strength, and physical performance, is frequently observed in patients with chronic kidney disease (CKD) and leads to poor clinical outcomes (1–3), as pathogenic factors—metabolic acidosis, inflammation, impaired insulin signaling, oxidative stress, accumulated uremic toxins, suppressed appetite, decline in satellite cells, and myostatin overexpression—accelerate skeletal muscle wasting as kidney disease progresses (4, 5).

Thus, sarcopenia is a major concern in patients with advanced-stage CKD. Moreover, protein restriction is usually implemented in patients with advanced-stage CKD to attenuate renal progression, and clinical supervision of nutritional status is highly recommended (6). Imaging modalities including bioelectrical impedance, ultrasound, dual-energy X-ray absorptiometry, computed tomography, and magnetic resonance imaging, as well as D_3-creatine dilution, are strongly recommended by expert panels as measurement tools for skeletal muscle mass (7–10), yet are not always available in outpatient settings. Therefore, surrogate markers for the early detection of sarcopenia in advanced-stage CKD are in demand.

Creatinine is a metabolite of creatine phosphate that is converted non-enzymatically from skeletal muscle, and the endogenous creatinine generation rate depends on skeletal muscle mass under steady renal function (11, 12). Due to its properties of free glomerular filtration and minimal tubular reabsorption, timed urine creatinine excretion (Ucr) is a reliable marker for predicting skeletal muscle mass in various populations (13–16). Unfortunately, the collection of 24-h urine samples is inconvenient. Moreover, under- or over-collection of urine samples is common.

To overcome the need of precise urine collection, 24-h filtered creatinine through the glomerulus can be estimated from the product of serum creatinine (Cr) and the glomerular filtration rate (GFR). Through the calculation of GFR from cystatin C (eGFRcys), a renal marker less affected by skeletal muscle mass than creatinine, Cr×eGFRcys has been proposed as a novel sarcopenia index (SI) (17). Our previous work demonstrates a significant impact of the low SI on overall mortality in non-dialysis CKD patients (18). However, whether this index is useful for the evaluation of skeletal muscle mass and strength in patients with advanced-stage CKD remains unexplored.

Thus, the study aimed to investigate the association of the novel SI with skeletal muscle mass, strength, and physical performance in patients with stage 3b-5 CKD and to establish its diagnostic validation for sarcopenia.

MATERIALS AND METHODS

Participants

This cross-sectional study was conducted in outpatient clinics at Hualien Tzu-Chi Hospital, a Medical Center in eastern Taiwan, between January 2018 and May 2021. Adult patients with non-dialysis stage 3b-5 CKD who have regularly been followed up at our CKD outpatient department were invited to participate in the study. Those younger than 20 years, with an estimated GFR of more than 45 mL/min/1.73 m^2, recent hospitalization within 3 months, a pacemaker, amputated limbs, active malignancy, wheelchair or bedridden, as well as those who refused to participate were excluded. CKD was defined as a decrease in renal function or the presence of kidney damage for more than 3 months (19).

A total of 297 patients with CKD were enrolled in this study. Basic information and comorbidities, including diabetes mellitus (DM), hypertension, chronic glomerulonephritis (GN), and cardiovascular (CV) disease, were collected through electronic medical records. CV disease comprised coronary artery disease, myocardial infarction, left ventricular hypertrophy, arrhythmias, or congestive heart failure.

All participants signed an informed consent approved by the Institutional Review Board of Tzu-Chi Hospital (IRB 108-219-A), and all methods were performed in accordance with the Declaration of Helsinki.

Anthropometric Analysis and Blood Pressure

Body mass index (BMI) was calculated as body weight (Kg) divided by height squared (m^2). In the standing erect position, waist circumference was measured at the shortest point between the lower rib margin and the iliac crest; hip circumference was measured at the level of greatest protrusion of the buttocks. Triceps skinfold (TSF) and mid-arm circumference (MAC) were measured at the midpoint between the acromion and olecranon using a skinfold caliper (QuickMedical, Issaquah, WA, USA) and flexible inextensible tape, respectively. The mean of the three TSF readings was accepted. Mid-arm muscular circumference (MAMC) was subsequently calculated as MAC (cm)-$\pi \times$ TSF (cm).

Systolic and diastolic blood pressure (BP) were measured using standard mercury sphygmomanometers after 10-min resting.

Skeletal Muscle Mass, Handgrip Strength, and Gait Speed

Skeletal muscle and fat tissue mass were assessed using a tetrapolar bioelectrical impedance device (Biodynamics® BIA 450 Bioimpedance Analyzer, Seattle, WA, USA), which delivers an electric current of 800 μA at 50 kHz. In the supine position, two electrodes were placed on the hand and wrist, and two were placed on the foot and ankle of the non-dominant side. Total skeletal muscle mass (SMM) was estimated based on a well-validated equation developed by Janssen et al. (20):

$$\mathrm{SMM} = \left(\frac{\mathrm{height}^2}{\mathrm{resistance}} \times 0.401\right) + \mathrm{age} \times (-0.071) + \mathrm{sex} \times 3.825 + 5.102$$

In this formula, height is input in centimeters; resistance in ohms; age in years; sex: female = 0, male = 1.

A hand-held dynameter (Jamar Plus Digital Hand Dynamometer, SI Instruments Pty Ltd, Hilton, Australia) was used to assess the handgrip strength (HGS). Patients were instructed to grip the dynamometer as tightly as possible in the standing position, with the arm at a right angle and the elbow at the side of the body. Three measurements were repeated in each hand, with a 1-min rest interval. The average value of both hands was adopted for analysis.

For the usual gait speed measurement, participants were instructed to walk at their usual speed for 6 m on a flat and straight path, and the gait speed was calculated accordingly. The gait speed test was not performed on 22 patients who reported dizziness or an unsteady gait on the test day.

TABLE 1 | Demographic and clinical characteristics of the study population.

Characteristics	Total ($n = 297$)	Sarcopenia ($n = 60$)	Non-sarcopenia ($n = 237$)	*P*-value
Age (years)	68.8 ± 12.9	77.0 ± 10.3	66.7 ± 12.6	<0.001*
Gender (male), *n* (%)	169 (56.9)	38 (63.3)	131 (55.3)	0.260
CKD stages, *n* (%)				
Stage 3b	25 (8.4)	9 (15.0)	16 (6.8)	0.121
Stage 4	177 (59.6)	33 (55.0)	144 (60.8)	
Stage 5	95 (32.0)	18 (30.0)	77 (32.5)	
Diseases, *n* (%)				
DM	155 (52.2)	25 (41.7)	130 (54.9)	0.068
Chronic GN	109 (36.7)	23 (38.3)	86 (36.3)	0.769
Hypertension	248 (83.5)	48 (80.0)	200 (84.4)	0.413
CV disease	88 (29.6)	21 (35.0)	67 (28.3)	0.308
Systolic BP (mmHg)	147 (131–163)	146 (132–166)	147 (131–162)	0.559
Diastolic BP (mmHg)	79 (70–86)	77 (68–85)	80 (70–87)	0.069
Anthropometry measures				
BMI (kg/m^2)	26.3 ± 4.4	23.5 ± 3.6	27.0 ± 4.3	<0.001*
Waist circumference (cm)	92 ± 12	87 ± 10	93 ± 12	<0.001*
Hip circumference (cm)	96 (92–102)	94 (89–97)	97 (92–103)	0.001*
MAMC (cm)	23 ± 3	21 ± 2	23 ± 4	0.001*
Fat tissue mass (kg)	19.7 (14.9–25.2)	19.9 (15.9–25.1)	19.7 (14.8–25.2)	0.799
Skeletal muscle measures				
SMM (kg)	23.2 (17.5–28.5)	19.5 (14.6–22.2)	24.6 (18.3–29.3)	<0.001*
HGS (kg)	23.8 ± 8.9	18.8 ± 6.7	25.1 ± 9.0	<0.001*
Gait speed (m/s)[a]	0.93 (0.73–1.11)	0.77 (0.56–0.98)	0.96 (0.79–1.14)	<0.001*
Laboratory data				
Hemoglobin (g/dL)	10.8 ± 1.8	11.0 ± 1.8	10.8 ± 1.9	0.298
Albumin (g/dL)	4.1 (3.8–4.3)	4.1 (3.9–4.3)	4.1 (3.8–4.3)	0.199
TCH (mg/dL)	146 (124–172)	143 (121–178)	146 (125–172)	0.692
Glucose (mg/dL)	106 (93–138)	107 (94–138)	106 (93–138)	0.866
BUN (mg/dL)	44 (32–58)	34 (25–55)	45 (35–59)	<0.001*
Creatinine (mg/dL)	2.8 (2.2–3.8)	2.3 (1.9–3.3)	2.9 (2.4–3.8)	<0.001*
Cystatin C (mg/L)	2.9 (2.4–3.6)	2.7 (2.2–3.5)	2.9 (2.4–3.6)	0.135
eGFRcre (mL/min/1.73 m^2)	20 (14–26)	24 (17–32)	19 (14–25)	<0.001*
eGFRcys (mL/min/1.73 m^2)	18 (13–24)	19 (13–25)	18 (13–24)	0.401
UPCR (g/g)	0.9 (0.3–2.1)	0.4 (0.2–1.3)	1.1 (0.4–2.4)	0.001*
SI	50.8 (42.5–61.8)	45.6 (37.0–52.1)	52.4 (44.8–63.1)	<0.001*
24-h Ucr (mg/day)[b]	921 (708–1,224)	734 (589–1,001)	974 (738–1,246)	<0.001*

Values for continuous variables are given as means ± standard deviations or medians and interquartile ranges. Categorical variables are expressed as numbers (%). CKD, chronic kidney disease; DM, diabetic mellitus; GN, glomerulonephritis; CV, cardiovascular; BP, blood pressure; BMI, body mass index; MAMC, mid-arm muscular circumference; SMM, skeletal muscle mass; HGS, handgrip strength; TCH, total cholesterol; BUN, blood urea nitrogen; eGFRcre, estimated glomerular filtration rate from serum creatinine; eGFRcys, estimated glomerular filtration rate from serum cystatin C; UPCR, urine protein/creatinine ratio; SI, sarcopenia index; Ucr, urine creatinine excretion.

[a] *Gait speed test was available in 275 patients.*

[b] *24-h urine sample was available in 265 patients.*

**P < 0.05 was considered statistically significant between sarcopenia and non-sarcopenia groups.*

Definition of Sarcopenia

The skeletal muscle index (SMI) was calculated as the SMM (kg) divided by height squared (m^2). Those with an SMI lower than 8.87 kg/m^2 in males and 6.42 kg/m^2 in females were classified as having a low SMI, based on two standard deviations below the mean of young Taiwanese adults (21, 22). Muscle weakness was defined as an HGS < 28 kg in males and 18 kg in females, whereas slow gait speed was 6-m gait speed < 1.0 m/s, based on the Asian Working Group for Sarcopenia (AWGS) 2019 consensus (23). Sarcopenia was defined as low SMI with either muscle weakness or slow gait speed.

Among 22 patients who did not perform the gait speed test, none of them had low SMI. Thus, all of them were classified as non-sarcopenic.

Serum SI and Laboratory Data

At the same visit, fasting serum samples were used for biochemical analysis within 1-h of collection. A standard autoanalyzer (Siemens Advia 1800, Siemens Healthcare

GmbH, Henkestr, Germany) was used to determine serum blood urea nitrogen (BUN), creatinine, albumin (bromocresol green method), total cholesterol (TCH), glucose, and urine protein/creatinine ratio (UPCR). Serum cystatin C levels were measured using a nephelometric Siemens immunoassay. The estimated GFR was calculated from serum creatinine (eGFRcre) and cystatin C (eGFRcys), based on the Modification of Diet in Renal Disease (24) and CKD-EPI Cystatin C equation (25), respectively. The stages of CKD in the study were based on the eGFRcys.

The novel SI, Cr×eGFRcys, was calculated as the product of serum creatinine (mg/dL) and eGFRcys (mL/min/1.73 m^2).

24-H Urine Creatinine Excretion

Detailed verbal and written instructions about the urine collection technique were provided to all participants. After discarding the first void in the morning, all participants were instructed to collect all urine throughout the following 24-h period, including the first morning void on the next day. The 24-h Ucr was calculated as the product of urine creatinine levels and 24-h urine volume. Among our participants, 265 (89.2%) completed 24-h urine sample collection.

Statistical Analyses

To detect a correlation coefficient of about 0.3 between SI and skeletal muscle measures in each gender, with an alpha level of 0.05 and a power of 90%, a total of at least 224 patients should be enrolled.

Continuous variables were expressed either as the mean ± standard deviation or as the median and interquartile range, based on the data distribution evaluated from the Kolmogorov–Smirnov test. The variables among sarcopenia and non-sarcopenia were compared by applying Student's independent *t* test or the Mann–Whitney U test. Categorical variables were expressed as absolute (*n*) and relative frequency (%) and were analyzed by the chi-square test. Scatter plots with Spearman's correlation coefficient were used to depict the correlations of SI and 24-h Ucr with SMM, HGS, and gait speed. Independency of SI with SMM, HGS, and gait speed was examined by multiple linear regression, adopting potential risk factors for sarcopenia.

To assess the diagnostic performance of SI and 24-h Ucr on sarcopenia, receiver operating characteristic (ROC) curves were constructed. The area under the ROC curve (AUC), cut-offs, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were established.

Statistical analyses were performed using SPSS (version 19.0; SPSS, Chicago, IL, USA). A *P*-value of < 0.05 was considered statistically significant.

RESULTS

Baseline Characteristics

Table 1 summarizes the baseline characteristics of 297 patients with CKD. Overall, the mean age was 68.8 ± 12.9 years, and 169 (56.9%) were male. The distribution of CKD stages was 8.4% stage 3b, 59.6% stage 4, and 32.0% stage 5. Among them, 52.2% had DM, 36.7% chronic GN, 83.5% hypertension, 29.6% CV disease. The prevalence of sarcopenia was 20.2%. Patients with sarcopenia were older ($P < 0.001$); had lower BMI ($P < 0.001$), waist circumference ($P < 0.001$), hip circumference ($P = 0.001$), MAMC ($P = 0.001$), SMM ($P < 0.001$), HGS ($P < 0.001$), gait speed ($P < 0.001$), BUN ($P < 0.001$), creatinine ($P < 0.001$), UPCR ($P = 0.001$); and had higher eGFRcre ($P < 0.001$). Notably, those with sarcopenia had significantly lower SI ($P < 0.001$) and 24-h Ucr ($P < 0.001$).

Association of SI With Skeletal Muscle Measures

As shown in **Figure 1**, SI was significantly lower in the sarcopenia group than in the non-sarcopenia group in both genders (46.0 ± 11.1 vs. 58.4 ± 14.4, $P = 0.001$ in males; 40.3 ± 5.5 vs. 48.9 ± 12.0, $P < 0.001$ in females) and in different CKD stages (49.4 ± 12.4 vs. 56.6 ± 13.6, $P = 0.002$ in stage 3b-4; 40.7 ± 8.0 vs. 51.0 ± 15.1, $P < 0.001$ in stage 5).

In **Figure 2**, SI was positively correlated with SMM ($r = 0.503$, $P < 0.001$), HGS ($r = 0.508$, $P < 0.001$), and gait speed ($r = 0.381$, $P < 0.001$). These moderate-intensity correlations were close to those of 24-h Ucr, which yielded correlation coefficients of 0.539, 0.582, and 0.351 with SMM, HGS, and gait speed, respectively. The correlations of other proposed SI with skeletal muscle metrics are also reported in **Table 2**.

Simple and multiple linear regression analyses of the SI in relation to SMM, HGS, and gait speed are shown in **Table 3**. In the unadjusted model (model 1), SI was significantly associated with SMM, HGS, and gait speed, the results of which were sustained after extensive adjustment for potential confounders, including age, sex, DM, hypertension, CV disease, BMI, waist and hip circumference, hemoglobin, albumin, TCH, glucose, eGFRcys, and UPCR (model 4).

Diagnostic Performance of SI on Sarcopenia

The AUC, cut-off values, sensitivity, specificity, PPV, and NPV of SI for sarcopenia are shown in **Table 4**. The SI had acceptable discriminative power in both males [AUC 0.646, 95% confidence interval (CI) 0.569–0.718, $P = 0.003$] and females (AUC 0.754, 95% CI 0.670–0.826, $P < 0.001$). In males, the best cut-off was 53.9, which provided 71.1% sensitivity, 58.0% specificity, 32.9% PPV, and 87.4% NPV; in females, the best cut-off was 45.8, which provided 81.8% sensitivity, 62.3% specificity, 31.0% PPV, and 94.3% NPV. The diagnostic performance of 24-h Ucr was also provided.

DISCUSSION

In our study, the novel SI, Cr×eGFRcys, was independently associated with skeletal muscle mass, strength, and usual gait speed in non-dialysis advanced CKD. The correlation coefficients with muscle measures and the discriminatory power for sarcopenia exhibited by SI were similar to the performance of 24-h Ucr.

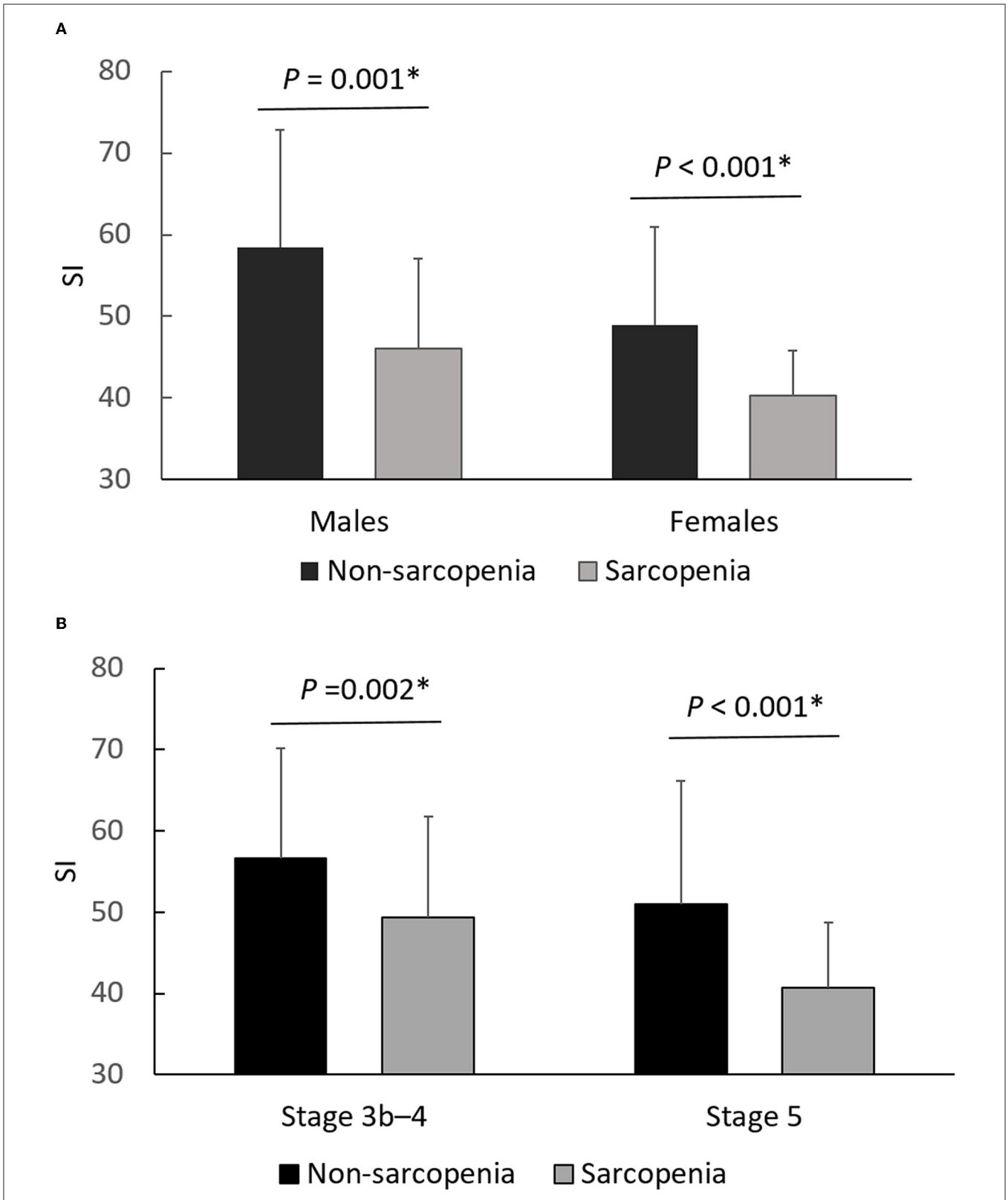

FIGURE 1 | Differences of SI between non-sarcopenia and sarcopenia, stratified by gender **(A)** and CKD stage **(B)**. *$P < 0.05$ was considered statistically significant.

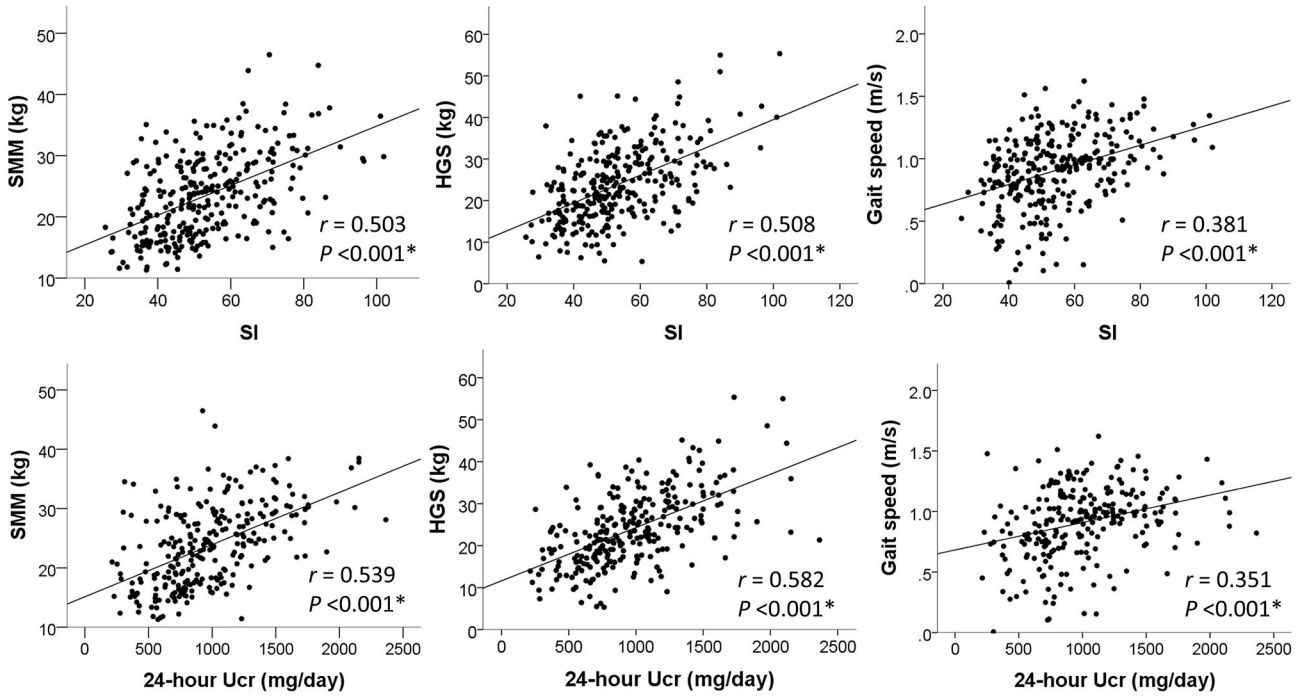

FIGURE 2 | Correlations of SI and 24-h Ucr with skeletal muscle measures. *$P < 0.05$ was considered statistically significant.

TABLE 2 | Spearman's correlations between different proposed sarcopenia indices based on creatinine and cystatin C with skeletal muscle mass, strength, and gait speed.

Sarcopenia indices	SMM (kg)		HGS (kg)		Gait speed (m/s)	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
Cr × eGFRcys	0.503	<0.001*	0.508	<0.001*	0.381	<0.001*
Cr/CysC	0.440	<0.001*	0.368	<0.001*	0.313	<0.001*
Serum Cr	0.362	<0.001*	0.101	0.083	0.179	0.003*
eGFRcys-eGFRcre	0.142	0.015*	0.169	0.003*	0.251	<0.001*
eGFRcys/eGFRcre	0.124	0.033*	0.167	0.004*	0.240	<0.001*

*SMM, skeletal muscle mass; HGS, handgrip strength; Cr, creatinine; eGFRcys, estimated glomerular filtration rate from serum cystatin C; CysC, cystatin C; eGFRcre, estimated glomerular filtration rate from serum creatinine. *P < 0.05 was considered statistically significant.*

Sarcopenia is concerning in patients with advanced-stage CKD; thus, assessing skeletal muscle health is of the same importance as monitoring renal function change. Considering the limited feasibility of imaging studies, several novel biomarkers, such as myokines, inflammatory and oxidative markers, have been emergently reported for skeletal muscle estimation (26, 27). Unfortunately, there is a gap between these results and their translation into real-world practice, given their weak correlations with skeletal muscle mass and high measurement cost. To our knowledge, 24-h Ucr, which was first validated in 1983, remains the most reliable and robust marker for the prediction of skeletal muscle mass (11). In patients with CKD, low 24-h Ucr is associated with reduced skeletal muscle mass, frailty, and enhanced mortality risks (28–30). Our study showed moderate correlations of 24-h Ucr with skeletal muscle mass and strength in advanced CKD, which were in line with a previous report from the large-scale Chronic Renal Insufficiency Cohort (CRIC), showing a correlation coefficient of 0.5 between 24-h Ucr and skeletal muscle mass, as evaluated by either BIA or DEXA in patients with CKD. This correlation was stronger in those with proper urine collection (30). Nevertheless, poor collection of urine samples is common in outpatient settings. As indicated in the CRIC cohort, up to one-third of urine samples were regarded as poor quality, which justifies the development of alternative surrogate markers to monitor skeletal muscle health in patients with advanced-stage CKD.

For the first time, we demonstrated that the novel SI calculated from creatinine and cystatin C, two widely used renal markers, independently predicts skeletal muscle mass, muscle strength, and physical performance in advanced-stage CKD, even after adjusting for potential confounders extensively. Cut-off values

for the novel SI yielded high NPV, which suggested its potential use for screening sarcopenia in patients with advanced-stage CKD. In addition, our previous work showed a close relationship between low SI and all-cause mortality in real-world cases using our CKD database (18). The patients in the low SI group conferred a three-fold increased mortality hazard after full adjustment for risk factors in comparison with those in the normal SI group.

Given the decreased serum creatinine, but not cystatin C, in patients experiencing muscle wasting, other indices based on these two renal markers, such as the creatinine-to-cystatin C ratio (Cr/CysC), eGFRcys-to-eGFRcre ratio (eGFRcys/eGFRcre), and the difference between eGFRcys and eGFRcre (eGFRcys–eGFRcre), have been reported for the assessment of sarcopenia in various populations (31–39). Our study demonstrated that, among these serum indices, Cr×eGFRcys exhibited the best correlations with skeletal muscle mass, strength, and gait speed. This observation was in accordance with results from two recent studies in cancer patients, which showed that Cr×eGFRcys outperformed Cr/CysC in predicting sarcopenia and postoperative complications (40, 41).

Our study is the first to explore the clinical utility of Cr×eGFRcys as an SI for predicting sarcopenia in advanced-stage CKD patients. The strength of the study was that 24-h Ucr was collected simultaneously, ensuring a direct comparison between the novel SI and 24-h Ucr. However, we report significant limitations. First, the sample size was relative limited, which precluded our further stratification by each CKD stage. The application of the gender-specific SI cut-offs in each CKD stage was shown in **Supplementary Table 1**. Second, the discriminatory power of SI in men was low, which limited its clinical utility to predict sarcopenia. Third, a single-frequency BIA was used to measure skeletal muscle mass, which could be overestimated by hydration status in patients with advanced-stage CKD. However, a strong correlation and good agreement between the BIA with dual-energy X-ray absorptiometry was demonstrated in dialysis patients (42). Fourth, non-renal factors other than skeletal muscle mass, including dietary protein intake, physical activity, inflammation, obesity, endocrine disease, and certain medications, affect creatinine or cystatin metabolism (43, 44) and account for variations. Thus, whether Cr×eGFRcys is useful for dynamic monitoring of skeletal muscle change longitudinally and for evaluation of response to intervention should be determined in future studies. Fifth, the quality of urine collection could not be ascertained. We propose that 24-h Ucr is the most reliable clinical marker for sarcopenia when an accurate collection of urine samples is ensured. Sixth, although the criteria for sarcopenia were well-developed in the geriatric population, there was a lack of agreement among patients with CKD. Finally, this is a single-center study in Taiwan; therefore, our findings should be extrapolated with caution, especially to other ethnic populations.

TABLE 3 | Independency of SI with SMM, HGS, and gait speed.

Variables	SI (per 1-SD increase)	
	β (95% CI)	*P*-value
SMM (kg)		
Model 1	3.44 (2.78–4.11)	<0.001*
Model 2	1.08 (0.53–1.62)	<0.001*
Model 3	1.11 (0.62–1.60)	<0.001*
Model 4	1.52 (1.02–2.01)	<0.001*
HGS (kg)		
Model 1	4.74 (3.87–5.60)	<0.001*
Model 2	2.78 (1.89–3.66)	<0.001*
Model 3	2.78 (1.89–3.67)	<0.001*
Model 4	2.77 (1.81–3.74)	<0.001*
Gait speed (m/s)		
Model 1	0.11 (0.08–0.14)	<0.001*
Model 2	0.06 (0.02–0.09)	0.002*
Model 3	0.06 (0.02–0.09)	0.002*
Model 4	0.05 (0.01–0.09)	0.014*

*Model 1, Unadjusted analysis. Model 2, Adjusted for age and sex. Model 3, Model 2 additionally adjusted for diabetes mellitus, hypertension, cardiovascular disease, body mass index, waist and hip circumference. Model 4, Model 3 additionally adjusted for hemoglobin, albumin, total cholesterol, fasting glucose, estimated glomerular filtration rate based on cystatin C, and urine protein to creatinine ratio. SI, sarcopenia index; SMM, skeletal muscle mass; HGS, handgrip strength. *P < 0.05 was considered statistically significant.*

TABLE 4 | Diagnostic validity of SI and 24-h Ucr on sarcopenia, overall and stratified by gender.

	Low SI					
	AUC (95% CI)	**Cut-off**	**Sen (%)**	**Spe (%)**	**PPV (%)**	**NPV (%)**
Overall	0.659 (0.602–0.713)*					
Male	0.646 (0.569–0.718)*	53.9	71.1	58.0	32.9	87.4
Female	0.754 (0.670–0.826)*	45.8	81.8	62.3	31.0	94.3
	Low 24-h Ucr[a]					
Overall	0.659 (0.599–0.716)*					
Male	0.688 (0.608–0.761)*	1022	72.4	61.8	30.9	90.5
Female	0.692 (0.599–0.776)*	710	80.0	64.5	32.7	93.7

SI, sarcopenia index; Ucr, urine creatinine excretion; AUC, area under the ROC curves; CI, confidence interval; Sen, sensitivity; Spe, specificity; PPV, positive predictive value; NPV, negative predictive value. [a]24-h urine sample was available in 265 patients.

**P < 0.05 was considered statistically significant.*

CONCLUSION

In conclusion, our study demonstrated that Cr×eGFRcys was independently associated with skeletal muscle mass, strength, and usual gait speed in non-dialysis advanced CKD. In addition to providing more accurate renal function estimates, measuring serum creatinine and cystatin simultaneously to generate the novel SI, Cr×eGFRcys, may be an easy-to-use approach for screening skeletal muscle health in patients with advanced-stage CKD. However, large-scale studies are encouraged to extend our findings.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hualien Tzu Chi Hospital (108-219-A). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Y-LL: conceptualization, methodology, formal analysis, and writing—original draft preparation. C-HW and I-CC: investigation. I-CC: data curation. B-GH: writing—review and editing and supervision. All authors reviewed the manuscript, contributed to the article, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.828880/full#supplementary-material

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Blood Plasma Metabolites in Diabetes-Associated Chronic Kidney Disease: A Focus on Lipid Profiles and Cardiovascular Risk

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Background: We investigated a cross-sectional metabolomic analysis of plasma and urine of patients with early and late stage diabetes associated chronic kidney disease (CKD), inclusive of stages 1–5 CKD, to identify potential metabolomic profiles between the two groups.

Methods: This cross-sectional study recruited 119 adults. Metabolomic biomarkers were quantified in 119 non-fasted plasma and 57 urine samples using a high-throughput proton Nuclear Magnetic Resonance platform. Analyses were conducted using R with the ggforestplot package. Linear regression models were minimally adjusted for age, sex, and body mass index and *p*-values were adjusted for multiple comparisons using the Benjamini-Hockberg method with a false discovery rate of 0.05.

Results: Apolipoprotein A1 concentration (ApoA1) was reduced (adj. $p = 0.04$) and apolipoprotein B/apolipoprotein A1 ratio (ApoB/ApoA1) was increased (adj. $p = 0.04$) in late CKD compared with early CKD. Low-density lipoprotein triglyceride (LDL-TG) had an increased concentration (adj. $p = 0.01$), while concentrations of high-density lipoprotein cholesterol (HDL-C) were reduced (adj. $p = 0.04$) in late CKD compared to early stages of disease.

Conclusion: Our results highlight the presence of abnormal lipid metabolism namely significant reduction in the protective ApoA1 and significant increase in atherogenic ApoB/ApoA1 ratio. The study also demonstrates significantly elevated levels of triglyceride-rich lipoproteins such as LDL-TG. We illustrate the significant reduction in protective HDL-C in individuals with diabetic CKD. It explores a detailed plasma lipid profile that significantly differentiates between the late and early CKD groups as well as each CKD stage. The study of complex metabolite profiles may provide additional data required to enable more specific cardiovascular risk stratification.

Keywords: chronic kidney disease, diabetes mellitus, metabolomics, cardiovascular disease (CVD), lipid metabolites

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INTRODUCTION

Diabetes and its associated complications have reached pandemic proportions, with a global prevalence of 537 million adults with diabetes (1). Unfortunately, numbers are predicted to rise to 783 million adults by 2045 (1). More than 40% of people with type 2 diabetes will ultimately develop chronic kidney disease (CKD), the leading cause of end stage kidney disease (ESKD) requiring renal replacement therapy (2). Diabetes-associated CKD is associated with an excess all-cause and cardiovascular mortality and is one of the most important causes of health care expenditure, disability, economic loss and mortality (3). Cardiovascular disease (CVD) is the leading cause of CKD-associated death, in part due to persistent low-grade inflammation and subsequent atherosclerosis, a result of atherogenic factors that constitute the metabolic syndrome (4, 5) and factors relating to the metabolic dysregulation of CKD. Diabetes-associated CKD is therefore considered a major global health issue (6).

Metabolomics is the large scale study of small molecules in a sample of biological fluid, such as blood, urine or saliva (7). Metabolites act as functional readouts of underlying physiological processes (8) and are increasingly being used to study kidney function and disease (9, 10).

The global scale and burden of diabetic-associated CKD, particularly its contribution to CVD, prompted us to explore the metabolomic profile in individuals with late CKD stage (Stage 3b−5) and early stages of the disease (Stage 1–3a). We also aimed to identify any potential metabolomic signatures that may have future utility for the early identification of CKD individuals at increased CVD risk.

MATERIALS AND METHODS

Participants

One hundred and twenty one adults with diabetes and CKD stages 1–5 at the time of their outpatient endocrinology clinic visit were recruited based on serum estimated glomerular filtration rate (eGFR as determined by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (11) with or without albuminuria. Where available, albuminuria was evaluated by measuring urinary albumin-to-creatinine ratio (ACR) in a spot urine sample. Microalbuminuria was defined as an ACR of 30–300 mg/g and macroalbuminuria defined as an ACR >300 mg/g (12). Participants qualified for inclusion if they were aged >18 years and were individuals with diabetes who had CKD for at least 3 months. Participants were divided into 2 distinct groups: "early CKD" and "late CKD". The early diabetic CKD group was defined as participants with diabetes who had stage 1, 2 or 3a CKD ($n = 83$), while the late diabetic CKD group was defined as participants with diabetes who had stage 3b, 4 or 5 CKD ($n = 38$). Participants were excluded if they were <18 years, had acute kidney injury, history of renal transplant, a single kidney, diabetes secondary to pancreatic pathology, steroid medication-induced diabetes, presence of non-diabetic kidney disease, active drug or heavy alcohol use, an active malignancy within the past 5 years, inflammatory bowel disease, were pregnant or breastfeeding or who had a Body Mass Index (BMI) < 20 or > 40 (13).

Data collection occurred only at one time point and included blood pressure, medical comorbidities, duration of diabetes, stage of CKD and associated complications, medications and pathology results. Anthropometric data was collected on the day of the clinic visit while the remainder of the patient's information was gathered from Austin Health's electronic medical record.

Blood Collection

5 mL of peripheral blood was collected in an Ethylenediaminetetraacetic acid (EDTA) anticoagulant tube and centrifuged at 3500 rcf at 4°C. The resultant plasma was separated into 0.5 ml aliquots. Samples were processed within 2 h of collection and aliquots stored at−80°C until thawed for metabolomic profiling.

Urine Collection

A spot urine collection was transported to the laboratory within 24–48 h of collection, centrifuged at 3500 rcf at 4°C, aliquoted into 5 ml tubes and stored at −80°C within 30 min of processing.

The research protocols that the authors developed for blood and urine collection have already been published (13). The methods outlined in the published protocol allow for replication studies.

Metabolomics Data and Statistical Analysis

Metabolomics Data

After quality control, metabolomic measures were quantified in 119 non-fasted plasma and 57 urine samples using Nuclear Magnetic Resonance (NMR) metabolomics (14, 15). A total of 225 metabolic measures within 14 subclasses were measured in plasma, while the urine NMR panel quantified 54 metabolites.

Statistical Analysis

Analyses were conducted using R (version 3.5.3) with the ggforestplot (v0.0.2) and WGCNA (v1.69) packages. The concentrations of all metabolomic measures were log-transformed and scaled to a standard distribution (SD units) to allow for comparison between metabolites across a range of concentrations. Principal-component analysis was performed, with pairwise Pearson's correlations calculated for each of the clinical and lifestyle variables with the initial five principal

Abbreviations: ApoA1, Apolipoprotein A1; ApoB, Apolipoprotein B; BCAA, Branched-chain amino acids; BMI, Body mass index; CKD, Chronic kidney disease; CVD, Cardiovascular disease; EDTA, Ethylenediaminetetraacetic acid; Egfr, Estimated glomerular filtration rate; ESKD, End-stage kidney disease; FDR, False discovery rate; GC-MS, Gas chromatography with mass spectrometry; GlycA, Glycoprotein acetyls; HDL, High-density lipoprotein; HDL-C, High-density lipoprotein-cholesterol; HREC, Human Research Ethics Committee; IDL, Intermediate-density lipoprotein; IDL-TG, Intermediate-density lipoprotein-triglyceride; LC-MS, Liquid chromatography with mass spectrometry; LDL, Low-density lipoprotein; LDL-C, Low-density lipoprotein- cholesterol; LDL-TG, Low-density lipoprotein-triglyceride; MCRI, Murdoch Children's Research Institute; M-HDL-C, Medium high-density lipoprotein cholesterol; NMR, Nuclear magnetic resonance; PC, Principal component; PCA, Principal component analysis; S-VLDL-C, Small- very low-density lipoprotein-cholesterol; TC, Total cholesterol; TG, Triglyceride; TGRL, Triglyceride-rich lipoprotein; VLDL, Very low-density lipoprotein; WHO, World Health Organization; XS-VLDL-TG, Very small- very low-density lipoprotein-triglyceride.

TABLE 1 | Clinical and biochemical characteristics of participants.

Patient characteristics	Mean early CKD (Group 1)	SD or %	Mean late CKD (Group 2)	SD or %	*P*-value
Age (yrs)	66.14	11.5	72.00	11.5	0.01
Male	50	60%	16.00	44%	0.16
Type of diabetes					0.24
Type 1	15	18%	3	8%	
Type 2	66	80%	33	92%	
LADA	2	2%	0	0%	
Duration of diabetes (yrs)	18.71	11.0	33.00	11.2	0.11
Hypertension	65	78%	34.00	94%	0.06
Diabetic retinopathy	32	39%	15.00	42%	0.91
Cardiovascular disease	30	36%	15.00	42%	0.72
Stroke/Transient Ischaemic Attack (TIA)	10	12%	4.00	11%	1.00
Peripheral vascular disease	12	14%	10.00	28%	0.14
Dyslipidemia	66	80%	31.00	86%	0.55
Depression	16	19%	4.00	11%	0.41
Smoking status					0.51
Non-smoker	46	55%	24	67%	
Ex-smoker	30	36%	10	28%	
Current-smoker	7	8%	2	6%	
BMI (kg/m^2)	29.44	7.9	28.53	7.9	0.58
SBP (mmHg)	109.58	49.7	121.09	50.0	0.26
DBP (mmHg)	63.38	27.2	66.20	20.1	0.58
Hb (g/L)	108.60	52.3	88.75	53.7	0.06
eGFR (ml/min/1.73 m^2)	61.17	22.8	23.89	12.0	<0.001
HbA1c (%)	7.51	1.8	7.66	1.7	0.69
TC (mmol/L)	4.00	1.1	3.74	1.0	0.25
LDL (mmol/L)	1.89	0.9	1.78	0.9	0.54

components. Scatterplots of the first and second principal components were used to visualize differences in the variance of samples on the basis of sex, CKD group (early/late), or CKD stage 1–5 (on a linear scale).

Linear regression models were used to estimate the association between CKD group or CKD stage as the exposure and each metabolomic measure as the outcome. Linear regression models were minimally adjusted for age, sex, and BMI. Potential confounders were iteratively added to models and retained in the final model if they altered any estimated coefficients by more than 10% (16). Linear regression model *p*-values were adjusted for multiple comparisons using the Benjamini-Hochberg method (17) with a false discovery rate (FDR) of 5%.

RESULTS

Clinical and Biochemical Characteristics

Data were available for 119 participants after quality control. The clinical and biochemical characteristics of this sample are shown in **Table 1**. Type 2 diabetes was present in the majority of participants ($n = 99$). There were 83 participants in the early diabetes-associated CKD group (stages 1, 2 and 3a; mean eGFR 61 ml/min/1.73 m^2, range 45 to 91 ml/min/1.73 m^2), and 36 participants in the late diabetic CKD group (stages 3b, 4 and 5; mean eGFR of 24 ml/min/1.73 m^2, range 4 to 43 ml/min/1.73 m^2). This distinction between early and late groups was made in light of the marked increase in death, cardiovascular events and hospitalizations observed as eGFR falls below 45 ml/min/1.73 m^2 (18). The mean age of 72 years in the late CKD group was significantly older when compared with the younger mean age in the early CKD group of 66 years ($p < 0.05$). There was a lower proportion of males (44%) in the late group compared with 60% males in the early group. The proportion of the 119 recruited participants in each stage of CKD is illustrated in **Figure 1**. Similar participant results are observed in a separate study (19) by the authors as the same patient group was used for both studies.

We used the Principal Component Analysis (PCA) method to transform the large set of variables in the metabolomic data set into a smaller set still containing all the relevant information. This analysis did not discriminate between early and late CKD groups (**Supplementary Figure 1**). There was also no discrimination between genders (**Supplementary Figure 2**).

The heatmap (**Figure 2**) demonstrated the correlation of the principal components to the clinical and biochemical variables in our participants. Principal component 1 (PC1) was most strongly correlated with lipid measures of total cholesterol (TC), low-density lipoproteins (LDL), high-density lipoproteins (HDL) and

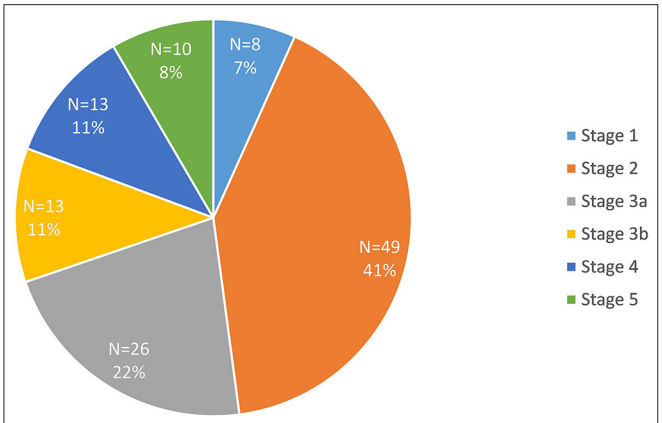

FIGURE 1 | Proportion of participants in each stage of chronic kidney disease (CKD).

triglycerides (TG). Principal component 2 (PC2) was also most strongly correlated with the same lipid measures.

A significant and expected rise in creatinine was observed in the late CKD group compared to the early CKD group and across each of the stages of CKD (**Figures 4A,B**). Mean creatinine levels increased from 59.9 umol/L in stage 1 CKD to 194 umol/L in stage 4 CKD in our patient study (**Figure 3**) and to 534.6 umol/L in stage 5 CKD. The rise in creatinine in ESKD was depicted by a higher median creatinine with a larger variation within the 25^{th}-75^{th} quartile as seen in **Figure 3**.

Metabolomic Variation Associated With Transition From Late to Early Stage CKD

After adjustment for age, gender and Body Mass Index (BMI), at least 7 metabolites within 6 classes were consistently and significantly associated with varying kidney function. Comparing late to early CKD groups (**Figure 4A**) there were lower concentrations of the metabolite valine in the amino acid class (**Supplementary Table 1A**, adj. $p < 0.05$) and as expected, significantly higher creatinine concentrations in late compared to early CKD (**Supplementary Table 1A**, adj. $p < 0.001$). The apolipoprotein class revealed significantly lower concentrations of protective apolipoprotein A1 (ApoA1, **Supplementary Table 1A**, adj. $p < 0.05$) and higher concentrations of atherogenic apolipoprotein B/apolipoprotein A1 ratio (ApoB/ApoA1, **Supplementary Table 1A**, adj. $p < 0.05$) in late compared with early CKD. The glycerides and phospholipids class demonstrated significantly higher concentrations of pathogenic low-density lipoprotein triglyceride (LDL-TG, **Supplementary Table 1A**, adj. $p < 0.05$) in late compared to early CKD. Cardioprotective high-density lipoprotein cholesterol (HDL-C) and HDL2-C concentrations were significantly lower in late compared to early CKD (**Supplementary Table 1A**, adj. $p < 0.05$). The lipoprotein subclasses showed statistically significant higher concentrations in a variety of pathogenic small very-low-density lipoproteins (S-VLDL) in late compared to early CKD

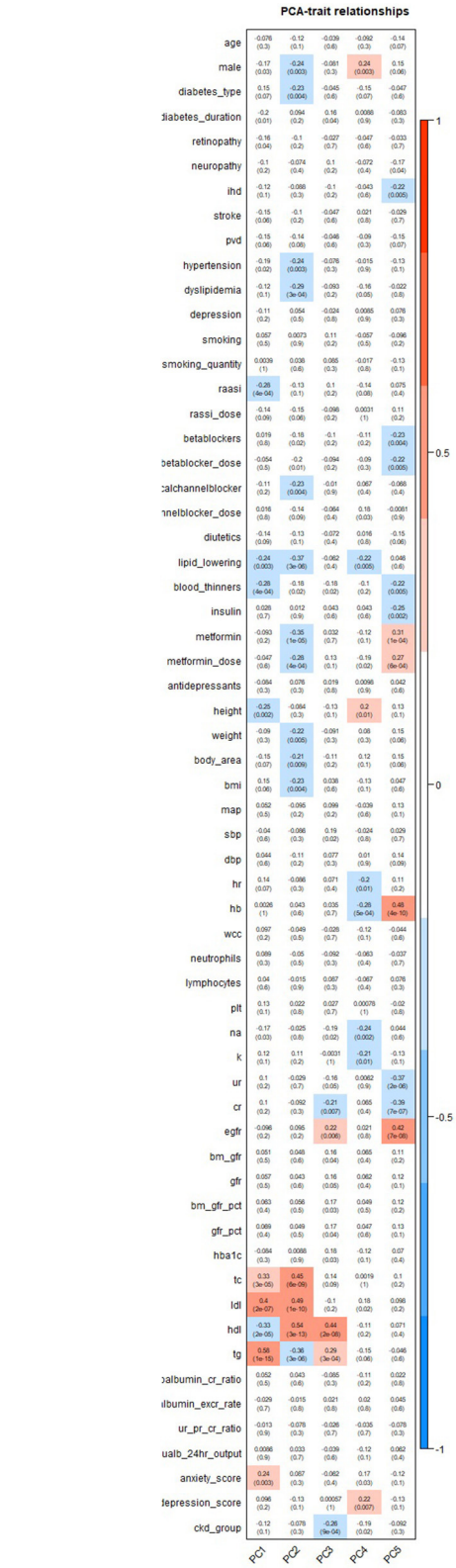

FIGURE 2 | Heatmap illustrating the correlation of principal components with clinical and biochemical characteristics.

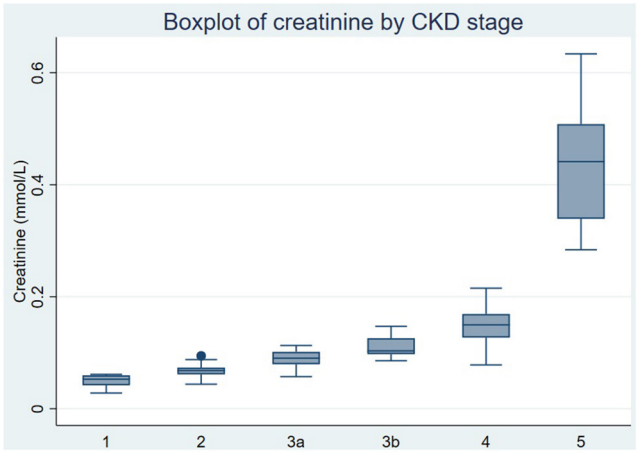

FIGURE 3 | Boxplot of creatinine by chronic kidney disease (CKD) stage.

including small very-low-density lipoprotein cholesterol (S-VLDL-C, **Supplementary Table 1A**, adj. $p < 0.05$) and very small very-low-density lipoprotein triglyceride (XS-VLDL-TG, **Supplementary Table 1A**, adj. $p < 0.05$). Significantly higher concentrations were also observed in intermediate-density lipoprotein triglyceride (IDL-TG, **Supplementary Table 1A**, adj. $p < 0.005$) and lower concentrations of a variety of medium high-density lipoprotein (M-HDL), including total cholesterol in medium high-density lipoprotein (M-HDL-C, **Supplementary Table 1A**, adj. $p < 0.05$) in late compared to early CKD. These differential metabolite profiles between late and early CKD are illustrated in **Figure 4A** and **Table 2**.

Evaluating progressive diabetes-associated CKD by stages (1–5), (**Figure 4B**, **Table 2**) we observed similar significant patterns of metabolite concentrations. Additionally, there was modest evidence for the amino acid glutamine having higher concentrations with progressive CKD stages, though this was not significant after correction for multiple testing (adj. $p = 0.06$) (**Figure 4B**, **Supplementary Table 1B**).

The metabolite Glycoprotein acetyls (GlycA), is a biomarker of chronic inflammation. Surprisingly, there was no evidence of significantly higher concentrations of GlycA, in late CKD compared with early CKD or with progressive CKD stage (**Figures 4A,B**, **Supplementary Tables 1A,B**).

Urine Metabolites in Early and Late Diabetes-Associated CKD Groups

In contrast to the significant metabolite changes observed in the plasma between late and early CKD, the urine metabolome did not demonstrate significant differences between late and early stages of CKD. Specifically, there were lower ratios of concentration of cis-aconitate to creatinine (caco/crea), citrate to creatinine (cit/crea) and indoxyl sulfate to creatinine (ind/crea) as well as lower concentrations of individual metabolites such as cis-aconitate, citrate, ethanol, glycolic acid, hippurate, indoxyl sulfate and trigoneline in late CKD compared with early CKD, but these differences were all non-significant after adjustment for multiple testing (**Figure 4C**, **Supplementary Table 1C**). Similarly, there were non-significant differences of higher ratios of concentration of dimethylamine to creatinine (dma/crea), formate to creatinine (form/crea), propylene glycol to creatinine (prgly/crea) and tryptophan to creatinine (trp/crea) were present in the urine of individuals with late CKD compared with early CKD (**Figure 4C**, **Supplementary Table 1C**).

DISCUSSION

Along the continuum of CKD, from microalbuminuria to end stage kidney disease, the risk of CVD increases exponentially (20). This study is the first to apply NMR-based metabolomics to both plasma and urine from a group of individuals with predominantly type 2 diabetes-associated chronic kidney disease, comparing late CKD with early CKD across all chronic kidney disease stages. Specifically, this study examined the lipid profile inclusive of apolipoproteins and lipoprotein subclasses and highlights a potential role of dyslipidaemia in relation to cardiovascular risk (21).

Plasma Metabolome

Validation of this NMR platform was confirmed by the step-wise increment in creatinine across stages 1–4 of diabetes-associated CKD. There was a marked increase in creatinine concentrations from CKD stage 4 to 5 (**Figure 3**) consistent with the exponential relationship between serum creatinine and eGFR and CKD stage.

Glycerides, Apolipoproteins and Lipoprotein Subclasses

Individuals with CKD have a high prevalence of hypertriglyceridemia (22), a consequence of increased production and reduced clearance of triglyceride-rich lipoproteins (TGRL) (23). Atherogenic triglyceride-rich lipoproteins comprise very-low-density lipoproteins (VLDL), chylomicrons and their remnants, intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) and lipoprotein(a) (24). ApoB is a large protein that is a component of all the atherogenic lipoproteins and provides structural integrity (24), whereas apolipoprotein A (ApoA1) is a component of high-density lipoproteins (HDL) which is anti-atherogenic (25). ApoB and ApoA1 can be directly measured in the blood and have been internationally standardized according to the World Health Organization and the International Federation of Clinical Chemistry (WHO-IFCC) (26).

In this study, we observed significantly higher concentrations of intermediate-density lipoprotein triglyceride (IDL-TG), atherogenic small very-low-density lipoprotein cholesterol (S-VLDL-C) and very small very-low-density lipoprotein triglyceride (XS-VLDL-TG) in late CKD compared to early CKD and across all stages of CKD (**Figures 4A,B**). Other atherogenic TGRLs included significantly higher concentrations of low-density lipoprotein triglyceride (LDL-TG) in late compared to early CKD as well as in every increment of CKD

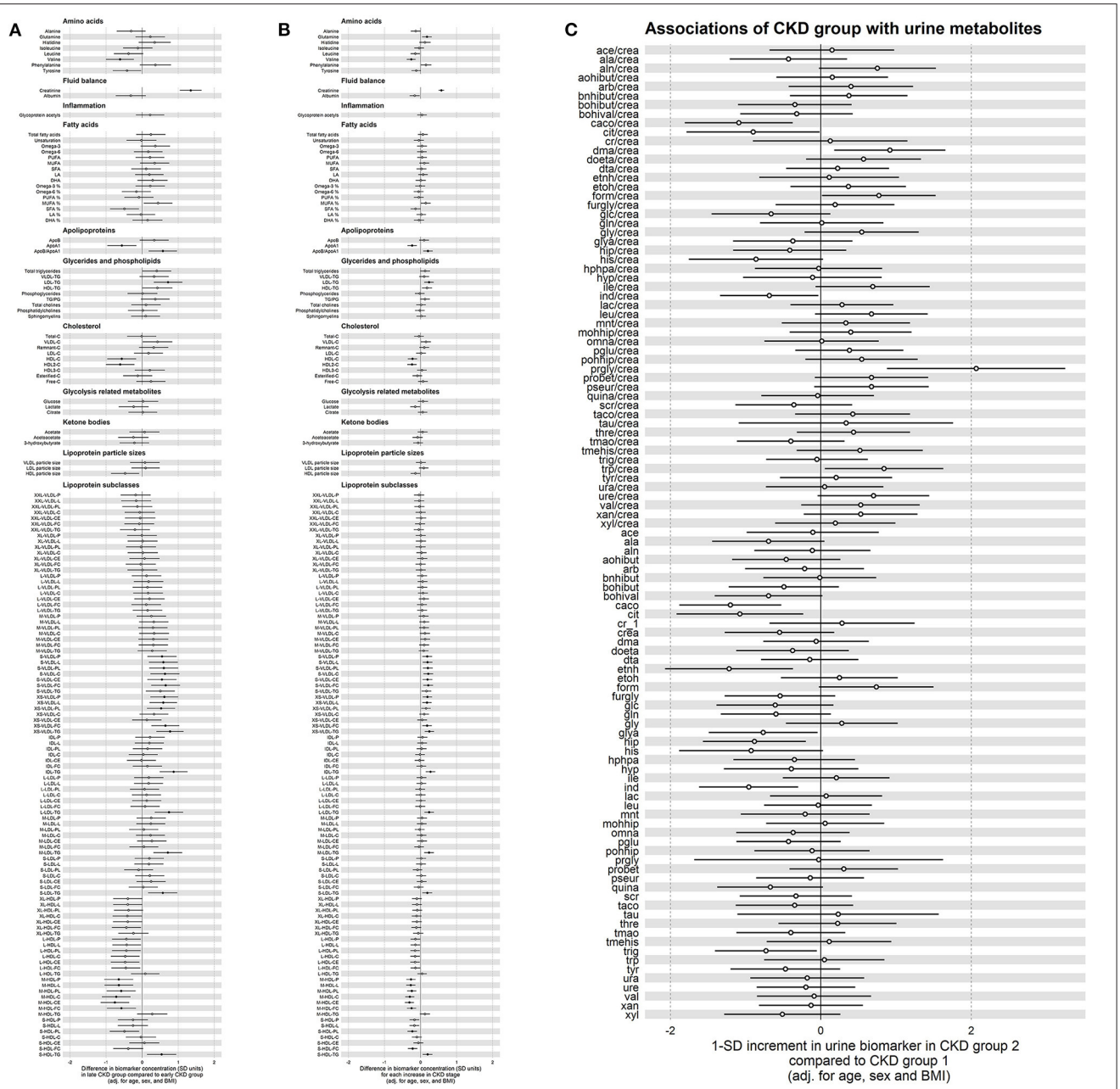

FIGURE 4 | (A) Forest plot comparing plasma metabolite concentrations between late and early chronic kidney disease (CKD) group. Black colored in circle signifies statistical significance. Values to the right of the midline are present in higher concentrations in late compared to early CKD group. Values to the left of the midline are present in lower concentrations in late compared to early CKD group. Model has been adjusted for age, sex and body mass index (BMI). **(B)** Forest plot comparing plasma metabolite concentrations at each stage of chronic kidney disease (CKD) when compared with the previous stage of CKD. Black colored in circle signifies statistical significance. Values to the right of the midline are present in higher concentrations at each stage of CKD when compared to previous stage. Values to the left of the midline are present in lower concentrations at each stage of CKD when compared to previous CKD stage. Model has been adjusted for age, sex and body mass index (BMI). **(C)** Forest plot comparing urine metabolite concentrations between late and early chronic kidney disease (CKD) group. White circle signifies no statistical significance. Values to the right of the midline are present in higher concentrations in late compared to early CKD group. Values to the left of the midline are present in lower concentrations in late compared to early CKD group. Model has been adjusted for age, sex and body mass index (BMI).

stage. These atherogenic TGRLs contribute to the progression of atherosclerosis and cardiovascular disease via intimal cholesterol deposition as well as being involved in enhancing proinflammatory, proapoptotic and procoagulant pathways (27). Evidence suggests that the sum of the total cholesterol carried by these atherogenic lipoproteins provides a better indication of cardiovascular risk than LDL-C, particularly in CKD patients with hypertriglyceridemia (27).

TABLE 2 | Summary table of differential plasma metabolites and their concentrations.

Class	Trait/metabolite Low concentration	Trait/metabolite High concentration
Amino acid	Valine	
Fluid balance		Creatinine
Apolipoprotein	ApoA1	ApoB/ApoA1
Glycerides and phospholipids		LDL-TG
Cholesterol	HDL-C, HDL2-C	
Lipoprotein subclass	M-HDL-C	S-VLDL-C
		XS-VLDL-TG
		IDL-TG

The ApoB/A1 ratio in our study was significantly higher in late CKD compared with the early CKD group and across progressive CKD stages (**Figures 4A,B**). These data are consistent with the known pathogenic processes that underpin progressive glomerular and interstitial lesions and kidney dysfunction (28). The ratio of ApoB/A1 is a well-studied risk factor for cardiovascular disease (29). The large Swedish Apolipoprotein-related MOrtality RISk (AMORIS) study measured ApoB and ApoA1 in more than 175,000 individuals prospectively followed for up to 25 years (30). The AMORIS study found a strong direct relationship between ApoB and risk of myocardial infarction (MI) and an indirect inverse relationship between ApoA1 and risk of MI. Interestingly, ApoB was a stronger risk factor than LDL-C, especially at low values of LDL-C (30). INTERHEART, was another example of a large case-control study that compared participants with a first MI to controls from 52 countries matched for age, gender and ethnicity (31). The results of this study showed that the ApoB/ApoA1 ratio in both sexes, was the strongest and most prevalent risk factor for MI when compared to other lipid measures and traditional risk factors (29, 31). The higher ApoB/ApoA1 ratio seen in our study of patients in late CKD compared to early CKD, is relevant given the magnified cardiovascular risk associated with progressive diabetes-associated CKD (18). The ATTICA study, investigated the risk stratification of apolipoprotein B, apolipoprotein A1 and the ApoB/ApoA1 ratio, in a random sample of adults, with an absence of cardiovascular disease, and showed that, using the area under the Receiver Operation Characteristic (ROC) curve, ApoB/ApoA1 was the best diagnostic marker of metabolic syndrome (32). Given the significant role metabolic syndrome plays in cardiovascular risk and most participants in our study demonstrate the metabolic syndrome, the use of ApoB/ApoA1 is most germane and adds more evidence for its use in the clinical setting.

In clinical practice, the more common measure of cardiovascular risk in the general population are LDL cholesterol concentrations, which are paradoxically not raised in individuals with CKD (22). This is in contrast to the significantly higher concentrations of TGRLs that exists with progressive kidney dysfunction, which is demonstrated in our study results. Our results also demonstrate that LDL cholesterol did not differentiate between the late CKD group compared with the early CKD group (**Figure 4A**) or across progressive CKD stages (**Figure 4B**). Another common clinical measure is the serum cholesterol assay, however, in individuals with CKD, it is generally within the normal range (33) and did not increase in this study (**Figures 4A,B**). Unfortunately, there is a paradoxical association between these low-normal serum cholesterol levels and high mortality in individuals with CKD. This anomaly is due to cardiovascular complications that arise due to increased systemic inflammation and oxidative stress which has the potential to increase oxidized LDL cholesterol levels without an increase in LDL cholesterol in CKD patients (34). It is difficult to shift the well-documented LDL-paradigm and convince committees and clinicians to accept that Apo ratios play a significant role as cardiovascular risk predictors and that these parameters need to be measured and taken into the clinical decision-making process in this group of susceptible individuals. This is reflected in the absence of an acknowledgment of apolipoprotein measurements in the most recent Kidney Disease: Improving Global Outcomes (KDIGO) lipid management guidelines (35).

The clinical implications of lipid profiles in individuals with diabetes and CKD have potential ramifications on patient management. There have been reports of novel TGRL lowering specific therapies, such as evinacumab, an angiopoietin-like protein 3 antibody that can reduce triglyceride levels by up to 70% (36). There may be a potential benefit in using these novel drug therapies beyond the standard of treatment with statins and/or cholesterol absorption inhibitors (ezetimibe) as per the current guidelines set out by KDIGO on lipid management in chronic kidney disease (37).

High-density cholesterol (HDL) is known to show anti-oxidant and anti-inflammatory characteristics as well as reduce the monocyte infiltration in artery intimal walls and thereby hinder the atherosclerotic process (34). Kidney impairment causes monocytosis and monocyte activation, which is an additional risk factor for the accelerated atherosclerosis in CKD (38). Furthermore, in individuals with CKD, there is HDL cholesterol deficiency and impaired HDL metabolism (39), which results in impaired anti-oxidant effects leading to increased oxidized LDL cholesterol formation, atherosclerotic risk and mortality (40). The results from our study underscore impaired HDL metabolism. Comparing late CKD with early CKD, as well as for every increment to CKD stage there is a significantly lower concentration of HDL-C as well as HDL-2 cholesterol (**Figures 4A,B**). This metabolomic pattern illustrates the dyslipidaemia and cardiovascular risk in this group of highly susceptible patients.

Amino Acids

The branched chain amino acids (BCAA) valine, isoleucine and leucine play a pivotal role in metabolism as precursors for the synthesis of proteins, fatty acids, regulators of protein

turnover and insulin release (41). We found a significantly lower plasma concentration of valine in the late CKD group compared with the early CKD group (**Figure 4A**) and across stages of CKD (**Figure 4B**) which was in keeping with results of other studies (42). Interestingly in a study of dialysis patients, plasma isoleucine and leucine were normal except in the malnourished patients, whereas valine was reduced more than would be expected from malnutrition alone (42). Ketoanalogues of amino acids (KA) are nitrogen-free analog supplements of essential amino acids (EAA) such as valine, leucine, isoleucine and phenylalanine (43). In individuals with CKD with impaired nutrition, as reflected by this imbalance of EAA, current evidence suggests that a low protein diet with KA supplementation should be included as part of the clinical recommendations for both the nutritional prevention and metabolic management of CKD (44).

Urine Metabolome

Urine is often considered a favorable biofluid for analysis as it is easily accessible, collected rapidly, non-invasively and cheaply. It is also chemically complex and this complexity has made it a difficult substrate to fully understand (45). Despite observing a lower concentration of metabolites such as cis-Aconitate, citrate, ethanol, glycolic acid, hippurate, indoxyl sulfate and trigoneline in late CKD compared to early CKD, these metabolites did not reach statistical significance after multiple testing (**Figure 4C**). Similarly, there were higher concentrations of particular metabolite ratios in late CKD compared to early CKD (**Figure 4C**), but these too were non-significant. This may be due to urine being highly reflective of the exposome and in contrast to plasma having greater fluctuations of metabolite levels making it harder to quantify molar concentrations of metabolites in such an unstable biological medium.

STRENGTHS AND LIMITATIONS

One of the strengths of this study is its investigation of both plasma and urine samples, using the NMR platform, across the spectrum of diabetes-associated CKD. To the best of our knowledge, it is the only study to evaluate the metabolomic profiles between late and early diabetic CKD as well as at every increment of CKD stage. It further explores a detailed plasma lipid profile that significantly differentiates between the late and early CKD groups as well as each CKD stage. One of the limitations however, is its small sample size, especially the presence of only 36 participants with the late stages (3b−5) diabetic CKD resulting in a potential loss of generalizability and lack of power to detect associations. Notwithstanding it is beneficial to have a larger number in the early group to identify possible metabolomic patterns at an earlier stage. Furthermore, the cross-sectional nature of this study meant patient samples were collected at only one time point and not studied longitudinally. Despite the limitations however, this study has shown some evidence of metabolomic variation in blood in association with diabetic CKD and hence provides the foundation for future directions for testing with a larger and longitudinal patient population.

CONCLUSION

This metabolomic analysis, inclusive of plasma and urine, provides novel insight into the metabolome of individuals with diabetes and varying degrees of kidney impairment. Our results highlight the presence of abnormal lipid metabolism namely significantly lower concentrations of the protective ApoA1, HDL-C, HDL-2 cholesterol and significantly higher concentrations of the atherogenic IDL-TG, S-VLDL-C, XS-VLDL-TG, LDL-TG and ApoB/ApoA1 ratio in late compared to early stages of CKD as well as in every increment of CKD stage compared with the previous stage of disease. Significantly, the finding of higher ApoB/ApoA1 ratio in late compared to early CKD reinforces the recommendation to use apolipoproteins, particularly ApoB/ApoA1 as novel tools for predicting lipid-related cardiovascular risk. Perhaps a new approach using the ApoB/ApoA1 paradigm is needed for future cardiovascular risk assessment in this high risk group of individuals, rather than current day clinical "standards" of serum LDL, HDL and cholesterol measures. Further research is needed to validate these findings as well as its potential role as biomarkers in diabetic CKD and CVD risk in longitudinal, large cohort studies.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article and its **Supplementary Materials**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Human Research Ethics Committee of Austin Health, Victoria, Australia (HREC/17/Austin/166) and the Human Research Ethics Committee of Deakin University, Australia. The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2013. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AL, EE, RS, and KD designed the study. AL processed initial samples. TM analyzed the data and made the figures. AL, TM, EE, RS, and KD drafted and revised the paper. All authors approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.821209/full#supplementary-material

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Association of Serum Adipokines and Resting Energy Expenditure in Patients With Chronic Kidney Disease

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Background and Aim: Metabolic disorders are prevalent in patients with chronic kidney disease (CKD) and may lead to protein energy wasting (PEW). Adipokines improve connections between PEW and energy metabolism. We aimed to determine the relationship between adipokine levels and resting energy expenditure (REE) in patients with CKD.

Methods: A total of 208 patients in non-dialyzed CKD stages 3–5 were enrolled in this cross-sectional study. Serum adipokines (leptin, adiponectin, and interleukin 6 (IL-6) were measured using enzyme-linked immunosorbent assay. Patient's REE was measured using indirect calorimetry. Fat mass (FM) and lean tissue mass (LTM) were measured using multiple-frequency bioimpedance analysis. Spearman correlation analyses and multivariate linear regression models were used to assess the association between serum adipokines and REE.

Results: The mean age was 52.7 ± 14.6 years, and 26.9, 26.4, and 46.7% of our participants had CKD stages 3, 4, and 5, respectively. The median values of serum adiponectin, leptin, and IL-6 were 470.4 (range, 291.1–802.2), 238.1 (range, 187.9–418.4), and 4.0 (range, 2.4–9.5) pg/mL, respectively. The male participants had significantly lower FM% ($P = 0.001$) and lower leptin levels ($P < 0.001$) than the female participants. After adjusting for age, diabetes, high-sensitivity C-reactive protein, intact parathyroid hormone, LTM, and FM, multiple linear regression analysis revealed that serum leptin levels were significantly positively associated with REE in men rather than in women ($P < 0.05$). Serum adiponectin levels were inversely associated with REE in men, but this association disappeared while FM was additionally adjusted. Adiponectin levels in women were not correlated with REE ($P > 0.05$). IL-6 was not significantly associated with REE in either men or women.

Conclusions: A sex-specific relationship between serum adipokines (leptin and adiponectin) and REE was observed in patients with CKD stages 3–5, which was partly confounded by FM.

Keywords: chronic kidney disease, resting energy expenditure, leptin, adiponectin, interleukin 6, adipokines, body composition

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INTRODUCTION

Individuals with chronic kidney disease (CKD) are predisposed to protein energy wasting (PEW) owing to various pathophysiological factors (1, 2), with the prevalence of 18 to 48% in patients with CKD stages 3–4 and reach as high as 75% in patients with CKD stage 5 (3), finally resulting in poor clinical outcomes and reduced quality of life (4). As a major component of wasting syndrome, altered energy expenditure is prominent and closely related to renal function, because kidney accounts for about 10% of REE (5, 6). Although key factors such as age, comorbidities, and body composition were evaluated, we cannot fully explain the individualized energy expenditure for a specific patient (7). More potential mechanisms for energy hemostasis deserve further investigation in the CKD population.

Adipokines, namely adipocyte-enriched regulatory peptides, are mainly secreted by adipose tissue (8). Adipokines are known to play an important role in energy metabolism. Elevated levels of leptin (9), adiponectin (10), and interleukin-6 (IL-6) (11), are markers of kidney injury and risk of disease progression as well as modulating factors of energy expenditure, appetite, glucose metabolism, and lipid metabolism (8, 12, 13). However, current evidence on the association of these adipokines with energy expenditure in the general population and individuals with chronic disease has shown inconsistent findings (14–18). Adipokines interventions have been studied to ameliorate weight loss-induced changes (19, 20), cachexia from CKD (21), and cancer (22, 23), but evidence, which is based on animal models or small-sample clinical trials, is still preliminary. Before adipokines are administered as a promising intervention to modulate wasting syndrome in CKD patients, we should fully explore its associations with resting energy expenditure (REE).

Therefore, we aimed to explore the independent relationship between circulating adipokines (leptin, adiponectin, and IL-6) and REE. Of note, body components including fat mass (FM) and lean tissue mass (LTM) are key contributors of REE (24). The distribution of body composition differs between sexes in CKD (25). More importantly, FM *per se* is closely associated with adipokines levels (26, 27). Thus, we constructed models for analyzing the association of adipokines and REE in male and female participants, respectively, using both FM and LTM as key confounders. Our results would be helpful to uncover the connection between adipokines and REE in CKD.

MATERIALS AND METHODS

Study Design and Patients

This is a *post hoc* analysis of the study on a novel equation for estimating REE in CKD patients (7). The study recruited outpatients with CKD according to the following inclusion criteria: age ≥18 years; non-dialyzed with CKD stages 3–5; consented to participate in all aspects of the study; willing to provide serum samples. Patients with the following conditions were excluded: abnormal thyroid function; a history of amputation; pregnancy; corticosteroid or immunosuppressive medication; comorbidities associated with protein catabolism, such as acute or chronic systemic infections, acute cardiovascular events, operations, trauma, an acute episode of gout within the previous 4 weeks, or tumors for which a patient had received radiotherapy or chemotherapy within 6 months; lung diseases that affected the measurement of gas exchange and body metabolism, such as asthma, chronic obstructive pulmonary disease, pneumothorax, and pleural effusion. The Ethics Committee of Peking University First Hospital approved the study protocol and adhered to the Declaration of Helsinki. Each patient provided written informed consent to participate in the study. This trial was registered at ClinicalTrials.gov (NCT03377413).

Demographic and Laboratory Measurements

Demographic and clinical data including age, sex, height, weight, primary renal disease, and diabetes mellitus (DM) were collected. Standing height was measured using a fixed stadiometer, and weight was measured using a calibrated digital scale.

Blood samples were collected following an overnight fast. Biochemistry data in relation to hemoglobin, serum albumin, lipids, glucose, uric acid, urea, creatinine, calcium, and phosphate were obtained using an automatic chemistry analyzer (Hitachi Chemicals). The estimated glomerular filtration rate (eGFR) was calculated using the Chinese equation for CKD patients (28). Serum concentrations of high-sensitivity C-reactive protein (hs-CRP) were measured using immune rate nephelometry (normal values, <3 mg/L). Serum intact parathyroid hormone (iPTH) levels were measured using a chemiluminescence assay (reference range, 15–65 pg/mL).

Adipokines Measurements

Serum concentrations of leptin and adiponectin were measured using enzyme-linked immunosorbent assay (ELISA) method (eBioscience, San Diego, CA, USA), with sensitivities of 6.4 and 56 pg/mL, respectively. IL-6 level was measured using commercially available ELISA kits (Beckmann Coulter Inc. Brea, CA, USA), with a sensitivity of 0.5 pg/mL. The normal IL-6 value is <6.5 pg/mL.

Body Composition

Multiple-frequency bioimpedance analysis was performed (BCM; Fresenius Medical Care, Bad Homburg, Germany); this procedure has been described in detail elsewhere (29), Briefly, with the patient positioned supine for a minimum of 10 min, standard tetrapolar electrodes were placed on the dorsal surface of the left wrist and on the anterior aspect of the left ankle. Three consecutive measurements were performed during a 2-min period, and the values of extracellular water (ECW), intracellular water (ICW), and total body water (TBW) were recorded. Based on these data, FM and LTM were estimated.

Abbreviations: BMI, body mass index; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; ECW, extracellular water; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; FM, fat mass; HF, heart failure; hs-CRP, high-sensitivity C-reactive protein; IC, indirect calorimetry; ICW, intracellular water; IL-6, interleukin 6; iPTH, intact parathyroid hormone; LTM, lean tissue mass; PEW, protein energy wasting; REE, resting energy expenditure; TBW, total body water.

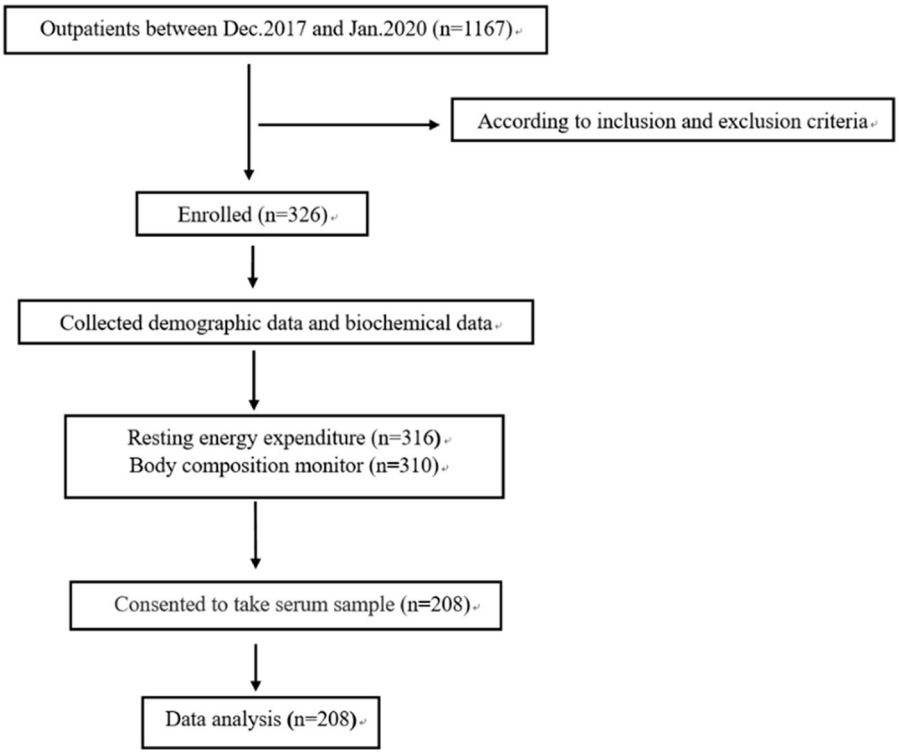

FIGURE 1 | Flow chart of the study.

Resting Energy Expenditure

REE was measured using indirect calorimetry (IC) with a VMax 29 n metabolic cart (CareFusion, Yorba Linda, CA, USA). The patients fasted overnight (>12 h). After 30 min of rest, they completed the measurements between 08:00 and 11:00 AM in a quiet, dimly lit room maintained at a constant humidity (room temperature, 20–25°C). During the test, the patients were instructed to lie supine for 15 min, breathe calmly, and avoid hyperventilation, fidgeting, or falling asleep. Oxygen consumption and carbon dioxide production were measured at 30-s intervals. Data were recorded only when the patients were in steady-state conditions, and the average O_2 and CO_2 volumes were used to calculate REE using the Weir equation (30).

Statistical Analysis

Normally distributed data are presented as mean ± standard deviation. Non-normal data are presented as median values with interquartile range. Categorical variables were expressed as percentages or ratios. Student's *t*, non-parametric, or χ^2 tests were used to compare the differences between male and female participants. Spearman correlation analyses were used to ascertain the relationship between various variables (all demographic and biochemical measurements) and REE; subsequently, the significant factors (age, DM, hs-CRP, and iPTH) were applied to a multivariate linear regression model to investigate the associations between serum leptin, adiponectin, and IL-6 and REE. Considering that the LTM and FM are components of body weight, we adopted three models to explore the independent effects of adipokines on REE: model 1 was adjusted for age, DM, hs-CRP, and iPTH; model 2 was adjusted for age, DM, hs-CRP, iPTH, and LTM; model 3 was adjusted for age, DM, hs-CRP, iPTH, and FM. Because IL-6 and hs-CRP are both inflammatory cytokines, hs-CRP was not adjusted in the three models with IL-6 as the independent variable. Because the distributions of serum adiponectin, leptin, and IL-6 levels were skewed, we used the log-transformed values of these variables in the regression analyses.

All probabilities were two-tailed, and the level of significance was set at 0.05. Statistical analysis was performed using SPSS for Windows software version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Basic Characteristics

Of the 326 patients recruited, 208 patients with stage 3–5 non-dialyzed CKD were included in the final study (**Figure 1**). The baseline characteristics of the patients are shown in **Table 1**. The basic characteristics showed that the mean age was 52.7 ± 14.6 years, 130 patients were men, and 72 (33%) had DM, which are proportional to the characteristics of the general CKD population in China published previously (31). The distribution of CKD stages was 26.9, 26.4, and 46.7% for stages 3, 4, and 5, respectively. The mean BMIs were 24.8 ± 4.2 kg/m^2, and 44.7% of the patients were overweight. The mean serum albumin

TABLE 1 | Demographic and clinical characteristics of CKD patients in male and female groups.

Variates	All ($n = 208$)	Male ($n = 130$)	Female ($n = 78$)	*P*-value
Age, y	52.7 ± 14.6	52.0 ± 15.2	53.8 ± 13.5	0.4
DM, *n* (%)	72 (33.0)	54 (41.5)	18 (23.0)	0.007
CKD stage, *n* (%)				0.881
3	56 (26.9)	33 (25.4)	23 (29.5)	
4	55 (26.4)	35 (26.9)	20 (25.6)	
5	97 (46.7)	62 (47.7)	35 (44.9)	
Height, cm	164.7 ± 8.46	169.2 ± 6.55	157.2 ± 5.36	<0.001
Weight, kg	67.7 ± 15.1	73.0 ± 15.0	58.8 ± 10.4	<0.001
BMI, kg/m^2	24.8 ± 4.20	25.4 ± 4.33	23.8 ± 3.79	0.007
Laboratory data				
Serum albumin, g/L	40.9 ± 4.44	40.8 ± 4.76	41.0 ± 3.87	0.775
Hemoglobin, g/L	116 ± 20.2	119.3 ± 21.1	110.4 ± 17.4	0.001
hs-CRP, mg/L	1.20 (0.50, 2.50)	1.17 (0.51, 2.5)	1.22 (0.6, 2.41)	0.767
Urea nitrogen, mmol/L	17.5 (22.9, 23.8)	19.9 ± 9.4	15.4 (10.3, 22.7)	0.022
Serum creatinine, μmol/L	319.5 (191.5, 542.6)	347 (230.8, 554.8)	260.5 (154.3, 523.3)	0.032
Serum calcium, mmol/L	2.29 ± 0.16	2.27 ± 0.17	2.31 ± 0.15	0.162
Serum phosphorus, mmol/L	1.44 ± 0.40	1.43 ± 0.43	1.46 ± 0.36	0.578
Serum sodium, mmol/L	140.3 ± 2.71	140.4 ± 2.41	140.1 ± 3.14	0.398
Serum potassium, mmol/L	4.57 ± 0.58	4.55 ± 0.59	4.58 ± 0.57	0.735
Total cholesterol, mmol/L	4.61 ± 1.19	4.39 ± 1.06	4.97 ± 1.31	0.01
Triglycerides, mmol/L	1.59 (1.16, 2.30)	1.64 (1.15, 2.36)	1.53 (1.19, 2.20)	0.738
iPTH, pg/mL	127.8 (72.5, 253.2)	133.5 (79.2, 256.0)	113.1 (65.2, 249.0)	0.957
eGFR, mL/min/1.73 m^2	16.4 (8.9, 30.5)	16.5 (9.28, 30.2)	16.4 (7.58, 32.4)	0.775

DM, diabetes mellitus; CKD, chronic kidney disease; BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; iPTH, intact parathyroid hormone; eGFR, estimated glomerular filtration rate.

Data are expressed as mean ± SD or median values with their lower and upper quartiles for numerical variables and percentage or ratio for categorical variables.

and hemoglobin levels were normal, with low hs-CRP values, representing a relatively stable status.

The height, weight, BMI, and percentage of DM were significantly higher in men than in women ($P < 0.01$ for all). Despite the comparable eGFR and distribution of CKD stages between sexes, the male participants had significantly higher hemoglobin, urea nitrogen, and serum creatinine but lower cholesterol values ($P = 0.001$, $P = 0.022$, $P = 0.032$ and $P = 0.01$, respectively).

Serum Adipokines, Body Composition, and REE

The distributions of serum adiponectin, leptin, and IL-6 were skewed. As shown in **Table 2**, the median values of serum adiponectin, leptin, and IL-6 were 470.4 (291.1, 802.2), 238.1 (187.9–418.4), and 4.0 (2.4–9.5) pg/mL, respectively, which were similar to those in other CKD samples (32). Men had slightly lower adiponectin and significantly lower leptin levels ($P = 0.054$ and $P < 0.001$, respectively), but their serum IL-6 levels were comparable with those of women ($P = 0.383$).

With regards to body composition, males had significantly higher ECW, ICW, TBW and LTM ($P < 0.001$ for all) but comparable FM compared with females ($P = 0.957$), a lower FM% calculated accordingly ($P = 0.001$). Due to a larger body size, REE values were also significantly higher in male patients ($P < 0.001$). Above findings are shown in **Table 2**.

Spearman correlation analyses showed that serum levels of log-transformed leptin were positively associated with REE in men ($r = 0.349$, $P = 0.000$) and women ($r = 0.284$, $P = 0.012$) (**Figures 2A,B**). Log-transformed serum adiponectin levels were negatively correlated with REE in men ($r = -0.201$, $P = 0.022$) (**Figure 2C**), but no significant difference was found in women ($P = 0.251$) (**Figure 2D**). Log-transformed IL-6 was not significantly associated with REE in either men or women (**Figures 2E,F**) ($P > 0.05$). **Table 3** listed the correlation coefficients between other variables and REE.

We performed multivariate linear regression analyses to examine the relationships between log-transformed serum adiponectin, leptin, and IL-6 and REE (**Table 4**). For men, adiponectin was negatively associated with REE in models 1 and 2 ($P = 0.02$ and $P = 0.013$, respectively). Nevertheless, when FM was introduced into the regression model, the effect of adiponectin levels on REE disappeared. There was a positive association between leptin level and REE after adjusting for age, DM, hs-CRP, and iPTH ($P < 0.001$). This association did not change even after further adjusting for LTM or FM ($P < 0.001$ and $P < 0.031$, respectively). For women, only leptin, rather than adiponectin, showed a significantly positive association with REE in models 1 and 2 ($P = 0.024$ and $P = 0.002$, respectively).

TABLE 2 | Serum adipokines, body composition and REE in male and female groups.

Variates	All ($n = 208$)	Male ($n = 130$)	Female ($n = 78$)	*P*-value
ECW, L	16.3 ± 3.53	18.0 ± 3.13	13.5 ± 2.13	<0.001
ICW, L	19.2 ± 5.16	21.6 ± 4.97	15.3 ± 2.32	<0.001
TBW, L	35.5 ± 8.16	39.6 ± 7.29	28.8 ± 4.11	<0.001
LTM, kg	39.5 ± 10.3	44.5 ± 9.06	31.1 ± 5.85	<0.001
LTM%	59.8 ± 13.0	63.3 ± 12.5	54.1 ± 11.8	<0.001
FM, kg	19.6 ± 9.2	19.6 ± 10.6	19.6 ± 7.70	0.957
FM%	28.5 ± 13.7	26.3 ± 15.6	32.2 ± 8.70	0.001
Adiponectin, pg/ml	470.4 (291.1, 802.2)	416.6 (266.9, 722.3)	553.8 (346.1, 854.9)	0.054
Leptin, pg/ml	238.1 (187.9, 418.4)	216.4 (180.0, 287.2)	350.7 (215.7, 609.7)	<0.001
IL-6, pg/ml	3.98 (2.42, 9.52)	4.09 (2.44, 10.1)	3.76 (2.32, 7.31)	0.383
REE, kcal/d	1374.2 ± 296.8	1498.2 ± 290.2	1167.5 ± 163.0	<0.001

ECW, extracellular water; ICW, intracellular water; TBW, total body water; LTM, lean tissue mass; FM, fat mass; IL, Interleukin; REE, resting energy expenditure.

When FM was introduced into the model, leptin did not retain its significant association with REE ($P = 0.186$). For both men and women, IL-6 levels were not associated with REE in any of the models ($P > 0.05$).

DISCUSSION

This study explored the association between serum adipokines, such as leptin, adiponectin, and IL-6 levels, and REE values measured by IC, the gold standard of REE, in patients with CKD stages 3–5 through a single-center cross-sectional study. Our data indicated that serum leptin and adiponectin were closely correlated with REE in all or only in male participants. The association of serum leptin, adiponectin and REE was in part confounded by the FM. Serum IL-6 levels were not associated with the REE in either unadjusted or adjusted models.

As shown in our data, serum leptin exerted an independent effect on REE after multivariate adjustment in our male participant. The correlation coefficients of serum leptin and REE are similar to those reported in the general population (14). As a peripheral signal that informs the brain of the metabolic state, it is believed that leptin increases REE through its effects on the cardiovascular system and brown adipose tissue thermogenesis *via* the hypothalamus (33). A positive association between REE and leptin was observed in cross-sectional studies involving 40 to 50 patients with chronic obstructive pulmonary disease (COPD) (17) or heart failure (HF) (27). Leptin administration in obese or normal participants increased energy expenditure and food intake. Conversely, leptin receptor antagonists reduced REE in patients with lipodystrophy (34). CKD-associated cachexia and PEW in rats were ameliorated by a leptin antagonist (21, 35), or by blockade of the leptin receptor (36). Our data support an independent relationship between serum leptin and REE in CKD, especially in men. In this context, we hope the relationship of leptin with energy expenditure in CKD patients will provide clues for blocking leptin activity as a novel therapeutic strategy for PEW in CKD. Of note, about half patients were overweighted in our study, supporting special phenomenon of obese sarcopenia in CKD population. We should observe if leptin antagonists would exacerbate obesity while energy catabolism is corrected in this population.

In contrast, our data did not show a significant independent association between serum adiponectin and REE in our CKD patients. Adiponectin is considered to play a health-promoting role in metabolic homeostasis, such as increasing fatty acid oxidation in the liver and skeletal muscle or reducing liver gluconeogenesis and inflammation (37), which suggests a potential modulator role in energy expenditure. Adiponectin injection in mice has been reported to stimulate food intake and reduce energy expenditure (38). However, observational studies involving patients with COPD, HF, and obesity have shown inconsistent findings on the association between REE and adiponectin values (17, 27, 39). On the basis of myriad pathologies of diseases, we need to explore whether using adiponectin as an agonist or antagonist can modulate energy expenditure in each specific disease. Although our data did not support an independent effect of adiponectin on energy expenditure, we still cannot exclude the possibility that adiponectin plays a role in energy balance through its modulating effect via other pathways (e.g., food intake) in CKD.

Our analysis suggested that a sex-specific relationship between serum adipokines and REE exists in CKD patients. The association between energy expenditure and serum adipokines (leptin and adiponectin) was much weaker in female patients. To the best of our knowledge, there is a different complex distribution of adipokines across sex, which could lead to distinct downstream biological effects (40–42). In addition, sex hormones may influence the biological roles of adipokines. For example, one study indicated that estradiol (43) potentiates the anorexigenic action by enhancing leptin sensitivity within the brain, whereas androgen 5α-dihydrotestosterone seems to operate in the opposite manner (44). Another study suggested that testosterone may have direct effects on the modulation of production, complex formation, and clearance of adiponectin (45). Whether sex hormones can influence the systemic effects of adipokines via specific receptors in remote target organs has

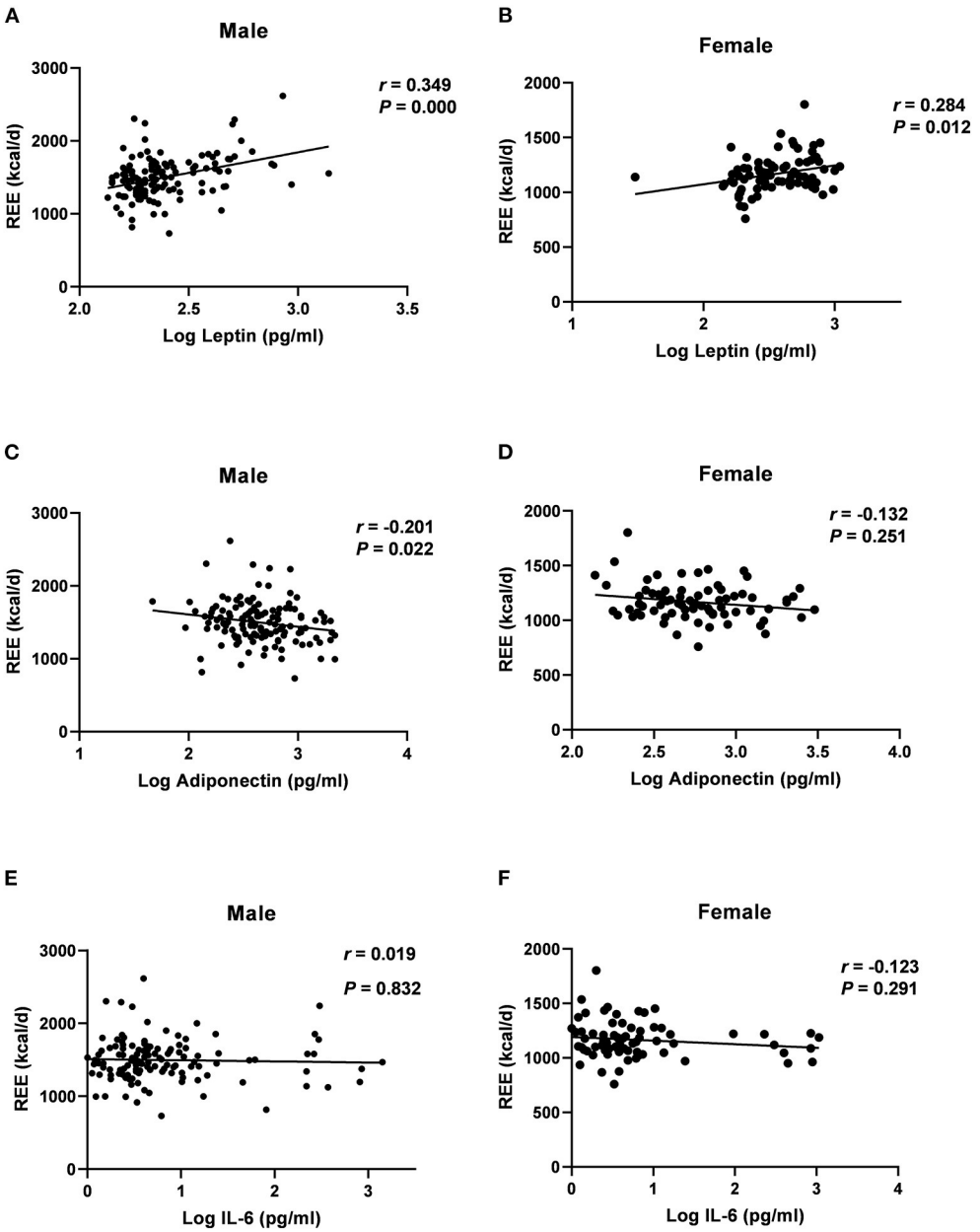

FIGURE 2 | Correlations of log-transformed adipokines level with REE in males and female groups, assessed by Log leptin **(A,B)**; Log adiponectin **(C,D)**; Log IL-6 **(E,F)**; REE, resting energy expenditure; IL, Interleukin; r, Spearman's rank correlation coefficient.

not been determined. Further research is required to explore how sexual dimorphism influences the effects of adipokines on energy expenditure.

As shown in our data, FM is a key confounder in the association between serum adipokines and REE. FM is closely linked to the concentration of adipose-derived hormones, including serum leptin and adiponectin (46, 47), which may in turn disturb the effect of adipokines on REE. In men, adiponectin was found to be negatively associated with REE, but this association disappeared after additional adjusting for FM. In women, leptin had a significantly positive association with REE, which was weakened after adjusting for FM again. Our findings are in line with previous reports that the inverse relationship between adiponectin and REE disappeared after adjusting for FM in adult women (48) and COPD patients (17). Similarly, a study showed a positive association between leptin and REE only in COPD patients before adjusting for FM (17). Given that leptin and adiponectin are the only two peptides that are selectively expressed in adipocytes (8), the impact of leptin on modulating the REE

independent of FM, as shown in our data, can be proven as the endocrine hormone that exert systemic biological effects in CKD.

Inflammation is closely associated with increased REE in patients with CKD (49–51). The relatively high levels of inflammatory cytokines, such as IL-6, in patients undergoing hemodialysis (median, 7.1 pg/mL; range, 2.2–163.5 pg/mL) (52), those with lung cancer (mean, 30.3 ± 40.2 pg/mL) (15), and those with Crohn disease (mean, 13.8 ± 13.4 pg/mL) (53), show a positive correlation with REE. However, IL-6 appeared not to be associated with REE in our cohort, as has been reported in patients with HF (27). We consider that a relatively lower IL-6 level (median, 4.0 pg/mL; range, 2.4–9.5 pg/mL) in our cohort, compared with patients with lung cancer (15) and inflammatory-bowel disease (53), represents a stable clinical state, which may mask a potential relationship between IL-6 and REE.

This study had several strengths. For the first time, the relationship between adipokines, including leptin, adiponectin, and IL-6, and REE was analyzed in patients with CKD stages 3–5. Our findings provide preliminary evidence on the potential benefits of adipokines-related interventions on energy expenditure in this population. In addition, REE was measured using IC, the gold standard of REE. Another strength of our study was that the data of men and women were analyzed separately due to different fat mass distributions. The confounding effects of LTM and FM on the relationship between adipokines and REE were further evaluated in men and women, respectively. The sex difference in the regulation of adipokines in REE will encourage further exploration of sexual dimorphism in the association between adipokines and energy expenditure.

There are some limitations to our study. First, our cross-sectional study could not determine whether associations between adipokines and REE represent a causal process. The causal relationship between REE and adipokines needs to be examined in prospective and interventional studies. Second, body compositions (FM and LTM) were estimated using the BCM instead of dual-energy X-ray absorptiometry. We did not measure visceral or subcutaneous FM using CT or MRI, which is not helpful for differentiating the effect of regional FM on serum adipokines. The complex cross-talk among FM, serum adipokines, and REE still needs to be explored in further research.

TABLE 3 | Correlation Coefficients between variables and REE.

Variable	*Total*
Age	−0.24**
DM	0.20**
LTM	0.72**
FM	0.34*
Hemoglobin	0.09
Serum albumin	−0.05
Urea nitrogen	0.15*
Serum creatinine	0.21**
Serum potassium	0.03
Serum calcium	−0.07
Serum sodium	−0.08
Serum phosphate	0.10
Triglycerides	0.28**
Total cholesterol	−0.10
hs-CRP	0.19**
iPTH	0.14*

REE, resting energy expenditure; DM, diabetes mellitus; LTM, lean tissue mass; FM, fat mass; hs-CRP, high-sensitivity C-reactive protein; iPTH, intact parathyroid hormone.
**P < 0.05 and **P < 0.01 for correlation coefficients between variables.*

TABLE 4 | Multiple linear regressions of serum adipokines and REE for males and females.

Variable	Male			Female		
	β	95%CI	*P*	β	95%CI	*P*
Leptin						
Model 1	0.33	(0.17, 0.48)	<0.001	0.25	(0.04, 0.47)	0.024
Model 2	0.28	(0.15, 0.43)	<0.001	0.28	(0.10, 0.45)	0.002
Model 3	0.17	(0.02, 0.33)	0.031	0.15	(−0.08, 0.38)	0.186
Adiponectin						
Model 1	−0.19	(−0.36, −0.03)	0.020	−0.12	(−0.36, 0.12)	0.329
Model 2	−0.18	(−0.33, −0.04)	0.013	−0.13	(−0.33, 0.07)	0.211
Model 3	−0.08	(−0.23, 0.07)	0.275	0.01	(−0.24, 0.24)	0.995
IL-6						
Model 1	0.01	(−0.16, 0.17)	0.930	−0.18	(−0.41, 0.06)	0.136
Model 2	0.01	(−0.14, 0.16)	0.904	−0.14	(−0.34, 0.05)	0.152
Model 3	−0.04	(−0.18, 0.10)	0.592	−0.21	(−0.43, 0.02)	0.067

Model 1 was adjusted for age, DM, hs-CRP, iPTH.
Model 2 was adjusted for age, DM, hs-CRP, iPTH, LTM.
Model 3 was adjusted for age, DM, hs-CRP, iPTH, FM.
hs-CRP was not adjusted in 3 models with IL-6 as the independent variable; Adiponectin, leptin, IL-6 were log-transformed for analyses.

CONCLUSION

Our results suggest that serum leptin levels are positively associated with REE in male patients with CKD stages 3–5, providing evidence for the role of leptin in energy metabolism in this population. Moreover, a sex-specific relationship between serum adipokines (leptin and adiponectin) and REE was observed, which was, in part, confounded by FM. Further research is needed on how sexual dimorphism and the distribution of body composition influence the effects of adipokines on energy expenditure.

DATA AVAILABILITY STATEMENT

Data described in the manuscript, code book, and analytic code are not readily available because the Management of China's Human Genetic Resources does not allow sharing this information. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking University First Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JD and XX: research idea and study design. NA, XX, ZY, and TM: data acquisition. NA, XX, and JD: statistical analysis and manuscript drafting or revision. JD: supervision or mentorship. All authors: read and approved the final manuscript.

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Reduced Growth, Altered Gut Microbiome and Metabolite Profile, and Increased Chronic Kidney Disease Risk in Young Pigs Consuming a Diet Containing Highly Resistant Protein

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High-heat processed foods contain proteins that are partially resistant to enzymatic digestion and pass through to the colon. The fermentation of resistant proteins by gut microbes produces products that may contribute to chronic disease risk. This pilot study examined the effects of a resistant protein diet on growth, fecal microbiome, protein fermentation metabolites, and the biomarkers of health status in pigs as a model of human digestion and metabolism. Weanling pigs were fed with standard or resistant protein diets for 4 weeks. The resistant protein, approximately half as digestible as the standard protein, was designed to enter the colon for microbial fermentation. Fecal and blood samples were collected to assess the microbiome and circulating metabolites and biomarkers. The resistant protein diet group consumed less feed and grew to ∼50% of the body mass of the standard diet group. The diets had unique effects on the fecal microbiome, as demonstrated by clustering in the principal coordinate analysis. There were 121 taxa that were significantly different between groups (adjusted-$p < 0.05$). Compared with control, plasma tri-methylamine-N-oxide, homocysteine, neopterin, and tyrosine were increased and plasma acetic acid was lowered following the resistant protein diet (all $p < 0.05$). Compared with control, estimated glomerular filtration rate ($p < 0.01$) and liver function marker aspartate aminotransferase ($p < 0.05$) were also lower following the resistant protein diet. A resistant protein diet shifted the composition of the fecal microbiome. The microbial fermentation of resistant protein affected the levels of circulating metabolites and the biomarkers of health status toward a profile indicative of increased inflammation and the risk of chronic kidney disease.

Keywords: resistant protein, microbiome, metabolomics, protein fermentation, inflammation, kidney function

INTRODUCTION

Protein is an essential macronutrient in the human diet needed for tissue growth and maintenance, the synthesis of enzymes and hormones that drive multiple integrated systems and functions, and as a source of energy (1). The recommended dietary protein intake for adults is 0.75–0.84 g/kg/day for women and men, respectively (2). Dietary protein is obtained from animal products, grains, cereals, legumes, nuts, and pulses (3). When consumed, protein undergoes enzymatic digestion and absorption in the small intestine followed by the microbial fermentation of unabsorbed portion in the large intestine. Enzymatic digestion by gastric and intestinal enzymes requires their interaction with specific amino acid recognition sequences and yields a mixed hydrolysates, such as large polypeptides, smaller peptides, and free amino acids (4). Progressive hydrolysis leads to the absorption of most dietary protein as amino acids in the jejunum (4). However, protein products, particularly larger peptides, that are resistant to enzymatic digestion (5, 6) are transported into the large intestine and undergo fermentation by the gut microbiota (4, 7).

Resistance to enzymatic digestion may result from certain conformations, natural ligands, and substituents that sterically protect otherwise digestible proteins (5). Alternately, resistance can arise from food processing. Heat-related chemical modifications that impose the loss of enzymatic recognition, such as the Maillard reaction, reduce digestibility (5, 6) and bioavailability of proteins (5, 8), and invoke microbial fermentation. Plant proteins are inherently less digestible than animal proteins, which is further attenuated by thermal processing in the presence of carbohydrates (Maillard chemistry), leading to the loss of digestibility (8). There are a number of converging factors that have increased exposure rates to less digestible, resistant forms of protein, in Western-style diets. These include the consumption of ultra-processed foods [such as, baked goods, reconstituted meat products, fast food, and many snack foods (9)], high-protein weight loss (such as, ketogenic) diets, the processed forms of infant formulae, and the popularity of "meat" substitutes made from processed plant proteins.

It is normal for a small proportion of dietary protein to be fermented in the large intestine. On average, 6–18 g of protein reaches the large intestine per day, a mixture of dietary and endogenous proteins (10) that is roughly proportional to total protein intake (11–13). However, process-modified resistant proteins increase the amount of dietary protein being fermented in the large intestine (14), which may result in health risks associated with the elevation of unhealthy protein fermentation products (7). Protein fermentation is associated with shifts in the composition, diversity, and/or relative abundance of gut microbial species, favoring nitrogen-utilizing, proteolytic microbes (11, 15–18). This poses a potential health risk when the microbiota are polarized toward nitrogen-utilization, and protein fermentation leads to the production and absorption of toxic levels of neuroactive, sulfide, aromatic and amine metabolites (7).

The metabolic pathways of protein fermentation yield multiple products capable of exerting independent effects on host tissues (7, 18, 19). Products include short- and branched-chain fatty acids, amines, ammonia, phenols, cresols, thiols, indoles, and sulfides, as well as neurotransmitters (e.g., gamma-aminobutyric acid (GABA), norepinephrine, dopamine, histamine, and serotonin) and other neuroactive compounds (e.g., tryptamine and phenethylamine) (7, 20–23). The normal levels of protein fermentation are of benefit to the host, for example, the fermentation process is required for the localized production of dopamine and serotonin that exert important signaling functions in the gut nervous system (20). However, we hypothesize that a diet high in resistant protein, and increased fermentation of resistant protein, contributes to poorer health status *via* several mechanisms related to gut microbiota composition, the production of neuroactive metabolites, and the promotion of inflammation. This pilot study aimed to examine the effects of a standard vs. highly resistant protein diet, fed at a normal protein level (21% w/w) on growth, gut microbiome, metabolomic profiles, and the biomarkers of disease risk, to identify the outcomes of interest for further study. Here, pigs are used as an appropriate animal model for human digestion and metabolism.

MATERIALS AND METHODS

Animals

Ethical approval for the project was granted by the Monash Animal Research Platform-1 Animal Ethics Committee (Approval No. 17533). This work was carried out in accordance with the Australian code for the care and use of animals for scientific purposes (24) and is reported in accordance with the ARRIVE guidelines (25). A pig model of human digestion and metabolism was used for this study as pigs have a similar diet (omnivorous) and digestive system to humans. The study involved two groups of four male weanling pigs (Large White cross Landrace). One extra animal for each group was kept in the circumstance where an animal could not continue the project due to ill health or injury, making a total of five pigs per group. The pigs were 3–4 weeks old and newly weaned on the commencement of study acclimatization period. Each group was from a single litter and was housed together for the study duration (12 h natural light/dark cycle, ambient temperature 15–20°C, with straw bedding). One group was fed a standard diet (control) and the other group was fed a resistant protein diet (intervention), each containing 21% w/w protein. Feed was administered in controlled quantities to meet 100% of the pigs' energy requirements (∼530 kJ digestible energy/kg bodyweight/day), and water was given *ad libitum*. Pigs were acclimatized on their respective diets for 7 days and monitored daily throughout this period. Following acclimatization, individual feeding was conducted two times daily in metabolic cages throughout the study, and pigs were retained in the cages on the morning of day 20 until a fecal sample was produced (**Figure 1**). For the duration of the study, pigs were monitored 3 times per week for condition; feed intake was recorded on a daily basis and body weight (to nearest 0.5 kg) was recorded two times per week. The resistant protein diet group had lower feed intake than the standard diet group, thus their diet was substituted by 25% with the standard diet from day 18 onwards.

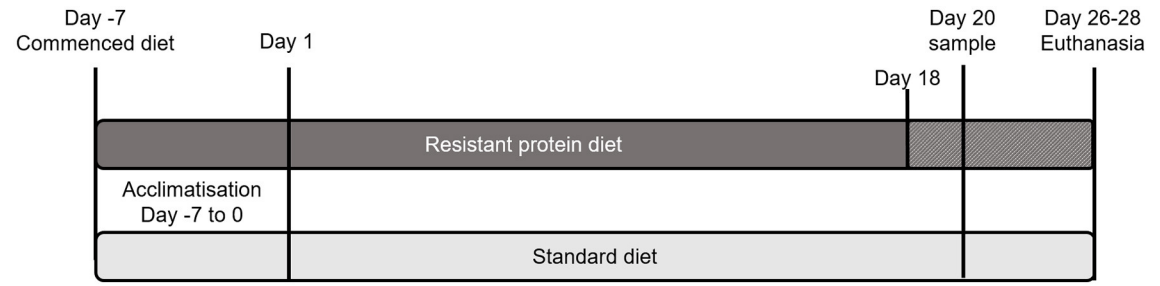

FIGURE 1 | Schematic of the study design showing the period of acclimatisation and the schedule of standard and resistant protein diets, including supplementation of resistant protein with the standard diet from Day 18 and sampling on day 20.

Sample Collection, Biochemistry, and Hematology

Fecal samples, collected on day 20, were snap frozen (dry ice) and stored at −80°C. Venous blood samples were collected as a terminal sample immediately prior to euthanasia (6–8 days after day 20 sample collection). Animals were anesthetised *via* the mask inhalation of Sevoflurane and blood samples were collected from the anterior vena cava into plain tubes (10 ml) for biochemistry, fluoride oxide tubes for glucose evaluation, and ethylenediaminetetraacetic acid (EDTA) tubes for hematology (10 ml). Samples were stored at 4°C before transport and analysis for general hematology and biochemistry markers (Gribbles Veterinary Pathology, Glenside, South Australia), such as plasma alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), urea, and creatinine. Kidney function marker, estimated glomerular filtration rate (eGFR), was calculated from body weight and the measures of plasma urea and creatinine, using a formula appropriate for swine (26).

Characterization of Diets

The standard and resistant protein diets were matched for energy, macro-, and micro-nutrient composition (**Supplementary Table 1**). The standard diet was a laboratory pig weaner diet (SF18-148 Specialty Feeds, Glen Forrest, WA) containing wheat, barley, lupins, soya meal, calcium carbonate, salt, dicalcium phosphate, lysine, and a vitamin and trace mineral premix; digestible energy 13.3 kJ/g. The resistant protein diet was a skim milk powder pig weaner diet (SF18-147 Specialty Feeds) containing barley, skim milk powder (supplied by Tatura Milk Industries, Tatura, Victoria), soya meal, canola meal, calcium carbonate, salt, dicalcium phosphate, and a vitamin and trace mineral premix; energy 13.3 kJ/g. The resistant protein diet was treated by autoclave heating (15 h at 70°C followed by 20 min at 121°C) to drive the Maillard chemistry of proteins and carbohydrates and confer digestive resistant status to the protein. The resistant protein was expected to have low enzymatic digestibility and to preferentially undergo colonic fermentation. The effect of heat treatment on micronutrients was measured by the analysis of thiamine (vitamin B_1) content (PathWest Laboratory Medicine, Nedlands, WA). The heat treatment of the resistant protein diet was designed to simulate the high heat processing that many ultra-processed food products undergo, to create a (somewhat extreme) model for a diet high in ultra-processed foods and resistant proteins.

The *in vitro* digestibility of proteins in the standard and resistant protein diets was measured by the method reported in Wu et al. (27), which simulates adult digestion conditions using gastric and intestinal enzymes and a standardized ratio of enzymes to protein nitrogen. Briefly, the method involved gastric digestion using pepsin (60 min, 0.5 ml, and 7 mg/ml) followed by duodenal digestion using a pancreatin-bile solution (180 min, 10 mg/ml pancreatin, and 60 mg/ml bile salt). Dried feed samples were dispersed in simulated gastric buffer (0.15 M sodium chloride, pH 2.5) at 1.25 mg total nitrogen/ml. Samples, such as simulated gastric buffer controls (no test protein), were preheated to 37°C and pepsin was added to initiate digestion. Sampling (125 μl) was conducted every 15 min for 60 min, with the inactivation of enzymes achieved by the addition of an equal volume of 0.25 M NaOH solution. After 60 min, the pH was adjusted to 7.5 (1 M sodium hydroxide) and pancreatin-bile solution in simulated duodenal buffer (150 mM sodium chloride, 1.0 mM sodium bicarbonate, and pH 7.5) was added. The simulated duodenal digestion proceeded for 180 min, with samples taken every 30 min (125 μl), followed by the chemical inactivation of enzyme. All samples were stored at −20°C until analysis of the degree of hydrolysis using derivatisation with o-phthaldialdehyde, monitored by a microplate reader (Excitation = 340 nm and Emission = 450 nm) (27). A standard curve was prepared using L-serine and the results of duplicate sample analysis are reported as the equivalents of L-serine.

Analysis of Fecal Microbiome

Fecal samples, collected on day 20, from three pigs in each group were sequenced for diversity profiling to compare the microbiome between the two groups. Polymerase chain reaction (PCR) amplification and sequencing were performed by the Australian Genome Research Facility (Melbourne, VIC). PCR amplicons were generated using the 341F and 806R primers to amplify the V3-V4 region of the 16S gene. Thermocycling was completed with an Applied Biosystem 384 Veriti and using Platinum SuperFi mastermix (Life Technologies, Australia) for the primary PCR. The first stage PCR was cleaned using magnetic

beads, and samples were visualized on 2% Sybr Egel (ThermoFisher). A secondary PCR to index the amplicons was performed with TaKaRa Taq DNA Polymerase (Clontech). The resulting amplicons were cleaned again using magnetic beads, quantified by fluorometry (Promega Quantifluor) and normalized. The eqimolar pool was cleaned a final time using magnetic beads to concentrate the pool and then measured using a High-Sensitivity D1000 Tape on an Agilent 2200 TapeStation. The pool was diluted to 5 nM and molarity was confirmed again using a High-Sensitivity D1000 Tape. This was followed by sequencing on an Illumina MiSeq (San Diego, CA, USA) with a V3, 600 cycle kit (2 × 300 base pairs paired-end).

16S rRNA Gene Bioinformatics Analyses

The DADA2 R package (28) was used for reads pre-processing and denosing, and the phyloseq R package for further analyses (29). For FASTQ trimming and filtering, "filterAndTrim" function was used to form the packages, setting "trimLeft" option to 20 bases for both R1 and R2 reads to remove adaptor sequences at the 5 prime of the reads, and "truncLen" option to 267 and 222 for R1 and R2, respectively, to truncate reads length at the 3 prime of the reads due to low base quality, "maxEE" was set to 2 for both R1 and R2 reads to remove reads that have too high expected error. In addition, auxiliary functions were used to dereplicate samples, to merge paired-end reads, and to estimate and correct for read errors, before applying the "Dada2" function to pick amplicon sequence variants (ASVs) with "pool" option set, which aggregates all samples together for the greater sensitivity of ASV selection. After the filtering, ASV selection and chimeric reads removal, there were between 21,875 and 51,408 unique reads that formed 2,916 unique ASVs. For *de novo* ASVs annotation, a greengenes classifier (version gg_13_8_train_set_97.fa.gz) with "assignTaxonomy" function was used. In some of the downstream analyses, ASVs were collapsed into corresponding phylum or genus bins. Additionally, a phylogenetic tree was generated using phangorn R package (30), "optimal.plm" function using GTR model, and nearest neighbor interchange rearrangement, to perform phylogenetic tree-based filtering, to remove ASVs that appeared outliers on the tree and were not of bacterial 16S rRNA origin, according to the BLAST cross referencing.

To understand individual sample α-diversity, data were analyzed using Chao1, Shannon, and Simpson's indices and plots were generated with "plot_richness" function from phyloseq package. To assess β-diversity, datasets were visualized using unweighted UniFrac distance with "ordinate" function and visualized with "plot_ordination" function. Moreover, we used the same "ordinate" and "plot_ordination" function, but with two different distances types, Bray–Curtis dissimilarity and weighted UniFrac, as a function of either diets or treatment groups. Both metrics gave very similar results estimating β-diversity. Additionally, to test the significance of the separation of the clusters between diet groups, we used a permutational multivariate analysis of variance (PERMANOVA) test, implemented in "Adonis" function, from vegan R package (31, 32) on weighted UniFrac distances with default, 999 permutation to form pseudo F-distribution.

For differential taxa occurrence, we used DESeq2 R package (33). Raw taxa counts were normalized for library size with counts per million and we used "DESeqDataSetFromMatrix" function to form a DESeq object and fitted with emodels with "DESeq" function using the Wald test and parametric fit type. Significance was determined as false discovery rate (FDR) <0.05 (34).

Analysis of Metabolites in Plasma

The quantification of metabolites was undertaken using an Agilent 1200 series high-performance liquid chromatography (HPLC) system (Agilent 255 Technologies) coupled to tandem mass spectrometry (API 3200, AB SCIEX) with electrospray ionization following previously validated protocols (35–40). All study samples were processed, run, and analyzed as a single batch. For short- and medium-chain fatty acids and ketosis markers, a chromatographic separation of processed plasma samples was conducted using a Kinetex EVO C18 analytical column (100 × 2.1 mm, 100 Å, 5 μm, Phenomenex Inc., CA, USA) under binary gradient conditions (mobile phase A: 0.1% formic acid in milliQ water containing 10 mM ammonium formate, pH 3, and mobile phase B: 0.1% formic acid in 9:1 methanol:isopropanol solution), as derivatives of benzyloxyamide (40–42), and 2-ethyl butyric acid as internal standard. For amino acids and neurotransmitters, chromatographic separation was achieved by Luna Omega Polar C18 analytical column (100 × 2.1 mm, 100 Å, 3 μm, Phenomenex Inc., CA, USA) under binary gradient conditions using mobile phase A (as above) and mobile phase B (0.1% formic acid in acetonitrile). Plasma samples with D9-Choline as internal standard were processed and derivatised as benzoyl chloride derivatives using benzoyl chloride in acetonitrile buffered at alkaline pH with volatile ammonium carbonate for MS compatibility (43). For all other metabolites, chromatographic separation was implemented by Ascentis Express HILIC column (150 × 2.1 mm, 100 Å, 2.7 μm, Supelco) under binary gradient conditions using mobile phase A (as above) and mobile phase B (as above) along with D9-Choline and 1-(4-fluorobenzyl)-5-oxoproline as internal standards for positive and negative mode, respectively. All experimental data processing and analysis were performed by Analyst (AB SCIEX, USA, version 1.6.2) and Multiquant software (AB SCIEX, USA, version 2.0).

Statistical Analysis

This was a pilot study, designed to discover the outcomes of interest in relation to high resistant protein diets, and therefore was not powered based on any one outcome measure. Data were tested for normality using Shapiro–Wilk test. Data for eGFR and liver function markers were analyzed using an independent *t*-test. Differences between the groups for feed intake, body mass, and thiamine intake on each day were assessed using the Mann–Whitney *U*-test for independent groups, with a significance threshold of $p < 0.05$. Data from targeted metabolomics analysis were mean-centered and divided by the standard deviation (SD) of each analyte. Significance was identified by a fold change threshold of 2 and a FDR (q) of 0.1, using Metaboanalyst (version 5.0).

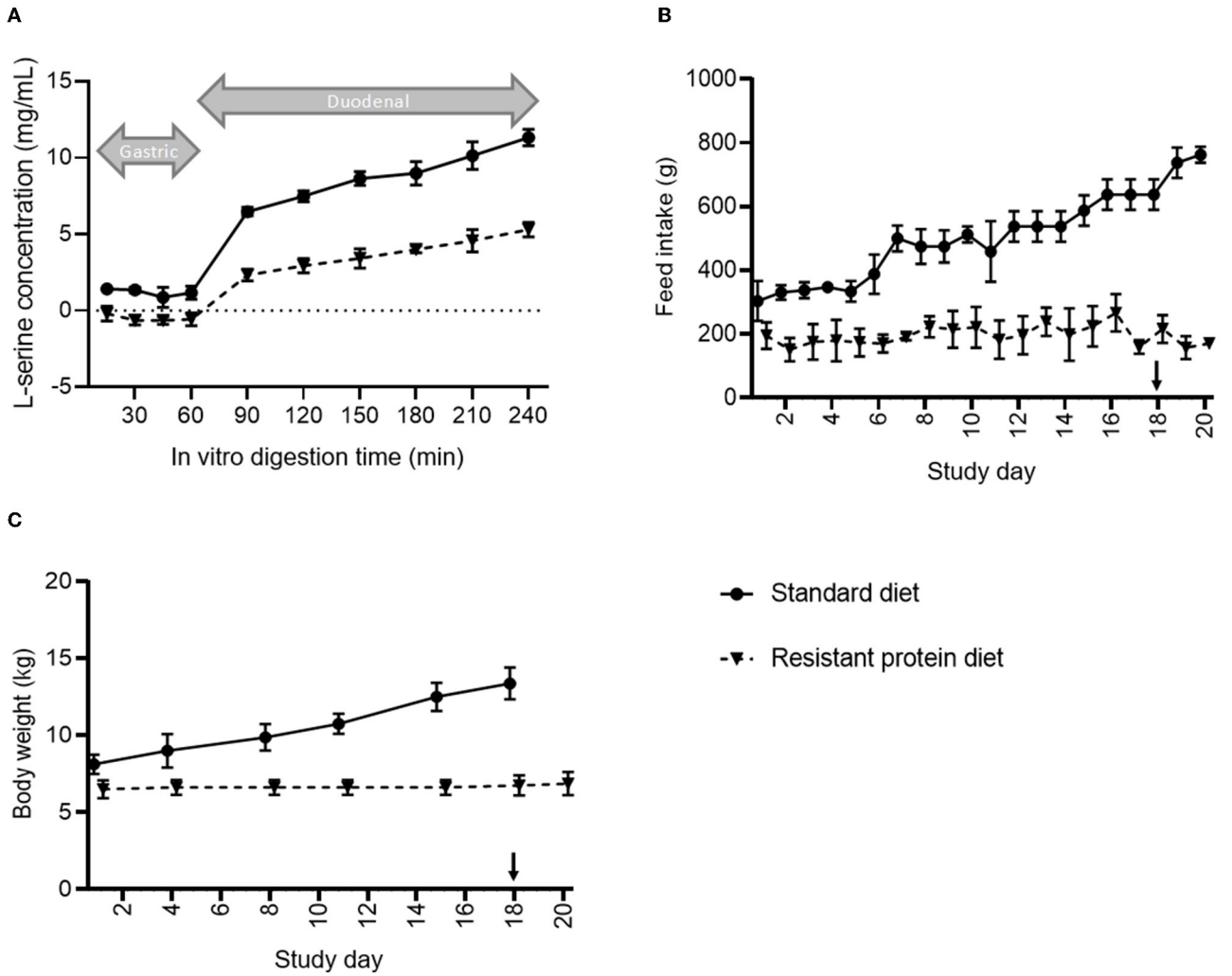

FIGURE 2 | Comparison of **(A)** *in vitro* digestibility, **(B)** average daily feed consumption, and **(C)** changes in the body mass of pigs fed standard vs. resistant protein diets, where '↓' indicates where the resistant protein diet was supplemented with 25% of the standard diet. Results represent the mean and SD of data for $n = 4$ pigs per group. From day 1 onwards, feed intake and body weight were significantly different between groups ($p < 0.05$).

RESULTS

Protein Digestibility

Following sequential *in vitro* treatment with gastric and intestinal enzymes, the digestibility of the resistant protein diet was found to be approximately 50% that of the standard diet (**Figure 2A**). This indicates that the upper gut bioavailability of proteins in the resistant protein diet was lower than that for proteins in the standard diet.

Effect of Diets on Pig Health

The mean bodyweights for the pigs in the resistant protein diet group (mean ± SD, 5.8 ± 0.7 kg) and standard diet group (8.3 ± 1.4 kg) were significantly different at baseline ($p < 0.01$), and their feed intakes and bodyweights were significantly different for the duration of the study (**Figure 2**). Although the difference in baseline mean bodyweights between the groups may have contributed to the differences in subsequent growth, it was clear that the resistant protein diet group ate significantly less of the resistant protein feed from day 1 onwards (**Figure 2B**, $p < 0.05$), which can account for their lower rate of growth (**Figure 2C**). The reduced feed intake may also have indirectly affected other markers assessed. Due to the lack of growth and initial signs of condition loss among the resistant protein diet pigs, the resistant protein feed was supplemented with 25% of the standard diet from day 18 onwards. The thiamine (vitamin B_1) content was significantly reduced in the resistant protein diet (40.5 vs. 111 μg/g, resistant vs. standard protein) indicating nutrient loss associated with autoclaving. The feed intakes of the resistant protein diet group produced a nutritional deficiency in thiamine (meeting 45–85% of recommended daily intake).

The biomarkers of liver function were similar between the two groups (ALP and GGT $p > 0.05$, **Figures 3A,C**). Compared

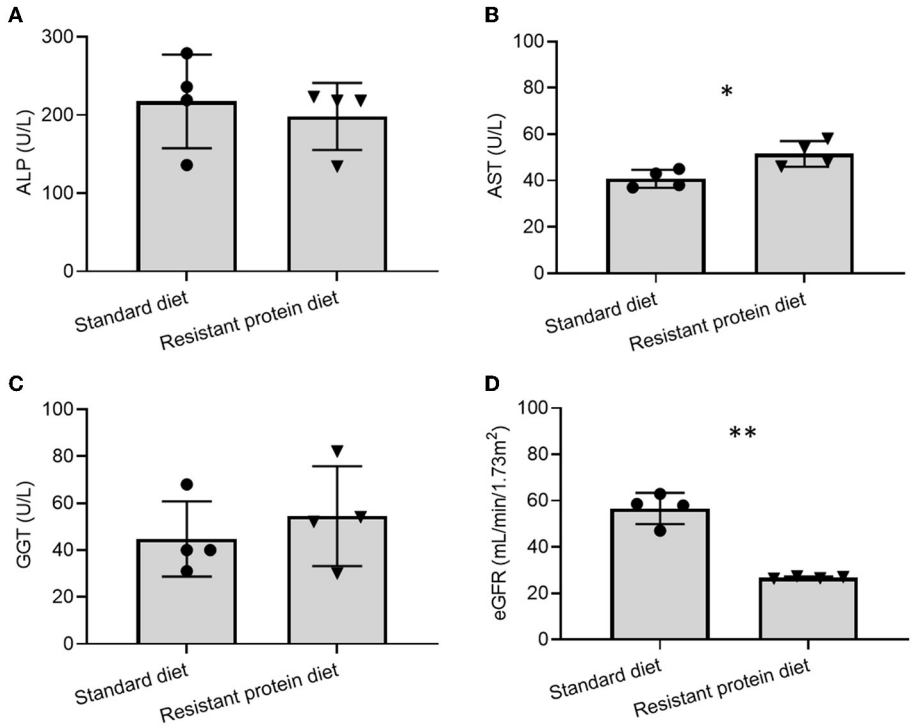

FIGURE 3 | Comparison of effects of standard and resistant proteins diets on the biomarkers of liver and renal function, showing results for **(A)** alkaline phosphatase (ALP), **(B)** aspartate aminotransferase (AST), **(C)** gamma-glutamyl transferase (GGT), and **(D)** estimated glomerular filtration rate (eGFR). Results represent the mean and SD of data, with dots indicating individual data points. Statistical difference between groups is designated as follows: $^{*}p = 0.019$, $^{**}p < 0.001$.

with the standard diet group, the resistant protein diet group had slightly increased AST ($p = 0.019$, **Figure 3B**), which could suggest a worsening liver function in that group, however, the levels of AST in both groups were within the reference range of 5–60 U/L. The kidney function of the resistant protein diet group, indicated by eGFR, was significantly lower than the standard diet group (**Figure 3D**, $p = 0.001$). Other hematology and biochemistry markers indicated similar functional and health status between the two groups (**Supplementary Table 2**).

Effect of Diets on Pig Fecal Microbiome

The effect of standard and resistant protein diets on fecal microbiome diversity and composition were assessed. The α-diversity indices, such as Observed, Chao1, Shannon, and Simpson representations, each indicated equivalent numbers of taxa and no differences in diversity or richness between the diets (**Figure 4**, all $p > 0.05$). However, the difference in the microbial composition between the groups was evident in the analyses of β-diversity (**Figure 5**), where there was a large separation between the groups in unweighted and weighted UniFrac analyses as well as Bray-Curtis distance (all $p < 0.05$). In the unweighted UniFrac principal coordinate analysis plot, the first dimension explained 41% of the total variability in the data, suggesting that different bacteria were present according to diet (**Figure 5A**). In the Bray–Curtis (**Figure 5B**) and weighted UniFrac (**Figure 5C**) principal coordinate analysis plot, the first dimension explained 52.3–66.2% of the total variability in the data, respectively, suggesting that the abundance of certain bacteria differed between the groups.

The most abundant taxa were clearly different between the two diet groups, with the standard diet group being dominated by bacteria from the phylum Firmicutes, such as *Kandleria, Butyrivibrio, Streptococcus, Catenibacterium*, and *Megasphaera* genus. In contrast, the resistant protein diet produced a high prevalence of *Bacteroidetes* phylum, such as *Prevotella*, and Firmicutes phylum, such as *Lactobacillus* sp and *Blautia* genus. Indeed, when a differential taxa occurrence analysis was performed, there were 121 ASVs that were significantly different between diets, after adjustment for multiple comparisons ($q < 0.05$, **Supplementary Table 3**). These findings were validated by an independent linear discriminant analysis. Both groups shared similar prevalence of the phyla Firmicutes, *Bacteroidetes*, Euryarchaeota, *Actinobacteria*, *Proteobacteria*, *Spirochaetes*, and *Tenericutes*. Whereas, WPS-2, TM7, and *Cyanobacteria* (although this may represent ingested chloroplasts, contamination or related phylum, such as *Melainabacteria*) were only observed in the standard diet group (**Figure 6**).

Effect of Diets on Plasma Metabolites

Compared with the standard diet group, metabolites analysis identified that tyrosine, homocysteine, tri-methylamine-N-oxide (TMAO), and neopterin were significantly increased, and acetic acid was significantly lowered in the resistant protein diet group (**Figure 7**, and **Supplementary Table 4**).

Several additional biomarkers trended towards differentiation (**Supplementary Figure 1**) but statistical significance was not reached likely due to the limited ($n = 3$) replicates analysed for each treatment.

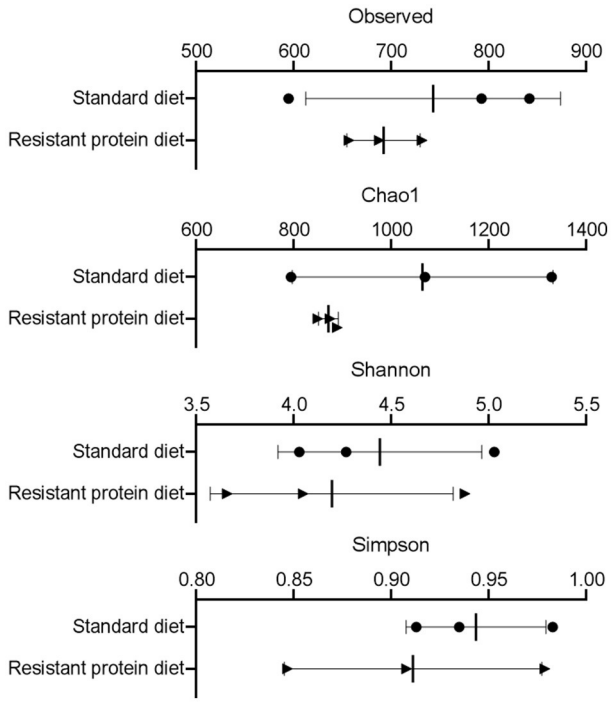

FIGURE 4 | The effects of standard and resistant protein diet on pig microbiota at day 20 showing effects on scores of α-diversity by measures of Observed taxa, Chao1, Shannon, and Simpson indices ($n = 3$ pigs per treatment). Results represent the mean and SD of data, with dots indicating individual data points. There was no significant difference between diets on α diversity (all $p > 0.05$).

DISCUSSION

This study aimed to determine the effects of substituting a standard form of dietary protein with a resistant form of protein, produced by the high-heat treatment of the feed. In both diets, the total protein was 21% of total solids, as required for the developmental stage of life of the pigs. As such, the study did not compare the effects of a high with standard protein diet, but modelled the acute response to colonic fermentation of indigestible, resistant protein (**Figure 8**), which is present in many processed protein-containing foods, albeit in lower proportions. Here we begin to address the missing links between a high resistant protein diet, the fermentation of resistant proteins, and adverse health outcomes.

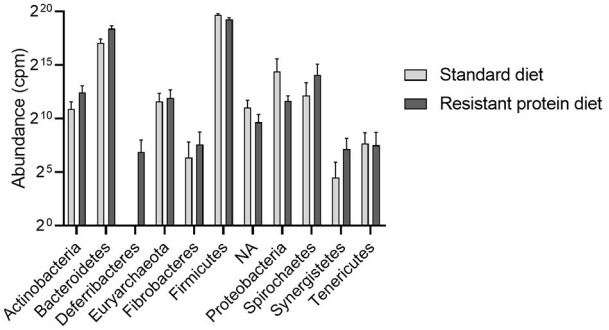

FIGURE 6 | The effects of standard and resistant protein diet on the distribution of phylum abundance in pig faecal microbiota at day 20 showing the relative abundance of the dominant phyla. Data represent the mean and SD ($n = 3$ per group). There were no significant differences between groups for any phyla. NA, phylum unspecified; cpm, counts per million.

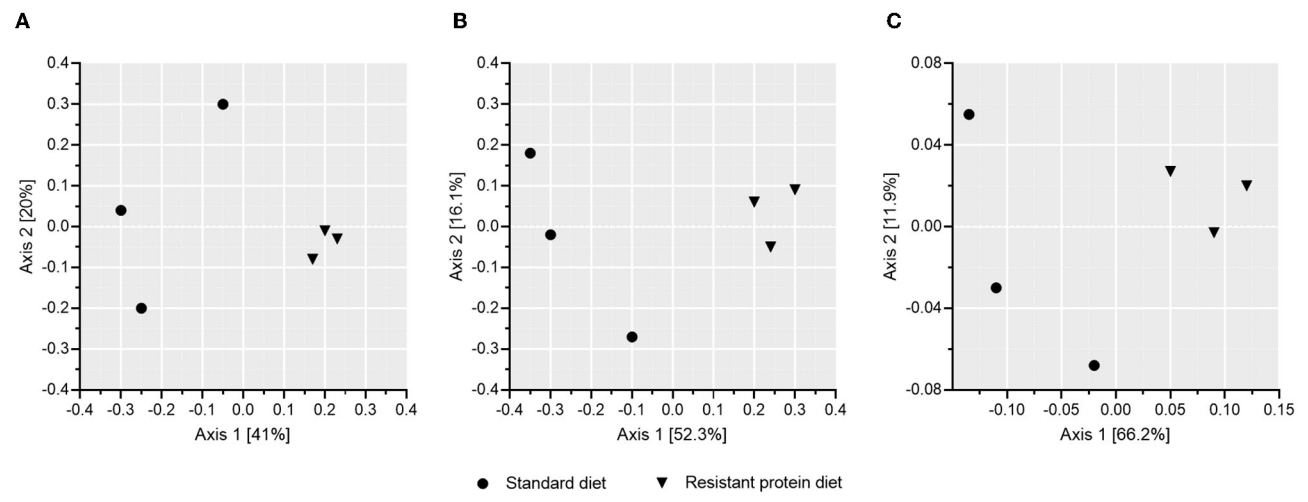

FIGURE 5 | The effects of standard and resistant protein diet on pig microbiota at day 20 showing effects on β-diversity according to mean scores of **(A)** unweighted Unifrac, **(B)** Bray–Curtis, and **(C)** weighted UniFrac analyses (presence/abundance of taxa), which indicate significant differentiation of the microbial composition due to diets ($n = 3$ pigs per treatment, all $p < 0.05$).

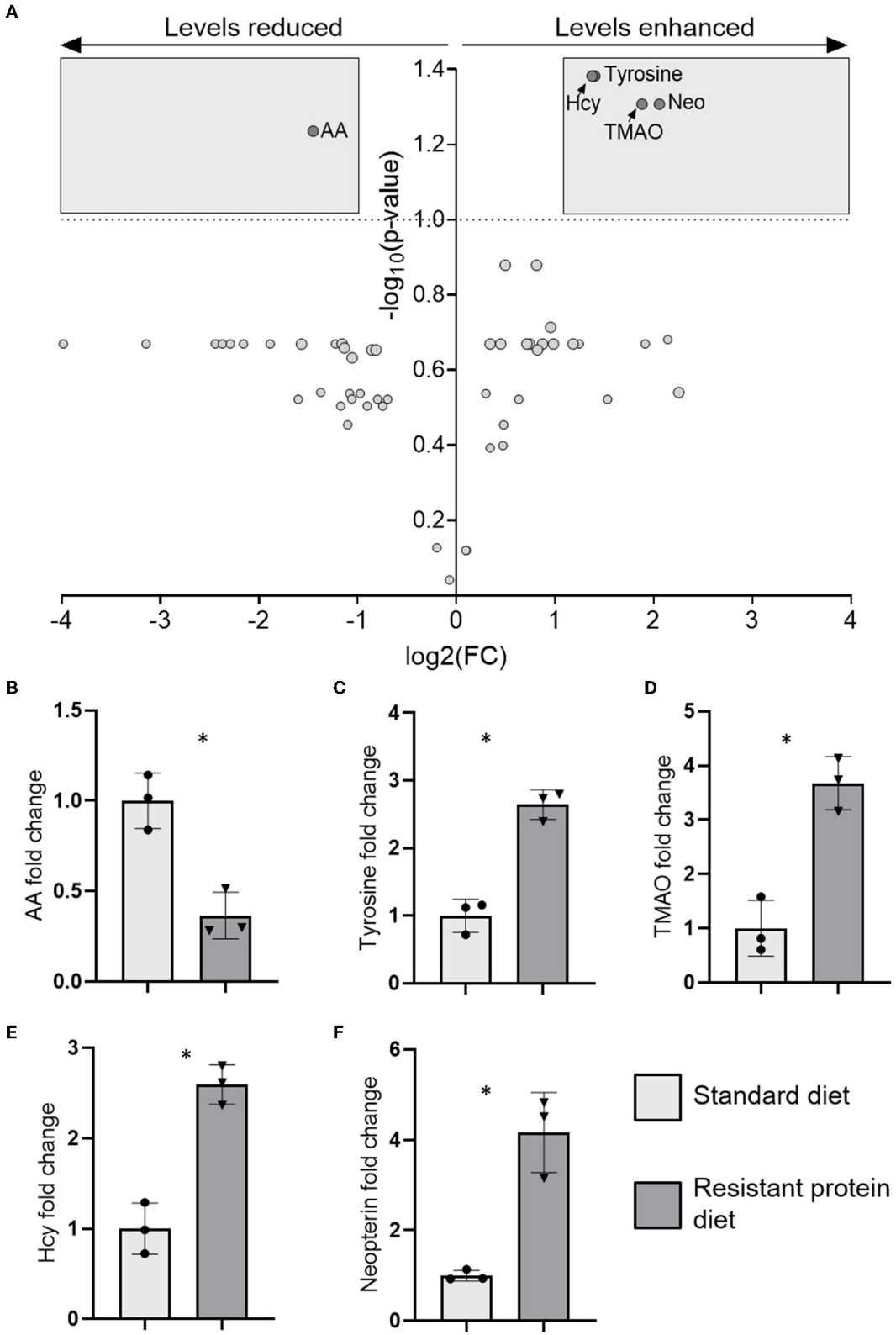

FIGURE 7 | The results of metabolomics analysis in plasma showing **(A)** volcano plot of five significantly differentiated metabolites after applying a fold-change threshold of 2 and false discovery rate (FDR) (q) of 0.1, and the fold change of those individual metabolites **(B)** acetic acid (AA), **(C)** tyrosine, **(D)** tri-methylamine-N-oxide (TMAO), **(E)** homocysteine (Hcy), and **(F)** neopterin (Neo). Data represent the mean and SD, with individual data points identified as circles (standard diet) and triangles (resistant protein diet). Statistical difference between groups is designated as follows: $^{*}p < 0.05$.

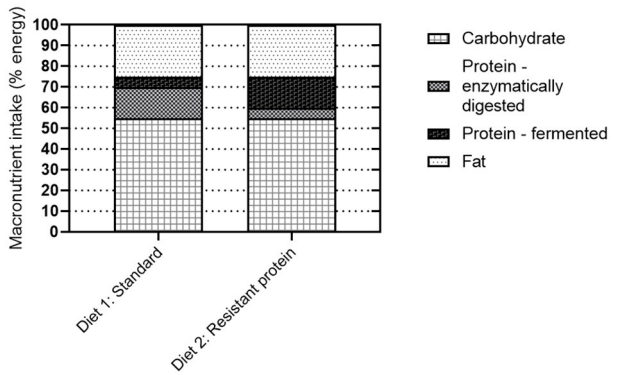

FIGURE 8 | Schematic demonstrating the proportion of protein that is enzymatically digested vs. fermented by the gut microbiota as a function of protein resistance (standard or resistant protein).

Digestibility and Bioavailability of Resistant Protein

Standard food processing techniques, such as heating, are known to compromise enzymatic digestibility and promote protein digestive resistance. For example, the prolonged heating of pre-cooked tuna (160–180°C for up to 3 h) caused significant time and temperature-dependent reductions in the enzymatic digestibility of protein (44). Similarly, the prolonged heating of a model protein, albumin, with formaldehyde or glucose caused significantly lowered protein digestibility, as demonstrated in rats and chicks (45). In the current study, the lowering of protein digestibility, i.e., digestive resistance, caused by heating was demonstrated by the loss of *in vitro* enzymatic digestibility (**Figure 2A**).

The high-heat processing of protein-containing foods results in reduced protein digestibility (resistant proteins) and the chemical products of Maillard reactions between proteins and carbohydrates, known as Maillard reaction products (MRPs) (8, 46). In a cohort of 20 adolescent boys fed a diet either low or high in MRPs for 2 weeks, 47% higher faecal nitrogen and 12% lower apparent nitrogen absorption was observed following the high-MRP diet (47). This indicates that the protein (nitrogen) from the high-MRP processed diet was resistant to enzymatic digestion and reached the colon where it could be fermented by the microbiota, and later excreted. While the excessive consumption of dietary MRPs (particularly advanced glycation end-products) is known to lead to an inflammatory phenotype (48), the effects of resistant protein fermentation on colonic or host health are less defined.

The concept of resistant protein that is incompletely digested in the small intestine and fermented in the colon, has been presented previously. While early reports suggested that protein fermentation was beneficial (5), the emerging consensus is that the fermentation of resistant protein is unfavourable for the reasons of (i) forfeited protein nutrition and (ii) fermentation metabolites, such as ammonia, phenols, indoles, sulphides, and biogenic amines, which are generally considered harmful (8, 10, 21–23). The current study confirmed that feeding resistant protein to young pigs had an adverse effect on body weight gain (**Figure 2B**), which may be partly due to reduced feed intake (**Figure 2C**). This demonstrates the forfeited protein nutrition that results from a resistant protein diet (**Figure 8**, diet 2) compared with a standard protein diet (**Figure 8**, diet 1). The following sections discuss the consequences of protein fermentation and the resulting metabolites on host health.

Effects of Resistant Protein on the Gut Microbiome

The focus of this research was to investigate the effects of resistant protein fermentation on gut microbiota composition, the resulting metabolites, and host health. To avoid the confounding effect of altering the ratio of dietary protein to carbohydrates, which can in itself affect the microbiota, energy content and macronutrient ratios were kept consistent between the diets. Therefore, shifts in microbiome and their metabolites were specific to altering the proportion of resistant to digestible protein (**Figure 8**). The colonic fermentation of protein is known to influence microbial biodiversity in the favour of species that catabolise proteins, peptides, and amino acids. In athletes, the use of a protein supplement (containing processed proteins) altered the composition of the microbiome, increasing the abundance of the *Bacteroidetes* phylum (49). Similarly, in the present study, the abundance of *Bacteroidetes* was higher (though not significantly) in the resistant protein diet group compared with the standard diet group (**Figure 6**).

However, more important than differences in certain phylum, are the differences in individual taxa. The capacity for pig gut microbiota to utilise protein has been previously demonstrated (50), and a correlation between total faecal bacteria (particularly faecal *Lactobacillus*) and nitrogen utilisation efficiency has been observed (50). Similarly, an elevation of the *Lactobacillus* taxa was observed in the resistant protein diet group in the present study (**Supplementary Figure 2**), suggesting an increased utilisation of nitrogen. However, the increased levels of *Lactobacillus* may also have been due to the milk powder in the resistant protein diet feed, which was absent from the standard diet.

The present study indicated a high prevalence of *Prevotella* genus among pigs on the resistant protein diet. A *Prevotella*-favouring enterotype has been associated with a low animal protein and saturated fat diet, and high carbohydrate and simple sugars diet. The *Prevotella* enterotype was associated with being vegetarian (51). While this initially appears to be in contrast with the present findings, it may actually support the idea that when dietary protein is more resistant to digestion (i.e., plant-based protein compared with the animal sources of protein), this encourages an expansion of the *Prevotella* genus. Similarly, data from Dong et al. (52) supports the conclusion that the increased microbial utilisation of protein is associated with an increase in *Prevotella.* They observed that faecal *Prevotella_7 spp* was depleted in people with adequate fibre intake and increased in people with higher protein intake (52). However, contrasting findings from a study on the effects of a high protein diet on the

microbiota of rats, showed a reduction in *Prevotella* among the rats on the high protein diet, compared with the normal protein diet (53).

Differences in β-diversity were observed between the diet groups in the present study (**Figure 5**), indicating the dissimilarity in microbial communities caused by the increased levels of indigestible protein reaching the colon in the resistant protein diet compared with the standard diet. Previously, shifts in β-diversity have been shown in response to high protein diets (16), supporting that bacterial communities readily adjust to utilise the substrate that is available to them. For example, compared with a standard protein diet fed to healthy mice (19.4% energy), a high protein diet (52% energy) altered several measures of β-diversity, without affecting α-diversity (16). Additionally, predicted metagenome analysis demonstrated increases in the urea cycle pathway with the high protein diet, providing an indication of increased nitrogen utilisation by the microbial community in response to the increase in dietary protein (16).

Effects of Resistant Protein on Plasma Metabolites

The microbial fermentation of protein is known to produce various compounds, such as biogenic amines, short- and branched-chain fatty acids, ammonia, phenols, cresols, indoles, and sulphides (10, 20–23). A problem with high intake of resistant protein is that many products of protein fermentation are toxic at increased levels (7). Here, we report plasma markers that reflect protein metabolites produced by the microbiota and absorbed into the bloodstream.

Biogenic Amines

It was expected that the fermentation of resistant protein by the gut microbiota would produce biogenic amines, as this has been previously demonstrated within the intestinal environment. Luo et al. (54) observed that, in comparison with a normal protein diet (20%), a low protein diet (14%) fed to piglets for 45 days reduced the levels of cadaverine (a biogenic amine) in the caecal contents. This suggests that the production of biogenic amines by the gut microbiota is proportional to the amount of protein available for fermentation. Similarly, a study in which piglets were fed diets consisting of 17, 19, or 23% protein, reported increased biogenic amines; putrescine, histamine, and spermidine, in the colonic contents of piglets on the 23% protein diet, compared with 17 and 19% protein (55). Furthermore, the level of tryptamine in the caecal contents of broiler chickens was significantly increased following a high resistant protein diet, compared with a low resistant protein diet, however, the level of total amines was lower following the high resistant protein diet (56). In the current study, tyramine trended towards elevation in plasma in the resistant protein diet group (**Supplementary Figure 1A**). Collectively, these results indicate that the fermentation of resistant protein in the colon results in the production of biogenic amines, which can be absorbed and enter the host's bloodstream.

Short-Chain Fatty Acids

The fermentation of resistant protein in the large intestine produces a range of short- and branched-chain fatty acids (57). In the present study, the plasma levels of all measured short-chain fatty acids trended to be lower, while acetic acid was significantly lower ($p < 0.05$), following the resistant protein diet compared with the standard diet (**Supplementary Figure 1C**). A similar effect was observed by Bryan et al. (56) in broiler chickens fed with high- or low-resistant protein diets, where the level of short-chain fatty acids was reduced following the high-resistant protein diet. The lower levels of acetic acid, a beneficial short-chain fatty acid, and one of the dominant short chain fatty acids produced by the fermentation of fibre in the gut (58), likely reflected the altered microbiota profile and altered fermentation substrate with the resistant protein diet. This was observed despite similar levels of fibre in the standard and resistant protein diets. Short-chain fatty acids, as well as providing energy to intestinal cells, play a role in preventing pathogenic bacterial growth, maintaining intestinal barrier function, supporting intestinal immune function, regulating fat and cholesterol synthesis in the liver, and suppressing weight gain by promoting glucagon secretion (58). The lowered levels of short-chain fatty acids observed following the resistant protein diet may, therefore, be associated with the poorer health status and increased risk of disease.

Uremic Solutes

The key indicators of health impact from protein-modified diets are the levels of amino acid-derived metabolites, which are associated with toxicity (7). The present findings indicated that plasma p-cresol, p-cresol sulphate, p-cresol glucuronide, and indoxyl sulphate were increased in the resistant protein diet group, although, likely due to the small sample size ($n = 3$), were not increased statistically (**Supplementary Figure 1D**). This is in line with a previous research, which demonstrated the production of indole and p-cresol compounds following the fermentation of proteins and peptones using faecal microbiota from healthy humans, in a model of the fermentative catabolism of intact protein (59). Indoxyl sulphate, a microbial metabolite of tryptophan (sulphated in the liver), is associated with accelerated glomerular sclerosis, endothelial dysfunction, enhanced monocyte adhesion to the vascular endothelium, and increased oxidative stress (60). Indoxyl sulphate and p-cresol sulphate (a microbial metabolite of tyrosine) both act as uremic toxins, with cytotoxic activity towards renal cells (11). In the kidney, p-cresol sulphate induces pro-inflammatory changes in cytokine mRNA expression and promotes proximal tubular cell death (61). The increased levels of p-cresol sulphate are associated with poorer clinical outcomes for patients with chronic kidney disease and were correlated with cardiovascular mortality (60). These findings indicate that resistant protein fermentation produces potentially toxic compounds, such as indoles and cresols that can enter the host's bloodstream.

Effects of Resistant Protein on Health and Disease Risk

The adverse effects of excessive colonic protein fermentation may be attributed to the development of a pathogenic and

pro-inflammatory microbial phenotype that lowers short-chain fatty acid production and increases the levels of amines, phenols, sulphides, indoles, and other bioactive derivatives of aromatic amino acids (7, 10). The following section describes the effect of the resistant protein diet on health and disease risk as indicated by the markers of systemic responses by the host.

Chronic Kidney Disease

Tyrosine, homocysteine, tri-methylamine-N-oxide, a uremic retention solute that is associated with renal dysfunction, inflammation, oxidative stress, and mortality in chronic kidney disease (CKD) (62), was increased in the resistant protein diet group (**Figure 7**). TMAO is formed through the microbial metabolism of carnitine (and choline) into trimethylamine, which is converted in the liver to TMAO (62). Similarly, neopterin, a marker of cell-mediated immunity, is also associated with renal dysfunction, inflammation, oxidative stress, and mortality in CKD (62), and was increased in the resistant protein diet group (**Figure 7**). A strong elevation of neopterin suggests a cellular immune response consistent with CKD in the pigs (63, 64). The metabolomics profile exhibited by pigs in the resistant protein diet group strongly indicated the pathology of CKD. The resistant protein diet group had worsened renal function as evidenced by lower eGFR (**Figure 3**).

Inflammation

Compared with the standard diet, several markers of pro-inflammatory status (homocysteine and neopterin) were increased in the resistant protein diet group (**Figure 7**). Chronic exposure to dietary MRPs is understood to contribute to multiple inflammation-driven degenerative diseases, *via* antagonism of the advanced glycation end-product receptor (65) and, more recently, due to the allergenicity of MRPs (66). In a similar study, rats fed with a high-MRP skim milk powder exhibited the increased levels of mediators associated with intestinal inflammation (67). In further support, an extreme high protein diet (52% of energy) fed to mice, produced the elevation of multiple plasma biomarkers of inflammation (nuclear factor-kappa B, monocyte chemoattractant protein-1, and tumour necrosis factor-α) and adversely affected both intestinal permeability and kidney function (16). Collectively, this data suggest that chronic exposure to excess process-modified protein (e.g., MRPs and resistant protein), and the microbial fermentation of that protein, contributes to chronic inflammation.

Limitations

This study demonstrated the novel effects of a diet high in resistant protein. While the design of the feeds and range of outcome markers measured were a strength of the study, we acknowledge several limitations. The sample size was small with 4 pigs per group and this was further reduced for certain analyses where only 3 pigs from each group were included due to the sample collection process or limitations in analysis capacity. The lower feed intake, due to an apparent and unforeseen dislike for the feed, in the resistant protein diet group was likely responsible for the reduced growth and weight gain, meaning this cannot be attributed to the poor bioavailability of the resistant protein. The lack of feed intake in this group may have indirectly affected other markers of metabolism and health status. Furthermore, the thiamine deficiency that resulted from the nutrient loss caused by the heating of resistant protein feed may also have negatively influenced the health status and feed intake of the pigs in the resistant protein diet group (68). Further investigation of the key findings from this work is required to gain an understanding of the health impacts of a diet high in resistant protein and the mechanisms by which they occur.

CONCLUSION

This study has demonstrated the effects of feeding a resistant protein diet, that modelled a highly processed and non-bioavailable form of protein, to pigs for 4 weeks on gut microbial composition, microbial metabolites, and markers of health status. The resistant protein was approximately half as digestible as the standard protein, with the lower bioavailability, reflected by the significantly lower gain of body mass. The consumption of resistant protein diet induced a shift in the composition of the gut microbiome and the elevation of protein-derived fermentation metabolites, confirming that the fermentation of resistant protein selects for a distinct microbial assemblage and promotes the production of nitrogenous metabolites. Plasma metabolomic analysis indicated that the levels of tyrosine, TMAO, homocysteine, and neopterin were increased, and the levels of acetic acid were reduced in the resistant protein diet group. In addition, responses to the resistant protein diet indicated reduced renal function and increased risk of CKD. In summary, the resistant protein diet appeared to invoke gut microbiome and host-mediated responses that contributed to risk factors for chronic diseases. Although the diet fed to pigs in the current study represented an extreme diet containing the high levels of resistant protein, there is some level of resistant protein present in most protein-containing processed foods and it is expected that habitual consumption over a longer period could drive the biological processes observed under these research conditions. The results justify the need to increase awareness and monitor the levels of resistant protein in processed foods and their potential relationship with adverse health outcomes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found here: https://www.ncbi.nlm.nih.gov/bioproject/798448.

ETHICS STATEMENT

The animal study was reviewed and approved by Monash Animal Research Platform-1 Animal Ethics Committee Monash University, Clayton 3800, Australia.

AUTHOR CONTRIBUTIONS

MM, SS-P, and LB: conceptualization. MM, AG, SS-P, GW, and LB: methodology. MM and VK: formal analysis. MM, AG, VK, MS, KT, TMW, TW, and LB: investigation. MC, FZM, and LB: resources. MM, MS, and KT: data curation. MM and LB: writing—original draft. MM, MC, AG, VK, FZM, SS-P, MS, KT, GW, TW, and LB: writing—review and editing. MM, MS, KT, TW, and LB: visualization. MC, FZM, GW, TMW, and LB: supervision. MM: project administration. MC, FZM, SS-P, TMW, and LB: funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Diet and Culture Among Chinese Patients Undergoing Hemodialysis: A Qualitative Study

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A growing body of research showed that diet management, such as promoting protein and vitamin intake and food restriction play a crucial role in extending time to morbidity and mortality in patients undergoing hemodialysis. However, the current dietary recommendations in nutrition guidelines lack examination of cultural factors. The study aimed to understand the diet influenced by culture in Chinese patients undergoing hemodialysis. Semi-structured interviews were conducted for 23 patients, aged 23–75 years, undergoing hemodialysis in a local tertiary hospital. Interview questions mainly focused on patients' real experience about diet, and their perceptions and attitudes toward diet. Each interview was digitally recorded, and conventional content analysis was used to attain information. The majority of patients reserved Chinese traditional dietary habits about salt and calcium intake. Although Chinese herbal medicine was not consumed, dietary therapy including brown sugar and dates was included in the participants' diet. Eggs, broth, and seafood were three prominent preferences and taboos profoundly impacted by culture. Additionally, Chinese social culture influenced patients' dietary behavior of eating at home and knowledge attainment. Diet in Chinese patients undergoing hemodialysis was still strongly influenced by culture. Culturally sensitive interventions regarding the improvement of diet intake are urgently needed.

Keywords: hemodialysis, culture, diet, nutrition, qualitative research

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BACKGROUND

Hemodialysis (HD) is the most widely available system of life maintenance for patients with end-stage kidney diseases and is reported to be used by 87% of all the patients undergoing dialysis (1–3). Nowadays, patients needing dialysis is estimated between 4.902 and 7.083 million worldwide (4). The prevalence of patients undergoing HD in China has been increasing remarkably in recent years. The number was only 3,85,000 in 2015 (5) and was close to 5,00,000 (6) in 2017. The high prevalence of HD in China places an increasing burden on both families and society due to the significant increases in the incidences of obesity and diabetes in the foreseeable future (7).

Malnutrition and abnormal serum nutrient concentrations that are strongly associated with diet have been commonly seen in patients undergoing HD with a high risk of increased morbidity and mortality (8, 9). In 2020, the Kidney Disease Outcomes Quality Initiative (KDOQI) proposed an updated global clinical practice guideline for nutrition in chronic kidney disease, which included some dietary recommendations regarding nutritional intake including protein, trace elements and minerals for patients undergoing HD (10). Protein intake of 1.0–1.2 g/kg body weight per day is

recommended to maintain a stable nutritional status in the KDOQI guide. However, daily protein intake (DPI) in most Chinese patients undergoing HD who also follow the guidelines was < 1.0 g/kg (11).

Although serum trace elements or minerals concentrations are only partly influenced by dietary intake in patients undertaking HD, it is required to alert Chinese patients' dietary habits and behaviors when facing undesirable serum concentrations compared to their counterparts in other countries. 54.5% of Chinese patients undergoing HD had serum phosphate levels > the normal range (1.7 mmol/L) (5). They had worse control of serum phosphate than their British counterparts, whose hyperphosphatemia accounted for 35.9% of the cohort (12). Higher calcium intake has previously been reported in European countries compared to the general population in China, which likely reflected different dairy intake between these countries (13). Dietary patterns may cause the higher proportion of patients with hypocalcemia (36.0%) in the Chinese patients undergoing HD (5), compared with the one in British patients (10.6%) who had calcium levels < 2.2 mmol/L (12).

Traditional Chinese Medicine with a history of thousand of years comprises of Yin and Yang theory, which represents two opposing and complementary forces and regulates the harmony between health and disease (14, 15). Food can also be categorized into Yin and Yang. Hence, the Chinese population generally believes that the preferences or taboos of food are of paramount importance to people's overall health and well-being, which influences the balance between Yin and Yang. The theory of dietary herbal medicines and dietary therapy are two crucial parts of the traditional Chinese medicine system as well. The pharmacological effects of dietary herbal medicines have been widely used to prevent or treat various diseases in China. Goji berry (*Lycium barbarum)* has always been described to work well in nourishing kidneys (16). Nowadays increasing studies showed that Lycium extracts, such as Europaeum and *Lycium barbarum* polysaccharides had the potential kidney protection (17, 18). Food is a diet providing necessary substances. In Chinese traditional culture, however, food is also regarded as medicine. Food therapy is the method of promoting health by using or adjusting food (19), which has been acknowledged by the Chinese population for more than 3,000 years (20). Additionally, Chinese people have always held a belief that animal organs correspond to human organs. If there is kidney injury, for example, then a pig's kidney can be eaten to strengthen the kidney function. Majority of Chinese health care professionals are often unaware of the influences of culture on diet management in patients undergoing HD as they are trained with western medical educational systems (14). Furthermore, Chinese patients undergoing HD generally follow the international consensus treatment rather traditional Chinese medicine. Therefore, establishing and implementing a culturally sensitive nutrition intervention requires a thorough understanding of the relationship between culture and diet in patients undergoing HD. This study aimed to understand the diet influenced by culture in Chinese patients undergoing hemodialysis.

METHOD

The qualitative study was conducted in a tertiary hospital in the east of China. The details of the study were explained to the potential participants, and then written consent was obtained from all the participants who were willing to participate in the study. Ethical approval was given by the Ethics Committee of the Affiliated Hospital of Nantong University (Ref.2015-12). The research team includes two doctoral researchers in charge of designing, implementing and analyzing the study, two graduate students assisting the participants' recruitment and one nephrology consultant providing guidance.

Participants Recruitment

Adult patients undergoing maintenance hemodialysis who participated in a larger quantitative study at a local tertiary hospital and expressed their willingness to participate in future studies were recruited. The exclusion criteria included age <18 years, receiving nutrition therapy, such as enteral nutrition or specific dietary restrictions, insufficient command over Mandarin or the dialect to give informed consent, or comply with the interview, and presence of speaking, hearing, and cognitive impairments. Maximum variation sampling (21) was adopted to increase the diversity of personal experiences within the given population (22). The participants were selected considering the wide range of demographics that are potentially associated with dietary intake or nutritional knowledge, including age, gender, residence, body mass index (BMI), education level, income, medical insurance, and living with family members.We explained the details of the consent form to patients who were willing to participate in the study and resolved their questions regarding the study. Recruitment was conducted until data saturation was achieved. Data saturation criteria referred to data replication and redundancy when additional interviews failed to uncover new ideas related to the study purpose (23).

Data Collection

A semi-structured interview pattern was applied to participants that comprised open-ended questions and prompt. The initial interview questions were developed by two first authors, based on the literature review of studies focusing on exploring the perceptions and attitudes to diet intake and the real experience about diet management among patients undergoing hemodialysis (24–26). Questions were then revised and added based on consultation with one health care professional. Two participants were randomly selected for a pilot interview, and the final interview guide was formed according to the interview results. All the interview questions were initially developed in Mandarin. Additionally, questions related to dietary culture were not initially included in the interview guide. However, researcher would make a detailed inquiry when patients spontaneously mentioned the topic. The interview guide included, "What do you think about protein for patient undergoing HD, and how do you have protein?", "What is your understanding of diet restriction in HD?", "Could you please name the foods that should be restricted as much as possible and tell me why you think it should be prohibited for patients?", "Will you

purposely choose any food?" and "How do you gain your dietary knowledge?" Some of the questions were slightly modified based on the participants' replies. For example, few participants perceived sodium control or calcium intake as a vital part of dietary management, and the researcher required them to express their views on sodium or calcium management. Furthermore, the researchers added a series of questions for successive interviews based on the content analysis results of previous interviews. These included, "How do you maintain your hemoglobin level in a normal range?", "Have you ever tried Chinese herbal medicine and what do you think of it?", and "Would you like to actively consult your doctors or nurses for dietary guidance?" Every interview was conducted with one participant and the first author in a meeting room in the hospital with the sign of "Interview in process; do not disturb" on the door. The interview times were chosen by patients as per their preference. Generally, it took participants 40 to 60 min for every interview, depending on the amount of information they wanted to share. All the interviews were digitally recorded and the transcripts were uploaded to NVivo 11.

Data Analysis

The two first authors independently read each transcript several times to gain insight into the individual participant's perception about diet by applying conventional content analyses (27). In the first cycle of coding, meaningful or recurring views of patients in the transcripts were coded to identify key points. The two primary authors used notes to keep a track of the rationale for coding. Their notes were double-checked with the first coding and were discussed by the two first authors and the corresponding author to clarify initial coding decisions by using triangulation. After a consensus was reached, a group-level analysis was conducted and the codes were combined into minor themes and then consolidated into major themes. The two first authors kept noting patterns and relationships between the categories (28), and the consensus pertaining to themes and their interpretations were discussed with all the authors.

RESULTS

Demographics of Participants

A total of 23 participants participated in this study. The mean age of participants was 44.9 years, ranging from 23 to 75 years old. 13 (57.5%) were male and 10 (42.5%) were female. The mean dialysis vintage was 6.2 years. Additionally, most participants received high school education (60.8%) and had job insurance (69.6%). The monthly income of 47.8% participants were more than 2,800 RMB and the majority of participants (87%) were living with families. **Table 1** shows the demographic details of the participants in the study.

Patients' real experience, perceptions and attitudes to diet intake were explored and their dietary habits and behaviors, dietary therapy and herbal medicine, diet preferences and taboos, and dietary knowledge attainment impacted by the Chinese culture were synthesized. **Table 2** presents examples of the themes in the study.

TABLE 1 | Demographics of participants ($n = 23$).

Characteristics	Categories	Mean ± SD/*n* (%)
Age		44.9 ± 14.6
Gender	Male/female	13 (56.5)/10 (43.5)
BMI		22.3 ± 4.0
Dialysis vintage (years)		6.2 ± 5.3
Residence	City/rural	16 (69.6)/ 7 (30.4)
Education level	Illiterate/Primary school/Junior high school/Senior high school/College/University	1 (4.3)/3 (13.0)/7 (30.4)/ 7 (30.4)/1 (4.3)/4 (17.4)
Medical insurance	Job insurance/Pension insurance/ Rural medical insurance/Paid by self	16 (69.6)/4 (17.4)/2 (8.7)/1 (4.3)
Income	>2,800 RMB/month/1,300-2,800RMB/month/<1,300 RMB/month	11 (47.8)/8 (34.8)/4 (17.4)
Living with family	Yes/No	20 (87.0)/3 (13.0)

SD, standard deviation; BMI, Body Mass Index.

Dietary Habits Influenced by Culture

Nearly no participants actively mention that salt or sodium should be restricted when describing food restriction management. Only two participants stated they avoided the intake of extremely salty food in their daily lives. "I tried not to eat any pickles as they are too salty" (woman, 31 years). "I know extremely salty food makes me thirsty. But I have meals with my family, and it is very hard for them to cook based on my needs for a long time" (man, 62 years). Only one participant believed that calcium fortification was crucial for them. None of participants actively mentioned the relationship between food and calcium intake. Bean products are Chinese popular traditional food. However, nearly all patients expressed that bean products were not suitable food for them. Patients were uncertain about the reason they rejected bean products. A couple of them stated, "Tofu contains a high quantity of protein that probably cannot be eliminated via hemodialysis" (woman, 32 years). A few patients believed bean products are detrimental for controlling hyperphosphatemia, and most participants stated, "I have no idea about the reason for its restriction. It probably contains high phosphorus" (man, 77 years). Participants were confused whether vegetables were edible for patients undergoing HD due to high potassium content in them. Some patients stated, "Chinese cabbage, leek, cabbage, and spinach should be restricted for us. They contain excess potassium" (man, 62 years). However, a few participants perceived the vegetables mentioned above as permitted and even beneficial for their health. One patient stated, "Chinese cabbage and dark leafy greens are vegetables that we can eat. They are as important as meat in providing nutrition" (man, 64 years). Similarly, although only six patients expressed that they preferred to consume

TABLE 2 | Quotations about diet influenced by culture in patients undergoing HD.

Major themes	Minor themes and coding	Exemplar quotations from patients undergoing HD
Dietary habits influenced by culture	Neglect of sodium restriction ①nearly no one mentioned sodium restriction ②Only restrict too salty food	"About diet restriction, I know we need to control phosphorus and potassium intake...both hyperphosphatemia and hyperkalemia are very dangerous." (man, 56 years) "I tried not to eat any pickles as they are too salty" (woman, 31 years)
	Maintaining low calcium intake ①bean products are perceived to be harmful ②not sure if vegetables are edible ③ dairy products were not popular in patients	"Tofu seems harmful to us. I don't know the exact reason, but I don't eat it." (male, 65 years) "I am not sure if I can eat dark leafy greens. It contains too much potassium, I think." (woman, 49 years) "I am not used to drinking milk. I heard that it contains high-phosphate levels as well." (man, 64 years)
Dietary therapy and herbal medicine	Red dates and brown sugar as excellent dietary therapy ①they are nourished ②they improve anemia	"Red dates are nourishing food. You can have several red dates and drink brown sugar to increase nutrition intake." (woman, 49 years) "Normal hemoglobin can be maintained by eating red dates but not in excess, although I am unsure of the reason. Brown sugar can maintain normal hemoglobin levels as well" (woman, 33 years)
	Dietary herbal medicine was not accepted ①it may impair kidney ②no potential effect on promoting health	"The toxicity of herbal medicine may impair kidney function." (male, 25 years) "I failed to see the effects of herbal medicine, you see, my disease has already progressed to hemodialysis" (man, 53 years)
Dietary preferences and taboos	Dietary preferences influenced by culture ①eggs as a preference for protein source ②broth as a preference for nutrition intake Dietary taboos influenced by culture ①seafood as a taboo due to its Fa-Wu characteristics	"I have an egg everyday, but only eat egg white." (man, 62 years) "the hospital provides us some food during the dialysis in the afternoon. I only have an egg." (man, 45 years) "nourishing substances are all inside the broth."(woman, 49 years) "we all know fish or fishy-flavor food is Fa-Wu. It may deteriorate our disease. " (woman, 65 years)
Dietary behaviors influenced by social culture	Dietary knowledge attainment impacted by culture ①deny to challenge experts ②feel ashamed of actively consulting with health care professionals No interest in dining out ①economic factor ②unhealthy diet	"Doctors and nurses know more about the disease than us, we don't have to disturb them with our questions. They will tell us about it anyway." (man, 64 years) "They have numerous patients in the unit. It is impolite to disturb them with my questions." (woman, 48 years) "I don't like dining out. There are too oily and too much ajinomoto in the dish. I am a patient, and the dish at the restaurant is not suitable for me" (woman, 49 years); "Eating at the restaurant is too expensive for me to afford. I have been doing hemodialysis for ten years, and the disease is a heavy economic burden for my family." (man, 45 years)

milk as a protein source, none of participants associated dairy with calcium source. The most prominent reasons for rejecting dairy products were "unpopular flavor" (woman, 30 years), "high-phosphate levels" (man, 64 years), and "excess fluid intake" (man, 65 years).

Dietary Therapy and Herbal Medicine

A few participants mentioned that protein intake could lead to an increase in hematocrit or hemoglobin levels when elaborating the benefits of protein intake. Furthermore, the patients also believed that besides protein intake, it is necessary to consume brown sugar and red dates to maintain normal hemoglobin levels. One patient stated "I know that keeping myself nourished is imperative to guarantee normal hemoglobin. Normal hemoglobin can be maintained by eating red dates but not in excess, although I am unsure of the reason. Brown sugar can maintain normal hemoglobin levels as well" (woman, 33 years). None of the participants mention that herbal medicine was included in their daily diet. When participants were asked to make a detailed inquiry about herbal medicine, one participant stated, "All medicine have toxicity to some degree, herbal medicine also has side effects. We have impaired kidney function, so toxicity can't be eliminated through kidneys" (man, 46 years). Some patients stated that they had used some herbal medicine when the kidney disease was diagnosed. However, "I failed to see the effects of herbal medicine, you see, my disease has already progressed to hemodialysis" (man, 53 years).

Dietary Preferences and Taboos

Most participants believed that consuming adequate amounts of meats, eggs and milk is an effective strategy for sufficient protein intake. However, nearly all participants reviewed eggs as the main and efficacious "protein supplement" in their daily lives. One 64-year-old male patient even stated, "An egg a day is enough for protein intake. I eat the yolk as well, and I fry eggs in excess oil." However, most patients did not consume the egg yolk; some patients ascribed it to the yolk being rich in phosphorus, and some participants stated that it is because of its high cholesterol level. Additionally, an overwhelming majority of the participants expressed being uncritical about diet or that no ingredient was prohibited in their recipes, except for seafood. Several patients expressed that they strictly avoided consuming seafood, and the rest mentioned that they only consumed a fairly small quantity of it. Their reasons were that seafood is "Fa-Wu (the food which triggers evil and causes health problems)" (man, 62 years) and "phosphorus-rich or itch-inducing" (man, 45 years; man, 48 years). Besides it, nearly all patients expressed their concern regarding broth intake. The most prominent reasons behind it were "excess fluid intake" (woman, 33 years), "high-phosphate levels" (man, 56 years), "fat-rich" (woman, 49 years), and "high-purine levels" (man, 62 years). However, there were still a few patients who believed that the nutrients of the food had been melted inside the broth. Hence, "we should drink broth due to its nourishing substances if it is possible" (women, 58 years).

Dietary Knowledge Attainment and Behavior Influenced by Social-Cultural Factors

The dietary knowledge source of the overwhelming majority of the participants was their experience after being diagnosed with kidney diseases, although a few participants expressed that health care professionals once provided dietary recommendations to them. Only three patients expressed that it would be possible for them to actively consult with their health care professionals about diet. The reasons they shared with us included, "Doctors and nurses know more about the disease than us, we don't have to disturb them with our questions. They will tell us about it anyway"(man, 64 years), "Doctors and nurses have numerous patients in the unit. It is impolite to disturb them with my questions" (woman, 48 years) and "They have hectic schedules to fail to provide dietary guidance" (man, 65 years). Concerning dining out, nearly no participants expressed that they were willing to eat at restaurants, and even others' homes. Most participants reported that they might occasionally eat out of home 1–2 times every month. Patients ascribed this low frequency to cost and unhealthy diet. A few participants stated, "I don't like dining out. There are too oily and too much ajinomoto in the dish. I am a patient, and the dish at the restaurant is not suitable for me" (woman, 49 years); "Eating at the restaurant is too expensive for me to afford. I have been doing hemodialysis for 10 years, and the disease is a heavy economic burden for my family" (man, 45 years). Even a patient reviewed eating out of home as wasting due to loss of appetite. He stated, "It is not economical for me to eat at a restaurant, because I can only eat a little bit of food due to my poor appetite" (woman, 33 years).

DISCUSSION

It has long been known that diet and nutrition are crucial to relieve systemic inflammation (29) and muscle wasting (30), improve quality of life (31), and decrease mortality risk in patients undergoing hemodialysis. A thorough understanding of diet in patients is a fundamental element in designing or conducting nutritional interventions. The evaluation system to choose lifestyle activities or not for people with chronic diseases is closely associated with their culture (32, 33). In light of the underlying relationship between diet and culture in patients undergoing maintenance hemodialysis, the qualitative study was conducted to understand Chinese patients' diet influenced by culture.

Nearly no participants actively mention the restriction of dietary salt and sodium when describing food restriction management. It potentially indicated that salt or sodium intake or restriction was not a concern for the patients in this study. The neglect of dietary salt restriciton may be ascribed to traditional Chinese diets, which are rich in salt. The mean daily salt intake of the Chinese population was 12 g in a national survey of nutrition and health status (34). Based on the data from the China National Nutrition and Health Surveillance (CNNHS) 2010–2012, the average sodium intake was 5,013 mg/day when being adjusted for energy to 2,000 kcal/day. 92.6% of adults' sodium intake exceeded the standard in the Chinese proposed intake for preventing non-communicable chronic diseases (PI-NCD) (35). This traditional diet habit may be profoundly grounded in the Chinese population, including Chinese patients undergoing hemodialysis, which was corroborated by some participants' perceptions that sodium could be restricted by only avoiding the intake of extremely salty food. Additionally, it was difficult for the Chinese patients undergoing HD to strictly restrict their salt or sodium intake. First, the group dining system has long been a tradition in Chinese. Second, caregivers in China believe that they are obligated to take good care of their sick family members by cooking for them (36). Almost none of the patients in the study needed to cook for themselves. The traditional dietary habit causes difficulties in adhering to strict salt or sodium restrictions for the whole family during their family member's long duration of hemodialysis.

Another dietary habit influenced by traditional Chinese culture is calcium intake. Chinese residence maintain low calcium intake for a long-term, which has been revealed based on nationally represented population surveys (37, 38). In western countries, calcium sources mainly include milk, cheese, legume and vegetables, in which dairy products are optimal and the predominant food rich in calcium (39). However, an investigation involving 9 provinces in China showed that vegetables (40%), bean products (20%) are main calcium sources for Chinese population (40). Although dairy products has been increased in Chinese recipe in the past decades of years, only 12% of calcium intake originates from dairy products (40).

Participants in the study, like most Chinese people (41), even Chinese immigrants living in other countries (42), may have low dairy consumption. A multicenter investigation across four countries has revealed that the lower dairy intake in the Chinese participants likely led to the lower calcium intake between these countries (13). Admittedly, not everyone is fond of dairy products, and Chinese patients undergoing HD can increase the intake of calcium through consuming vegetables and bean products. However, obviously, Chinese people failed to maintain sufficient calcium intake through vegetables and bean products (40). Especially, patients undergoing HD were concerned about vegetables intake due to their potassium contents. Furthermore, patients held a belief that bean products may impair kidney function due to high-purine or high-phosphorus. Therefore, traditional low calcium intake, including low intake of dairy, vegetables and bean products may partly explain the high levels (36.0%) of hypocalcemia reported in data from Chinese patients with hemodialysis (43) except for the influence of bone mineral disease or parathyroid hormone on serum calcium levels.

There was no concept of protein in traditional Chinese culture, leading to confusion between protein and nourishing substances in participants. Chinese people believe nutritious food is multifunctional, including strengthening the immunity system and improving anemia. It may lead to the finding in the present study in which a few patients undergoing hemodialysis still misunderstood the relationship between protein intake and hemoglobin. They considered protein and other nutritious food intakes as a guaranteed method of relieving anemia. Traditional Chinese beliefs about dietary therapy were corroborated by the evidence of the patients' awareness of the increase in red dates and brown sugar intake to improve anemia. According to the Compendium of Materia Medica, dates are considered to be a Chinese traditional medicine that nourishes blood (partly by improving anemia). Zhang et al. believed that the combined effect of polysaccharides, cyclic adenosine, vitamins, inorganic salts, and other active components of dates nourishes the blood (44). Meanwhile, an increasing number of questions have been raised about the relationship between dates and anemia. The iron content of 2.4 mg in 100 g dates is much lower than that contained in animal-based foods. There is insufficient evidence of the beneficial effect of dates on iron deficiency anemia, as stated in a systematic review (45). Similarly, the Compendium of Materia Medica also indicates that brown sugar is perceived as a superfood that provides energy and nourishes the blood. To date, it is widely used by women with menstrual disorders and new mothers. A review indicated many health effects of brown sugar related to immunology, anti-toxicity, and cytoprotective ability (46). However, few publications provided sufficient documentation or evidence of improving anemia. Additionally, the iron content of approximately 2 mg in 100 g brown sugar is incapable of meeting the 20 mg/d iron requirement of healthy adult women. In terms of dietary herbal medicines, although some participants in the study once had tried, all of them expressed that they didn't consume them at that moment. Two traditional dietary medicine consisting of herbal tea and herbal soups have been widely used for several centuries in China. However, it is mainly popular in the local populations of southern China. The participants in the study come from the east of China. Potentially, the impact of traditional culture is diverse on different regions. The study identified that Chinese culture failed to significantly influence the consumption of herbal medicine in patients undergoing hemodialysis in eastern China.

The patients undergoing HD in the study perceived the intake of protein to be crucial. They reviewed meats, eggs and dairy products as protein sources and ignored protein contributed by plant products. However, increasing studies showed that plant protein are associated with favorable kidney disease outcomes, and even with low mortality in those with estimated glomerular filtration rate < 60 ml/min/1.73 m^2 (47, 48). Additionally, participants' main source of protein in the study was egg rather than meat or other animal foods. Eggs long have been reviewed as one of the most important and nutritious food in Chinese diet culture. Its value is even utterly beyond other nourishing substances. A comparative study has shown that daily egg consumption (53.4%) was significantly higher in Chinese students compared to international students (32.8%) (49). It is also confirmed by the dietary habits of postpartum women in China. A comparative study regarding food intake during puerperium between civic and rural areas of Shandong Province found that the most popular food during puerperium were eggs in civic (96.17%) and rural (98.5%) women. Eggs were even regarded as staple food in rural postpartum women, accounting for the highest proportion (71.28%) of diet during puerperium (50). Additionally, most patients in the present study considered yolk as phosphorus-rich food. However, they still preferred egg white as the more effective strategy to promote protein intake. Restriction of the intake of the yolk is consistent that the phosphorus content in the yolk (586 mg/100 g) represents one of the highest concentrations of phosphorus in naturally occurring sources (51). Nevertheless, patients' attitudes toward egg yolk and egg white intake indicated the importance of encouraging patients to increase their protein intake sources and understand the common food sources of phosphorus, although egg white is one of the high-quality protein sources (52). The broth is a diet preference in a few of participants as well, although there were concerns due to its fluid overload, high fat and purine, and phosphorus-rich. These patients' beliefs to broth were in agreement with the Chinese proverb "Having soup is better than eating meat", which holds that all fine nutritious substances of food are in soup. Stewing soup considered as optimal diet optimal diet has been widely consumed in the south of China. However, modern medicine and studies have confirmed that the overwhelming amount of protein remained in the food rather than in soup. Furthermore, soup, especially broth contains large amounts of purine and nitrogen substances (53) that are harmful to health or disease progression in patients undergoing hemodialysis.

Seafood was restricted by the participants partly due to its high phosphorus or itch-inducing. The impact of phosphorous contents of seafood on patients undergoing hemodialysis is still full of debate. An investigation examining the nutrients included in 14 sea fish species found that the highest phosphorus contents per 100 g of fish were 227.52 mg and the lowest was 50.86 mg (54), which remarkably lower than ones in red meat

(311 mg/100 g) and egg yolk (586 mg/100 g). Increasing studies have shown seafood was potentially an excellent source of low-fat protein for patients undergoing HD (51). However, apart from phosphorus-rich of seafood, a few participants expressed concern about seafood as "Fa-Wu (stimulating food)". Fa-Wu is the food forbidden in sick or vulnerable people. It is one of the important compositions in TCM. The Fa-Wu belief holds the traits of promoting wind (roughly refers to pernicious influence factors that can invade bodies and become a destructive influence) and provoking Qi (roughly means energy), resulting in the disorders of Qi-blood in the body or deterioration of diseases. Fish or food with fishy flavor is the most commonly seen Fa-Wu in various Chinese medical literature (55), which was corroborated by the finding of the study, indicating the strong influence of the Fa-Wu belief on patients' perceptions or behavior about diet taboos.

A low frequency of dining out was reported in most participants in the study, which might be ascribed to the influence of social culture in the Chinese population. Chinese people are family-oriented and they are more willing to cook and eat at home. This explains the reason that it is hard to accept eating at the restaurant at the Spring Festival. A qualitative study exploring diet and culture found that even Chinese American children had been limited the frequency of eating at restaurants, ranging from once a week to only on special occasions (42). A systematic review revealed that eating out of home was linked with higher socioeconomic status (52), which is consistent with the participants' concern about cost. Especially for patients undergoing hemodialysis, long-term hemodialysis has been an economic burden in China. Additionally, the review also found that eating out of home was associated with higher energy and fat intake and lower micronutrient intake (52), which is similar to the participants' concern about the influence of dining out on health. Chinese people believe that food out of home is added more oil and ingredients, which is regarded as unhealthy and even pernicious. Traditional Chinese culture holds that the kidney is an organ for eliminating 'toxicity'. Hence, patients undergoing hemodialysis had more concern that their impaired kidney function was unable to get rid of the increased toxicity due to eating out of home. Another diet characteristic impacted by social culture was patients' social sensitivity when gaining dietary knowledge (14). It was shown that participants in the study would not actively consult with health care professionals or express their concerns. Chinese harbored a deep-seated evasive attitudes when it came to challenging experts. Addtionally, it is well known that Chinese population always hold implicit attitudes to some sensitive topics, which has been influenced by social culture for thousand of years as well.The evasive and implicit attitudes influenced by culture in the study may cause patients to fail to obtain dietary knowledge or follow the prescribed regimen, which in turn, lead to potentially suboptimal dietary intake.

CONCLUSION

Diet in Chinese patients undergoing HD were strongly influenced by culture, embodied in traditional dietary habits for salt and calcium intake, dietary therapy with brown sugar and red dates, dietary preference for eggs and broth, and dietary taboos for seafood. Additionally, Chinese social culture may affect the dietary behavior regarding eating at home and dietary knowledge attainment due to the rejection of challenging experts. It is urgently required for health care providers to appreciate the influence of Chinese culture on dietary management in patients undergoing HD. While providing appropriate counseling for patients on how to improve dietary intake, doctors and nurses need to be aware of culturally sensitive dietary education or interventions. Diet management and nutritional status can be potentially enhanced by understanding Chinese culture and how they influence dietary habits and behaviors in patients undergoing HD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Affiliated Hospital of Nantong University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YS and JW approached and recruited participants from hemodialysis units and conducted the interviews, independently transcribed each interview and completed the first cycle of coding. Their notes using to keep a track of rationale for coding were double-checked with the first coding and were discussed by YS, JW, and XC to clarify initial coding decisions. After a consensus was reached, further analysis including combination of the subcategories and consolidation of categories were completed by all authors in the study. Additionally, JW participated in the writing and editing English for the article. HL, XC, and MZ work at hemodialysis units and assisted the recruitment and explanation before the completion of consent forms. All authors contributed to the article and approved the submitted version.

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Corrigendum: Diet and Culture Among Chinese Patients Undergoing Hemodialysis: A Qualitative Study

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In the published article, there was an error in affiliation 3. Instead of "Department of Nephrology, Affiliated Hospital of Nantong, Nantong, China," it should be "Department of Nephrology, Affiliated Hospital of Nantong University, Nantong, China."

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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Nutritional Status and Other Clinical Variables Are Associated to the Resting Energy Expenditure in Patients With Chronic Kidney Disease: A Validity Study

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Background: Estimating energy requirements (ER) is crucial for nutritional attention to chronic kidney disease (CKD) patients. Current guidelines recommend measuring ER with indirect calorimetry (IC) when possible. Due to clinical settings, the use of simple formulas is preferred. Few studies have modeled equations for estimating ER for CKD. Nevertheless, variables of interest such as nutritional status and strength have not been explored in these models. This study aimed to develop and validate a model for estimating REE in patients with CKD stages 3–5, who were not receiving renal replacement therapy (RTT), using clinical variables and comparing it with indirect calorimetry as the gold standard.

Methods: In this study 80 patients with CKD participated. Indirect calorimetry (IC) was performed in all patients. The calorimeter analyzed metabolic measurements every minute for 15 min after autocalibration with barometric pressure, temperature, and humidity. Bioelectrical Impedance Analysis (BIA) was performed. Fat-free mass (FFM) was registered among other bioelectrical components. Handgrip strength (HGS) was evaluated and an average of 3 repetitions was recorded. Nutritional status was assessed with the subjective global assessment (SGA). Patients categorized as B or C were then considered as having malnutrition.

Results: We analyzed 71 patients and 3 models were generated. Model 1a included FFM; Model 2a included weight; Model 3c included handgrip strength (HGS). All other variables were stepwise, computer-selected with a $p < 0.01$ significance level; Malnutrition was consistently associated with ER among other clinical variables in all models ($p < 0.05$). The model that included BIA-FFM had $R^2_{adjusted} = 0.46$, while the model that included weight (Kg) had an adjusted $R^2_{adjusted} = 0.44$. The models had moderate concordance, LC = 0.60–0.65 with the gold standard, whereas other energy expenditure estimation equations had LC = 0.36 and 0.55 with indirect calorimetry.

Using these previously validated equations as a reference, our models had concordance values ranging from 0.66 to 0.80 with them.

Conclusion: Models incorporating nutritional status and other clinical variables such as weight, FFM, comorbidities, gender, and age have a moderate agreement with REE. The agreement between our models and others previously validated for the CKD patient is good; however, the agreement between the latter and IC measurements is moderate. The KDOQI lowest recommendation (25 Kcals/kg body weight) considering the 22% difference with respect to the IC for total energy expenditure rather than for REE.

Keywords: energy requirements, nutritional attention, indirect calorimetry, resting energy expenditure, chronic kidney disease, energy, equation, validity

INTRODUCTION

Estimating energy requirements (ER) is a crucial step in providing nutritional care to any population, especially to those at risk of malnutrition (1). Patients with chronic kidney disease (CKD) undergo a variety of physiological changes that may affect their ER and, thus, their nutritional status (2, 3). Historically, only a few studies have demonstrated a neutral energy balance with patients with CKD consuming approximately 30 kcal/day (4, 5). On the other hand, there is evidence of a lack of agreement between estimates of ER and actual resting energy expenditure (REE), which is frequently lower than estimated (6). Whenever it is possible, current guidelines recommend measuring ER using indirect calorimetry (IC) (7). Due to the complexity of clinical settings, simple formulas, and clinical criteria based on body composition, age, sex, comorbidities, and nutritional status, among others, such as inflammation, are also suggested for assessing ER. However, their quantitative contribution has not been established, and the general recommendation for estimating ER is the simple formula of 25–35 kcal/kg/day (7).

In 2021, two distinct studies (8, 9) published more complex and comprehensive formulas for estimating REE, both of which included body composition, gender, age, and comorbidities as indirect variables in linear regression models. However, none of the other variables suggested in the guidelines were studied. Subjective global assessment (SGA) is a valid tool for assessing nutritional status in patients with CKD (10).

Previous research has shown that SGA has an association with body composition when evaluated with BIA (11). Thus according to Steiber et al. (12) SGA classification may be a useful inclusion in an equation for estimating REE. On the other hand, while fat-free mass (FFM) is the most important variable associated with REE, current evidence suggests that FFM should be considered not only in terms of quantity but also in terms of quality due to the numerous clinical outcomes associated with it (including survival and improved QoL) (13). Thus, handgrip strength (HGS) as an overall functional-quality muscle mass may be of interest for estimating REE and providing better nutritional care.

Additionally, renal patients experience hydration changes and are more prone to overhydration, which has been shown in previous work to alter ER as measured by IC in peritoneal dialysis patients (14). Bioelectrical impedance analysis is not only useful for estimating FFM but also for studying patients' hydration status. Using vectorial bioimpedance analysis (VIBA), a qualitative analysis presented in figures based on the hydration status of a healthy population, (15) thus this study aimed to develop and validate a model for estimating REE in patients with CKD stages 3–5 who were not receiving renal replacement therapy (RTT) using clinical variables and comparing it with indirect calorimetry as the gold standard. Additionally, we aimed to compare our equation with previously validated equations using CKD as a congruent validation method for estimating REE.

MATERIALS AND METHODS

This was a validity study involving 80 patients with CKD, who were seen in the outpatient clinic of our institution. It was conducted in accordance with the established standards of good clinical practice and with the approval of the ethics and the research institutional review boards. Patients with a diagnosis of CKD stages 3–5 without RRT, an eGFR estimated using the CKD-epi equation, and any comorbidity (i.e., Diabetes Mellitus, Hypertension, Glomerulopaties, and others such as Lupus) were included if they consented to participate. Patients who were missing a limb or had a metal plate implanted in their bodies were excluded from the study. Those who agreed to participate in the study attended to the metabolic, body composition, and clinical tests after a minimum of 4 h of fasting.

Metabolic Analysis

All the patients underwent IC using a CardioCoach VO2 max (Korr Medical Technologies Inc., Salt Lake City, Utah). The patients wore a face mask connected to the calorimeter, and a computer recorded variables such as VO2, CO2, FEO2, FECO2, and heart rate. After autocalibration with barometric pressure, temperature, and humidity, as well as the respiration stabilization phase, the calorimeter analyzed the aforementioned variables in a computer interphase every minute for 15 min. The patients were placed in a supine position for 5 min prior to the start of the test. The calorimeter software was programmed with the user's weight, age, height, and gender. We considered data from the software from patients with stable calorimetry analysis defined as a respiratory coefficient between the physiological ranges [(QR) = 0.68–1.2] or having at least 1 period with less than 10% in coefficient variation (1).

Body Composition Tests

A Quadscan 4000 (Bodystat, Isle of Man), was used to conduct a bioelectrical impedance analysis (BIA). Following the IC, the patients were positioned supine. At 50 kHz, the reactance (Xc), resistance (R), and phase angle (PA) were measured and standardized by height. The FFM was determined in addition to other bioelectrical parameters such as phase angle.

Clinical Examinations

A hand dynamometer (Takei Scientific Instruments Co., Japan) was used to conduct strength tests. The patients were instructed and encouraged to squeeze as hard as possible and maintain their strength for 3–4 s. The average of three repetitions with the dominant arm was recorded.

Nutritional status was evaluated in 5 domains using SGA: (1) weight changes; (2) dietary changes; (3) functional capacity changes (i.e., daily activities); (4) muscle and fat storage changes; and (5) presence of edema or ascites. This tool categorizes a person's nutritional status into three groups: (A) appropriate nutrition; (B) mild to moderate malnutrition; and (C) severe malnutrition. Malnutrition was then considered to be present in patients categorized into the B or C group.

Recent laboratory data (within 1 month) were obtained from the medical record, including electrolytes, uremic waste products, and serum creatinine. The CKD-epi equation was used to calculate the estimated glomerular filtration rate (eGFR).

In order to determine construct validity as secondary objective, we compared previously validated formulas to our data and vice versa. The following formulas were analyzed:

- REE: 668 + (17.1*FFM-BIA kg)—(2.7*age years) – (92.7*sex) + (1.3*eGFR$_{CKD-epi}$) – (152.3 if having diabetes mellitus); sex: 0 = woman, 1 = male (8)
- REE: 854.5 + (7.4*weight kg) + (179.3*Sex) – (3.3*age years) + (2.1*eGFR$_{CKD-epi}$) + (25.6 if having diabetes mellitus); sex: 0 = woman, 1 = male (8)
- REE: 645.5 + (–4.7*age years) + (106*sex) + (13.1*weight kg) + (–51.6 if having DM) (9)
- REE: 25 kcal*actual weight (kg) (7)
- REE: Woman: 665 + (9.56 × weight Kg) + (1.85 × height cm) – (4.68 × edad)
- REE: Male: 66.5 + (13.75 × weight Kg) + (5 × height cm) – (6.78 × edad)

Sample Size

The sample size was determined using the Freeman equation (16), which states that ten people should be included for every K + 1 variable in a regression analysis, for both, qualitative and quantitative analysis [n = 10 (K + 1), where K is the model's number of variables]. We considered five variables to include in the model, which resulted in a sample size requirement of 60 people plus 20% of possible missing data, or 72 patients.

Statistical Analysis

The descriptive statistics were consistent with the variable distribution using the Kolmogorov-Smirnoff test. This study aimed to evaluate an *a priori* model proposed by an expert panel. We used a modified Delphi methodology to determine the content validity of a hypothesized renal-specific REE equation (data not published). Discussion took place during three rounds where a moderator gave feedback using all the comments and suggestions provided by the panel. Accordance was set as the case when 80% of the board agreed with the statement. It was agreed to include age, sex, and fat-free mass and evaluate SGA and HGS. The Delphi panel assessed the plausibility of the variables to be included in the model.

Correlations between quantitative and qualitative data were determined. We conducted a linear regression analysis with REE determined by calorimetry (IC-REE) as the dependent variable. The model was fitted stepwise, with a p-value of 0.1 for variable inclusion. Beta coefficients were calculated and used to predict fitted values, while standardized coefficients provided additional information. As suggested, the regression assumptions were analyzed. The model-fitted values were analyzed using the intraclass correlation coefficient (ICC), with IC measurements serving as the gold standard (convergent criterion validity). The concordance analysis was evaluated with Bland-Altman (BA) graphs accompanied by the Lins concordance coefficient (LCC). The BA method determined the differences between the IC-REE and fitted values. The data was single paired measurements (fitted values vs. gold standard). Acceptable limits of agreement were considered for 300 Kcal. The BA assumptions were analyzed with the normality of the differences from methods with q-q graphs and the Kolmogorov-Smirnoff test. At the same time, homoscedasticity was evaluated qualitatively with residuals graphs post-regressing the methods' means and their differences. If the assumptions were not satisfied, logarithmic transformation was considered. Proportional bias was analyzed using a regression line in the Bland-Altman figure. Any deviation from the zero line, indicating a linear trend, was considered proportional bias (where the variability of differences between methods increases as the magnitude of the measurement increase, or vice-versa). In such case, BA figures were presented as percentage differences with bias, and LoA's based on the regression analysis (17–19). As for the secondary objective, the same statistical method was applied, but comparing our models with other authors' models. STATA 15.1 (College Station, Texas) was used to analyze the data.

RESULTS

Eighty patients participated in the study, with 71 having an accurate IC measurement. **Table 1** contains descriptive statistics. Females constituted the majority of the patients, with a median eGFR of 33 (16–47) ml/min/1.73 m^2. Additionally, 16.9% of the study population identified by SGA had mild to severe malnutrition. The patients had a higher prevalence of hypertension than diabetes. The population had a mean BMI of 26.48 ± 4.92 kg per square meter, an impedance phase angle (PA) of 5.66 ± 1.14, and an HGS of 25 ± 9.6 kg per strength. By IC, the mean ER was 1386.23 ± 393.5 kcal/day, with a median respiratory quotient of 0.67 (0.64–0.69).

TABLE 1 | General characteristics of the study population.

Variable	Value *n* = 71
General characteristics	
Age (years)	53 (32–61)
eGFR (ml/min)	33 (16–47)
CKD stage *n* (%)	
3	38 (53.5)
4	18 (25.4)
5	15 (21.1)
Sex *n* (%) (female)	38 (53.52)
Diabetes mellitus *n* (%)	23(32.39)
Hypertension *n* (%)	31 (43.66)
Laboratory tests	
Glucose (mg/dl)	86 (81–102)
BUN (mg/dl)	38.7 (28.2–49.6)
Urea (mg/dl)	80.04 (60.35–101.65)
Creatinine (mg/dl)	2.14 (1.59–3.52)
P (mg/dl)	3.88 (3.47–4.25)
K (mg/dl)	4.64 (4.31–4.92)
Na (moll/l)	139 (138–141)
Body composition and nutritional measurements	
Weight (kg)	67.4 (54.5–79.2)
BMI (kg/m^2)	26.48 ± 4.92
Lean mass (kg)	47.07 ± 12.2
Fat mass (kg)	19.65 (14.9–26.6)
R/H (Ω/m)	330 (276–406)
R (Ω)	538.3 ± 114.8
Xc/H (Ω/m)	33.84 ± 10.64
Xc (Ω)	54 ± 16.7
PA°	5.66 ± 1.14
Subjective global assessment *n* (%)	
Normal	59 (83.1)
Mild to moderate	11 (15.5)
Severe	1 (1.4)
HGS right (kg/Strength)	25.02 ± 9.61
Indirect calorimetry parameters	
Energy kcal	1386.23 ± 393.48
Respiratory quotient	0.67 (0.64–0.69)
VO_2	201.24 ± 56.7
VCO_2	134.06 ± 37.08

eGFR, estimated glomerular filtration rate; Na, sodium; K, potassium; BUN, blood urea nitrogen; P, phosphorus; R/H, resistance adjusted from height; Xc/H, reactance adjusted from height; PA, phase angle HGS, handgrip strength; VO_2, oxygen rate; VCO_2, carbon dioxide production; BMI, body mass index. Data are expressed as mean and ± SD and median and P25-P75.

Figure 1 illustrates the correlations. The HGS, weight, and FFM all correlated positively with energy measured *via* IC, with the strongest correlation being FFM (r = 0.59; $p < 0.01$). Impedance components such as R and Xc, on the other hand, were negatively correlated with IC measurements. The correlation coefficient between the eGFR CKD-EPI and the IC measurement was 0.19, p = 0.106. The correlation coefficients for categorical and quantitative data were 0.39 for hypertension and 0.11 for diabetes mellitus and IC measurements, respectively, whereas malnutrition was negatively correlated with the ER at $r = -0.4$.

Linear Regression

The regression models are presented in **Table 2**, with Model 2a incorporating BIA-FFM, Model 2b substituting weight (kg) for FFM, and Model 3c replacing weight for dominant HGS. All other variables were computed-selected stepwise with a p = 0.01 significance level. The model that included BIA-FFM had an adjusted R^2 of 0.46, while the model that included weight (kg) had an adjusted R^2 of 0.44.

Validity: Concordance and Consistency

Additional concordance analysis is presented in **Figures 2**–**4**. The BA figures are shown in **Figures 2**–**4**. Some models (de Oliveira Fernandes et al. weight equation, Xu et al. equation, Harris-Benedict, and the KDOQI 25 Kcal/Kg/day) did not have parametric distribution on their differences with the gold standard and were log-transformed. Nevertheless, the normality did not improve. We decided to continue the analysis regardless of this limitation with the nature of the variable. All equations showed proportional bias and are presented in mean percentage difference. Most of the equations, except the Harris-Benedict and the KDOQI 25 Kcal/Kg/day, had non-significant percent mean differences, although all models had more significant than expected LoA. Our models had LCC values between 0.60 and 0.65 (**Figure 2**), whereas other energy expenditure estimation equations had LCC values between 0.36 and 0.55 (**Figure 3** and **Table 3**). Using these previously validated equations as a reference, our models had LCC values ranging from 0.66 to 0.80 (**Table 4** and **Figure 4**), and the percent mean difference was no greater than 10% between them. The only significant percent mean difference was observed in de Oliveira Fernandes et al. BIA-FFM equation and our model, including BIA-FFM.

The ICC is presented in **Table 4** to analyze consistency between our models and previously validated equations. In comparison to IC measurements, the ICC values for the models with BIA-FFM and weight were 0.66 (0.50–0.77) for both equations. The ICC values for previously validated equations were between 0.5 and 0.6 compared to our IC values (**Table 4**).

DISCUSSION

Estimating energy requirements is critical during the nutritional care process since it establishes a portion of the goals for treating the patient based on clinical evidence and critical thinking (20). Specifically for kidney patients, providing an appropriate and individualized nutritional treatment should consider the risk that this population has of developing malnutrition and attempt to avoid adverse outcomes associated with this phenomenon, even in the early stages of the disease (21). Today, various reference guides recommend estimating energy using the reference standard (CI) (1, 7, 22). However, since this method is not widely available, having validated energy estimation equations based on the reference standard is critical.

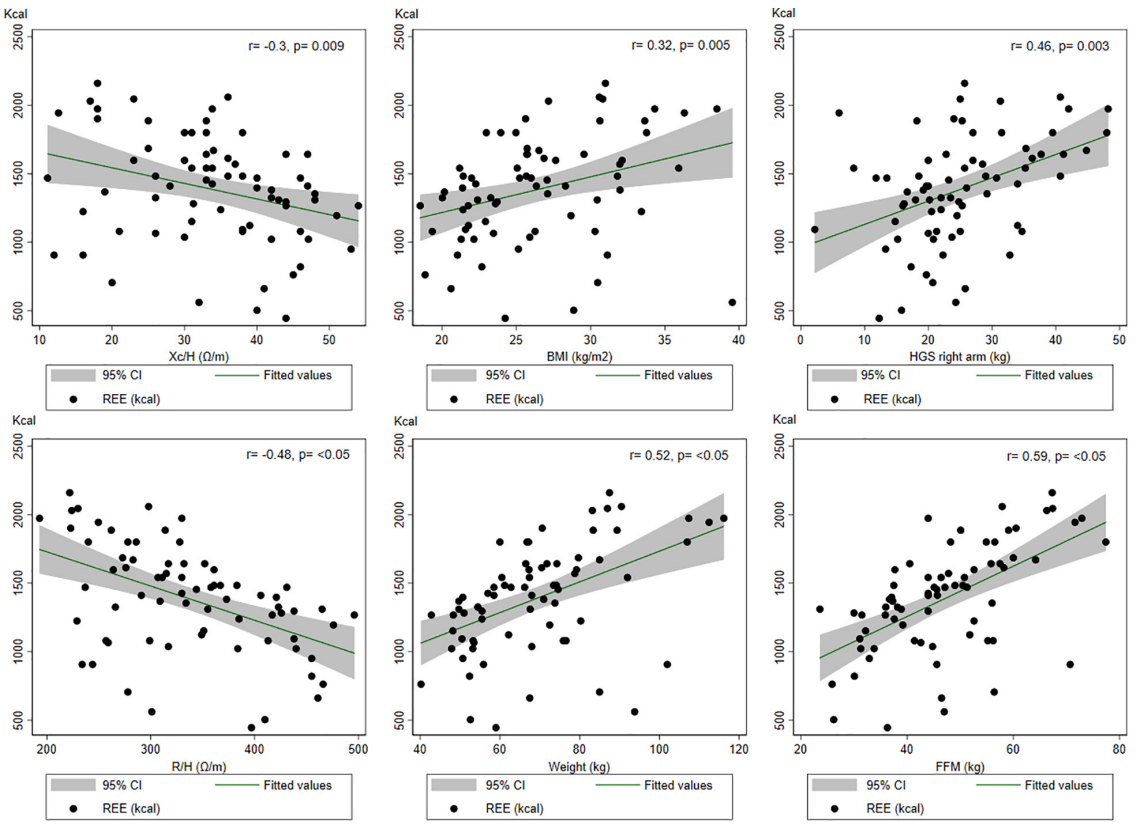

FIGURE 1 | Correlations between some variables of interest and the indirect calorimetry measurements.

We set out to develop and validate a model for estimating REE of kidney patients in stages 3–5 without RRT and compare our model's consistency and concordance with those developed recently and some others that are frequently used, such as Harris-Benedict and KDOQI Guidelines (23). We can emphasize the relationship certain variables have with REE, the most significant variable being the FFM determined by BIA (8, 9, 23, 24). These findings are consistent with previous research on body composition and REE, which indicates that, in addition to muscle mass, weight as the sum of all body composition compartments also has a moderate correlation. Around 80% of a person's REE is determined by body size, with lean body mass having the highest correlation.

According to de Oliveira Fernandes et al.'s model (8), weight in kg accounts for 21% of the variance in REE, and the addition of sex, age, kidney function, and diagnosis of diabetes increases the variance explained to up to 42% of REE. On the other hand, Xu et al. (9) demonstrated that the same model could account for 77% of REE variance with slightly larger sample size. Besides, de Oliveira Fernandes et al. (8) showed that FFM alone could account for between 33 and 36% of REE variability, depending on whether it is estimated using anthropometric measurements or bioelectrical impedance. It should be noted that many of the prediction equations for BIA-FFM used by the analyzers were developed in healthy populations, casting doubt on their validity for use in CKD patients. This is one of the primary reasons for encouraging validation studies of specific clinical tools for each of the pathological entities.

In our models, the weight and FFM estimated by BIA, combined with other variables, account for slightly less than 50% of the variance in REE. These additional variables include nutritional status, a diagnosis of hypertension, sex, and age. Interestingly, despite a modest correlation with REE (r = 0.46), The HGS is irrelevant for developing a predictive model. This could be because when other variables are considered, it loses significance in terms of REE. However, given the biological plausibility and proposal in the Delphi consensus described previously and the emphasis placed on evaluating muscle mass and its quality and strength, it is necessary to assess this possibility. Muscle strength loss has been linked to an increased risk of falls, loss of autonomy, and ultimately hospitalization and death, beginning as early as 30 years (25).

Although the guidelines suggest considering nutritional status as a variable when determining how many calories to indicate to the patient to avoid malnutrition, no specific recommendation is made (7). It is interesting to note that nutritional status, as determined by SGA, is a variable that remains statistically significant in all proposed models, lowering REE in the presence of malnutrition (B or C, as determined by SGA). This can be explained by the possible loss of muscle mass that people with this nutritional characteristic may experience; however, the evidence

TABLE 2 | Linear regression for indirect calorimetry measurements (Fat-Free Mass) (Weight), and (Handgrip strength).

Variable	Standardized beta	Coefficient beta	IC 95%	*P*-value
		A		
Fat free mass (BIA-Kg)	0.57	18.58	12.89–24.27	0.000
Nutritional status (SGA B or C)	−0.31	−325.55	−508.56 to −142.5	0.001
Hypertension (diagnosis)	0.21	167.31	28.42 – 306.21	0.019
Constant	–	489.2	212.1–766.31	0.001
		B		
Weight (Kg)	0.37	8.49	3.78–13.19	0.001
Nutritional status (SGA B or C)	−0.25	−265.34	−471.79 to −58.89	0.013
Sex (male)	0.24	195.24	36.01–354.46	0.017
Hypertension	0.27	212.93	64.62–361.24	0.006
Age (years)	−0.24	−6.05	−10.93 to −1.16	0.016
Constant	–	959.35	601.6–1317.1	0.000
		C		
Hand grip strength (Kg)	−0.04	−1.82	−14.1	10.5
Hypertension	0.32	258.5	103.8–413.2	0.001
Age (years)	−0.20	−5.1	−10.4–0.39	0.069
Height (cm)	0.29	5.2	0.94–22	0.033
Nutritional status (SGA B or C)	−0.25	−279.7	−521 to −38.3	0.001
Sex (male)	0.32	190	−37.1 – 417.21	0.100
Constant	–	−323.4	−1914.6 – 1268	0.686

A: $R^2_{adjusted}$ = 0.46; R^2 = 0.48; P_{model} = 0.000.
B: $R^2_{adjusted}$ = 0.44. R^2 = 0.48; P_{model} = 0.000.
C: $R^2_{adjusted}$ = 0.43; R^2 = 0.37; P_{model} = 0.000.
BIA, bioelectric impedance analysis; SGA, subjective global assessment.

TABLE 3 | Estimated calories and intraclass correlation coefficients between our models, and other authors, with indirect calorimetry measurements.

Equation *n* = 71	Estimated calories (REE)	ICC (95% CI)	LCC (95 % CI)
Indirect calorimetry	1,386 ± 393	ND	ND
BIA-FFM (Kg)	1,386 ± 275	0.66 (0.50–0.77)	0.65 (0.53–0.77)
Weight (Kg)	1,386 ± 275	0.66 (0.50–0.77)	0.65 (0.54–0.78)
Handgrip strength (Kg/strength)	1,386 ± 258	0.60 (0.43–0.73)	0.60 (0.47–0.73)
Xu et al, weight (Kg)	1,350 ± 255	0.51 (0.32–0.66)	0.51 (0.36–0.66)
De Oliveira et al (weight)	1,356 ± 204	0.45 (0.25–0.62)	0.55 (0.30–0.59)
De Oliveira et al (BIA-FFM)	1,293 ± 204	0.43(0.22–0.60)	0.43 (0.28–0.57)
Harris-Benedict	1,457 ± 270	0.52 (0.33–0.67)	0.52 (0.37–0.67)
KDOQI guidelines (25 Kcal/kg)	1,726 ± 426	0.36 (0.01–0.60)	0.36 (0.20–0.51)

BIA-FFM, fat-free mass determined with bioelectrical impedance; ICC, intraclass correlation coeffficient; LCC, lins concordance coefficient; REE, resting energy expenditure.

TABLE 4 | The intraclass correlation coefficient between previously validated equations, as a standard reference, and our models (*n* = 71).

Equations in comparison	ICC (CI 95%)	LCC (CI 95%)
BIA-FFM vs. de Oliveira Fernandes et al. BIA-FFM model	0.66 (0.43–0.79)	0.66 (0.54–0.77)
Weight vs. de Oliveira Fernandes et al. weight model	0.76 (0.64–0.84)	0.75 (0.66–0.84)
HGS vs. de Oliveira Fernandes et al. weight model	0.65 (0.49–0.76)	0.65 (0.52–0.78)
BIA-FFM vs. Xu et al. model	0.73 (0.60–0.82)	0.73 (0.62–0.83)
Weight vs. Xu et al. model	0.80 (0.70–0.87)	0.80 (0.72–0.88)
HGS vs. Xu et al. model	0.63 (0.46–0.75)	0.62 (0.48–0.76)

ICC, Intraclass Correlation Coefficient; LCC, Lins Concordance Coefficient; BIA-FFM, Fat-free mass determined with bioelectrical impedance; HGS, Handgrip strength.

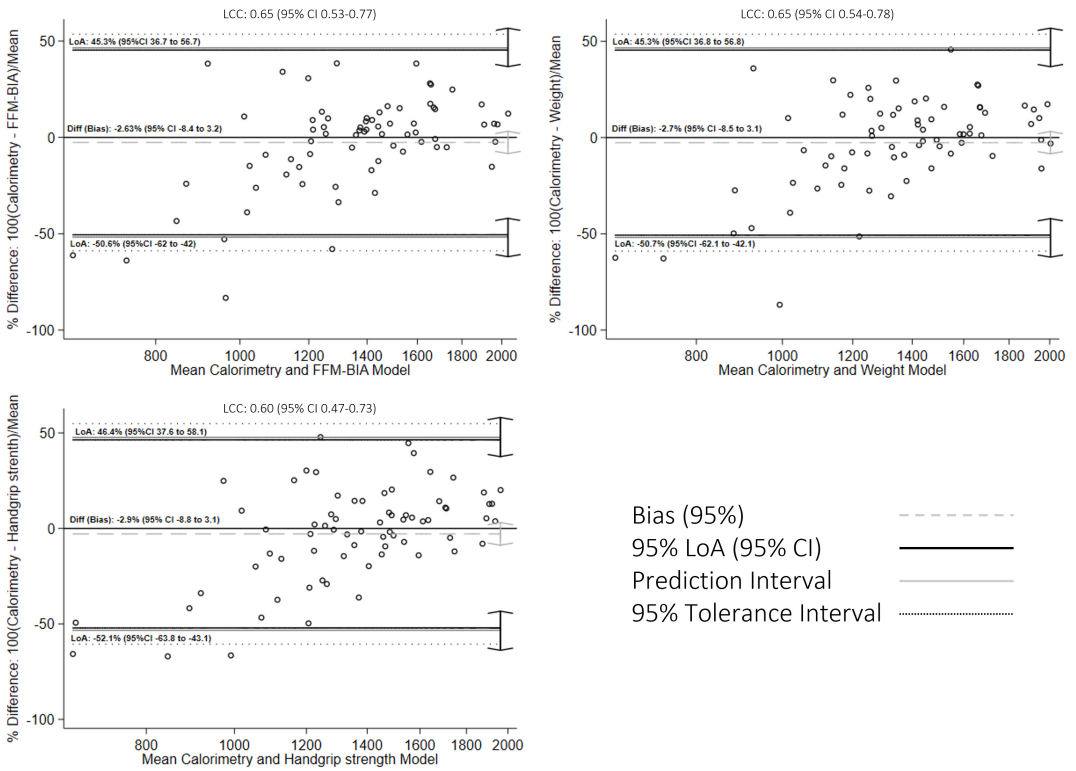

FIGURE 2 | Bland-Altman graph for concordance analysis between fitted values and indirect calorimetry measurements.

is changing since, while the phenomenon is similar in people with heart failure, the energy demands of people with malnutrition are typically increased in cancer patients (26).

It has been demonstrated that patients who do not receive dialysis may have lower REE levels comparable to healthy people (27, 28). It is suggested that comorbidities generally increase REE. While the diagnosis of diabetes mellitus does not affect the REE of our population, it is worth noting that only 32% of our patients had this diagnosis, compared to 43% who had hypertension, which contributed to the models having a standardized value greater than 0.20. Other proposed models incorporate DM into the equations (8, 9), increasing or decreasing the REE associated with the diagnosis, depending on the variables it interacts within the model. Various comorbidities may play a significant role in modifying REE in the CKD population i.e., catabolic conditions, poorly controlled diabetes, metabolic syndrome, and hyperparathyroidism are all included in these variables (2, 3, 7, 29, 30).

Age and gender are considered to be standard variables to consider when discussing REE (7). Since the beginning of human metabolism research, a strong correlation between these two variables and REE has been demonstrated, and this appears to be the case in renal patients. With increasing age, REE decreases, and it appears as though men require slightly more energy than women. When FFM is omitted from our models, gender, and age play a role.

The simplest method for calculating a person's energy requirements is to use estimation equations. The validity of the classic formulas is debatable, and the bias they may contain has been documented in several studies, with more than half of the population studied being over—or underestimated (6, 14). In our study, concordance between different models (including those created in this study) is moderate, despite the low mean differences that we found. The LoA are especially wide in those with lower REE in terms of means of methods, and percent means differences reached up to 50% in some equations in comparison with the gold standard. This may be in relation with the sample size, which was calculated for the modeling instead for the concordance and the BA analysis.

Interestingly, using previously validated equations as a reference standard, our models have a moderate to good ICC, particularly when compared to Xiao et al. model (ICC 0.80) (0.70–0.87). The concordance improved, having narrower LoA, however, when their models are applied to our population's reference standard (CI), the concordance is moderate. These findings are consistent with our observations about the agreement of other equations, such as Harris-Benedict. This may indicate that, while the equations are similar, the estimates vary among populations.

On the other hand, it is essential to note that the most significant difference we found is with the 25 Kcal/Kg/day of the KDOQI guidelines (7). However, we must mention that the

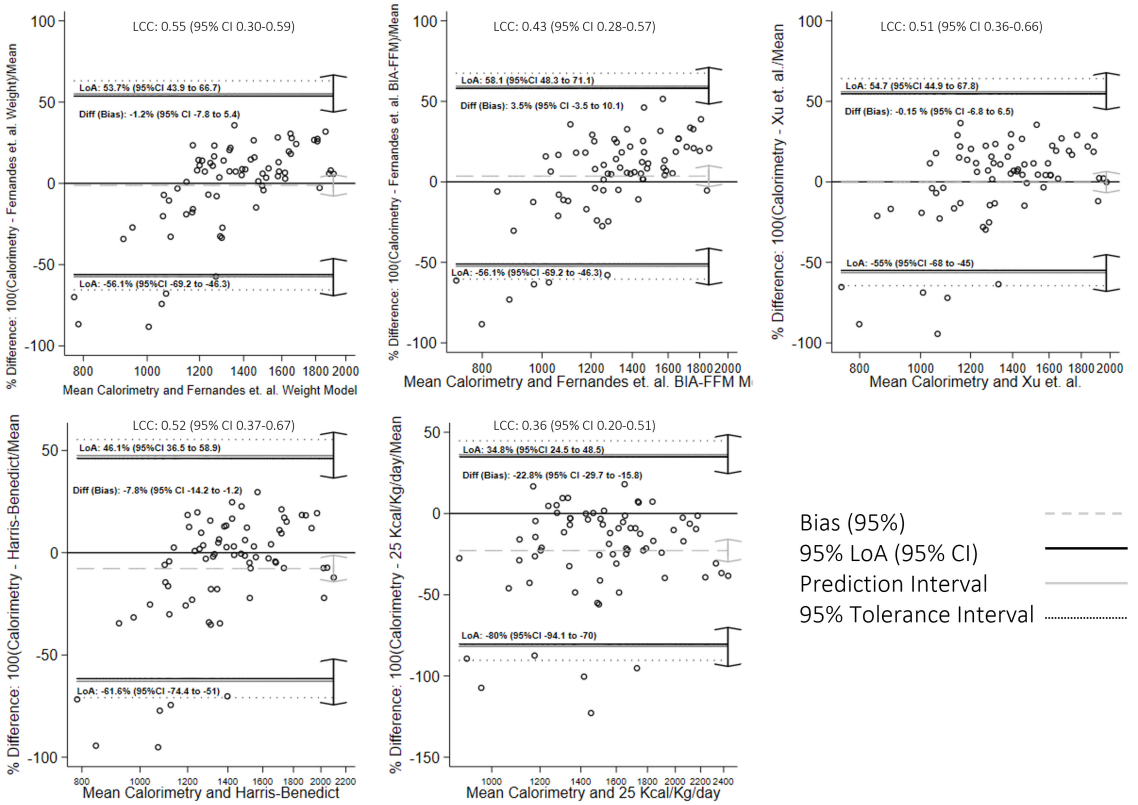

FIGURE 3 | Bland-Altman graph for concordance analysis between previously validated equations with indirect calorimetry measurements.

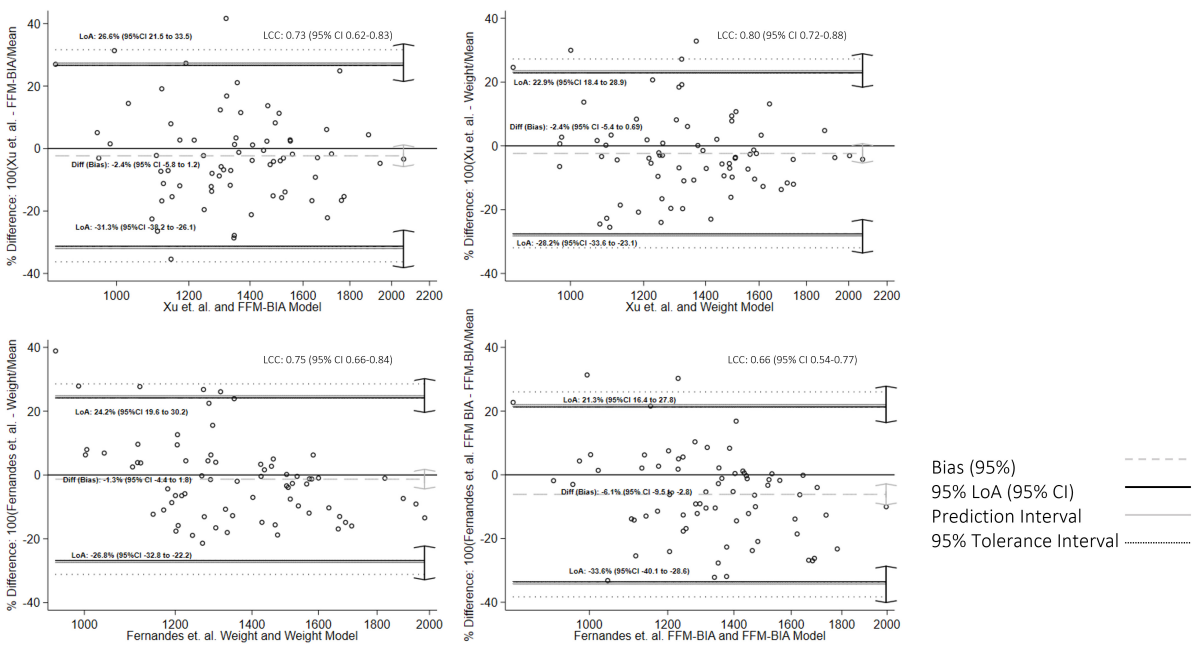

FIGURE 4 | Bland-Altman graph for concordance analysis between fitted values and predictions from previously validated equations.

22% difference found could mean the complementary calories to estimate GET instead of GER.

Although there is no acceptable range between calories estimated by prediction equations and calorimetry measurement, 10% variations are considered clinically significant. It is essential to mention that most of the formulas proposed by our team or other authors meet this characteristic in our population assessed with the mean percentage difference, nevertheless LoA are greater than 20% in all cases. The BA figures showed a bias toward overestimating the REE, for that measured by the IC, in people with averages < 1,200 Kcal, nevertheless great percentage of the population is within the LoA when the REE is > 1,200 Kcal.

Certain characteristics of kidney patients (changes in hydration and the bias this may represent in body weight, decreases in GFR *per se*, uremia, anorexia, inflammation, and insufficient physical activity) complicate studies on this subject. These variables are also proposed for consideration in the guidelines for estimating REE, nevertheless some of them are not feasible to in clinical settings (i.e., inflammation studied with PCR).

This study, has some limitations, including the difficulty of determining the external validity of our models due to the lack of an opportunity to apply the equations to a different sample from the one used to create the models. Another significant limitation of this research is the sample size. While we believe that the calculation used to create the regression model is sufficient, it is insufficient to perform finer stratifications or sub-analyses in terms of statistical power. Additionally, since hydration status was not explored in this study, weight may be skewed; however, other authors have proposed weight as a critical variable for measuring REE. It's also worth noting that the purpose of this study was to quantify REE, which means that exercise and physical activity were excluded by definition.

On the other hand, among the most significant strengths of our work, we can point out that it is one of the few that has been tasked with validating an energy estimation equation for CKD patients and that it also incorporates little-explored variables such as nutritional status and HGS, as suggested in the guidelines and expert consensus prior to this study (under review). Additionally, the work incorporates the models proposed to examine the concordance of their results in our population, indicating that, while the equations are similar, the estimates are not entirely concordant, possibly due to differences in populations. Nevertheless, those weight-based-equations seems to be the more consistent, and concordant and possibly the more valid to estimate REE in CKD patients in stages 3–5.

CONCLUSION

We can conclude that models incorporating nutritional status and other clinical variables such as weight, FFM, comorbidities, gender, and age have a moderate degree of agreement with REE measurements obtained *via* IC and have moderate validity. The concordance between our models and others previously validated for the CKD patient is high; however, the agreement between the latter and IC measurements is moderate, noticing that the concordance and dispersion of the data are significantly biased in those with energy expenditure below 1,200 Kcal, nevertheless for values > 1,200 kcal, the patients were in between the LoA. to the results of this study, we suggest the use of formulas based on body weight, as well as the use of the KDOQI lowest recommendation (25 Kcals/kg body weight) considering the 22% difference with respect to the IC for total energy expenditure rather than for REE.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Comité de Etica e Investigación del Instituto Nacional de Ciencias Médicas y Nutrición Salvaldor Zubirán No. 3045. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SR-A and LR-G participated in the research generation, carrying, collection, analysis of the data, and in writing the article. SL-C and AG-O participated in the interpretation of the data and revision of the manuscript. ÁE-C participated in study conception and design, revision, analysis of the data, writing the manuscript, revision, and approval of the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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The Feasibility and User-Experience of a Digital Health Intervention Designed to Prevent Weight Gain in New Kidney Transplant Recipients—The ExeRTiOn2 Trial

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Half of kidney transplant recipients (KTRs) gain more than 5% of their body weight in the first year following transplantation. KTRs have requested support with physical activity (PA) and weight gain prevention, but there is no routine care offered. There are few high-quality studies investigating the clinical value of diet, PA or combined interventions to prevent weight gain. The development and evaluation of theoretically informed complex-interventions to mitigate weight gain are warranted. The aims of this mixed-methods randomized controlled trial (RCT) were to explore the feasibility, acceptability and user-experience of a digital healthcare intervention (DHI) designed to prevent post-transplant weight gain, in preparation for a large multi-center trial. New KTRs (<3 months) with access to an internet compatible device were recruited from a London transplant center. The usual care (UC) group received standard dietary and PA advice. The intervention group (IG) received access to a 12-week DHI designed to prevent post-transplant weight gain. Primary feasibility outcomes included screening, recruitment, retention, adherence, safety and hospitalizations and engagement and experience with the DHI. Secondary outcomes (anthropometrics, bioimpedance, arterial stiffness, 6-minute walk distance and questionnaires) were measured at baseline, 3- and 12-months. 38 KTRs were screened, of which 32 (84.2%) were eligible, and of those 20 (62.5%) consented, with 17 participants (85%) completing baseline assessment (Median 49 years, 58.8% male, Median 62 days post-transplant). Participants were randomized using a computer-generated list (n = 9 IG, n = 8 UC). Retention at 12-months was 13 (76.4%) (n = 6 IG, n = 7 UC). All *a priori* progression criteria were achieved. There were no associated adverse events. Reflexive thematic analysis revealed four themes

regarding trial participation and experience whilst using the DHI. Halting recruitment due to COVID-19 resulted in the recruitment of 40% of the target sample size. Mixed-methods data provided important insights for future trial design. A definitive RCT is warranted and welcomed by KTRs.

Clinical Trial Registration: www.clinicalTrials.gov, identifier: NCT03996551.

Keywords: web-based intervention, weight gain prevention, physical activity, kidney transplantation, behavior change

INTRODUCTION

Weight gain within the first year of receiving a kidney transplant is a critical health issue (1) and occurs in both obese and non-obese kidney transplant recipients (KTRs) (2). Over half of KTRs gain more than 5% of their body weight within the first year of transplantation (3, 4). Post-transplant weight gain is usually accompanied with an increase fat mass, not lean tissue mass (3). There is a positive association with an increase in adipose tissue (visceral and sub-cutaneous) with insulin resistance in KTRs (5). Factors underlying post kidney transplant weight gain include reduced physical function (6) and physical activity (PA) (7), increased appetite, (3) steroid medication use (8), and the lifting of dietary restrictions (9). Whilst "triple therapy" anti-rejection regimes including steroid medication are current practice to reduce the risk of graft failure (10–12), they have been found to increase both the severity and incidence of cardiovascular risk factors (13). In addition, these medications effect bone health, weight gain, hypertension, abnormal glucose mechanism and the development of post-transplant diabetes mellitus (10, 11). They have also been associated with appetite stimulation and changes in nutrient partitioning that favor fat deposition (14). Therefore, interventions to address weight gain and modifiable risk factors such as physical activity and healthy eating behaviors are warranted (15).

KTRs have asked for support for both PA and healthy eating behaviors post transplantation (9, 16, 17). Despite national clinical practice (18) and workforce practice guidelines (19) that recommend access to both kidney physiotherapists and dietitians, these healthcare professionals (HCPs) are not routinely represented in all transplant centers (20). Whilst COVID-19 has seen an increase in virtual kidney services (21), and the creation of online PA and well-being interventions for people living with chronic kidney disease (22), there remains no recognized intervention to prevent weight gain in new KTRs (15).

A recent systematic review and meta-analyses (15) revealed that there was no evidence that dietary, exercise, or combined interventions led to significant changes in body weight or body mass index (BMI) in a pooled sample of participants within the first year of receiving a kidney transplant. Limitations of the review include small number of randomized-controlled trials (RCTs) with significant methodological variation, and variable quality study design (17). Future studies would benefit from healthcare digital behavior change intervention guidance (23) such as the use of the behavior change techniques (24, 25) and behavior change wheel (26, 27) to explore and report intervention components, and context. There is therefore a need for quality, theory informed RCTs to investigate complex interventions that include dietary counseling, PA interventions and behavior change techniques to address the multifactorial problem of weight gain post kidney transplantation.

The usability and experience of a personalized digital health intervention (DHI): ExeRTiOn (Exercise and weight gain prevention in renal transplant online), which was co-designed with KTRs and transplant healthcare professionals (HCPs) to aid weight gain prevention after kidney transplantation has already been reported (16). The results from this initial study (16) were used to facilitate iterative patient-led refinements and improve the acceptability of the ExeRTiOn DHI in preparation for its use in this feasibility RCT. The aims of this current mixed-methods RCT were to explore the feasibility, acceptability and experience of using the ExeRTiOn DHI, and participating in the trial in preparation for a large multi-center trial.

METHODS

Trial Design

Mixed-methods feasibility RCT with 1:1 allocation ratio.

Participants

KTRs were approached during routine transplant clinics at both King's College Hospital NHS Foundation Trust and Guy's and ST Thomas' Hospital NHS Foundation Trust. Participants were included if they were $\geq$18 years, had received a single organ kidney transplant within <3-months, had access to an internet compatible device, and had a BMI $\geq$ 18.5 kg/m^2. Exclusion criteria included active pregnancy, a medical condition preventing PA participation (e.g., unstable angina), a cognitive impairment preventing engagement with the DHI, or if they were unable to complete the DHI in English.

Study Procedures

Ethical approval was sought, and obtained, from the London Dulwich Research Ethics Committee (19/LO/1138). The trial was registered (www.clinicalTrials.gov; no: NCT03996551). Eligible KTRs were provided with approved patient information sheets and given $\geq$24 h (or at the participants convenience) to consider participation. Participants provided written consent, attended a baseline assessment, and were then randomized with a computer-generated list (28). They were allocated to either the 12-week ExeRTiOn intervention group (IG) or usual care (UC) by a member of the research team. The trial physiotherapist and participants were not blinded.

Participants attended the King's National Institute for Health Research Clinical Research Facility for assessment of secondary outcomes at baseline, 3-, and 12-months. Medical history and hospital admissions were reviewed. Assessments were booked around clinical appointments with a window of 14 (±7) days. A purposive sample of participants from both groups were invited to complete individual semi-structured interviews, conducted over the telephone or face-to-face.

Interventions

The ExeRTiOn DHI

Participants in the IG were provided with access to the ExeRTiOn DHI. The ExeRTiOn DHI has been previously reported (16). The design, development and evaluation of the ExeRTiOn DHI was iterative, and was informed by the Medical Research Council Framework for complex interventions (29), the combined intervention design approach (30), the person-based approach (31), evidence and theory, guidance for digital healthcare development (23, 32), the behavior change wheel (26), recognized behavior change techniques (24, 25), principles of self-efficacy (33), motivational interviewing (34), patient and public involvement, and input from research and clinical experts such as the renal-specific weight management team (35–37).

In summary the ExeRTiOn DHI was password protected, had both a patient-facing and back-end website monitored by a trial physiotherapist, with a secure encrypted two-way message function between participants and the trial physiotherapist. IG participants were provided with a brief one-to-one orientation session with the trial physiotherapist and were then able to complete the 12-weekly sessions independently with any internet compatible device. As the ExeRTiOn DHI was designed utilizing a reactive website, participants could choose to view the DHI with their smart phone, laptop, tablet or computer. DHI content and functionality included kidney transplant specific education from health care professionals, tips from kidney transplant recipients, an optional home exercise diary, a resource page, graphical displays of self-reported physical activity minutes and body weight, and the secure two-way message function (16). Intervention participants were encouraged to set physical activity and healthy eating goals, and were prompted to self-monitor physical activity minutes and body weight weekly (16). Food intake was not captured.

Personalized "trigger messages" were sent by the trial physiotherapist to the IG participants when two sessions in a row were not completed. Automated reminder emails, and personalized messages were provided as per the research protocol. The physiotherapist who supported the DHI engagement was trained in motivational interviewing principles (34, 38, 39), and had experience working in both weight management and exercise services for people living with a kidney transplant. After completion of the 12-week DHI, IG participants were able to revisit completed sessions until the 12-month visit.

Usual Care

Usual care at both sites involved routine inpatient physiotherapy input, the provision of a "healthy eating after kidney transplant" leaflet by a renal dietician during transplant surgery admission, and encouragement to be physically active, and follow a healthy diet from outpatient transplant nephrologists and nurses.

TABLE 1 | Feasibility outcomes and a priori progression criteria.

Criteria	Pre-set cut offs
Screening of potential participants	≥50% deemed eligible approached to do the study consider progression to a definitive trial If <50% and no significant valid reasons provided, consider not progressing to a further study
Recruitment rate	≥50% consider progression to a definitive trial 40–49% TMG to discuss trial, and if valid modifiable reasons identified, the study may progress ≤30% and there are no significant valid reasons provided, the study will not progress to a definitive trial
Retention rate at 12-months	≥60% progress research 50–59% discuss with TMG. If valid reasons identified, the study may progress ≤40% do not consider further research
Intervention adherence	≥60% of the intervention completed (≥7 out of the 12 sessions) If <60% adherence, with no valid reasons from discussions with the TMG, the study may not progress
Safety and hospitalizations	Capture and report any harms e.g., Slips/trips Capture and report unplanned hospitalizations Capture and report any associated adverse events Non-related serious adverse events were defined as unplanned and unrelated hospitalizations (≥24 h).

TMG, trial management group.

Primary Feasibility Outcomes

Primary feasibility outcomes included screening, recruitment, retention, adherence to study visits, safety and hospitalizations, engagement and experience whilst using the DHI, and the feasibility and experiences taking part in the study. This would allow the assessment of the feasibility of the DHI but also the feasibility of running a RCT in preparation for a definitive RCT. Feasibility was assessed by a set of *a priori* progression criteria. "Stop" and "go" criteria (40) were decided prior to the intervention by the study team, Trial Management Group, KTRs, HCPs, researchers, and review of published literature (41). Feasibility outcomes and progression criteria are found in **Table 1** below. In addition, the fidelity of the ExeRTiOn DHI was assessed.

Secondary Outcomes

Anthropometric measures included body weight (kilograms) waist circumference (centimeters), hip circumference (centimeters) and BMI (kg/m^2). Blood pressure and heart rate were recorded three times on each occasion and averaged. Bioimpedance analysis was assessed using the Fresenius body-composition Monitor (Fresenius BCM) (42, 43), a CE marked device (44). Fat mass and lean tissue mass were recorded.

Functional exercise capacity was assessed by the 6-minute walk test, using a standardized protocol (45). Pre and post resting heart rate, and total 6-minute walk distance (meters) was recorded.

Arterial stiffness was measured by pulse wave velocity and augmentation index, using the Vicorder system (Skidmore Industries, UK). Standardized procedures (46) and calculations of arterial path length (47) were used. Pulse wave velocity and augmentation index were measured three times, and then averaged for a final score.

A number of questionnaires were completed at each study visit. The General Practice Physical Activity Questionnaire which has been validated in people living with kidney disease (48) classified PA into four categories: inactive, moderately inactive, moderately active and active (49). The Nutrition Self-Efficacy Scale and the Physical Exercise Self-Efficacy Scales (50) assessed self-efficacy. Higher scores indicated greater likelihood to change the targeted behavior (e.g., PA) (50). The Euro-QOL 5-Dimension-5-level questionnaire (EQ-5D-5L) (51), which has been validated in KTRs as a measure of health status (52), assessed health-related quality of life. The EQ-5D-5L visual analog scale, and the EQ-5D-5L index value were collected (53). The EQ-5D-5L index value was calculated using the van Hout et al. (54) method, using a downloadable calculator (55). Fatigue symptom severity was assessed by the Chalder Fatigue Scale (56), which included sub-scales for physical and mental fatigue, and a total fatigue score (from 0 to 33) (56). Permission was obtained to use the Chalder Fatigue Scale and EQ-5D-5L. Participant and transplant characteristics were collected from medical records. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI creatinine equation (measured in ml/ min/1.73m^2) (57), and the CKD-EPI calculator (58). Serum creatinine blood results (μmol/L) from routine transplant clinic blood tests that were conducted on the same day as the study visits were used.

Sample Size

As recommended by the Consolidation of Reporting Trials (CONSORT) guidelines for feasibility trials (59), formal power calculations were not completed. The initial target sample was 50 participants. A sample size between 24 and 50 has been recommended to estimate standard deviations for use in a sample size calculation for a follow-up trial (60–62).

Nested Qualitative Sampling

A purposive sample (63) of trial participants were invited for individual semi-structured interviews to explore the experiences of participating in the trial, and the experiences using the ExeRTiOn DHI. To capture both groups experiences regarding the feasibility of taking part in this trial, participants from both groups were sampled for the qualitative interviews. A range of age, gender, and adherence with the DHI were included in the qualitative sampling framework. The final qualitative sample size was informed by the inductive reflexive analysis (64), information power (65), and the meaning and themes derived from the analysis (66). *A priori* analysis estimated sample size of 5 to 10 rich interviews would be sufficient to uncover common patterns and themes from across the dataset.

Statistical Analysis Plan

Since this was a feasibility study (59), no significance testing was performed. Descriptive statistics are presented with corresponding two-sided 95% confidence intervals using SPPS© for Mac (Version 27). Summary statistics were presented using Medians and interquartile ranges (IQRs). Analysis followed the intention-to-treat principle i.e., all participants with a recorded outcome were included in the analysis according to the treatment group to which they were randomized regardless of treatment actually received.

All qualitative interviews were recorded, transcribed, and imported into NVIVO for MAC© Version 12 for analysis. Data quality and richness was assessed using information power (65). Reflexive thematic analysis (64, 67), from a pragmatic philosophical standpoint (68) was performed.

A convergent mixed-methods analysis was used (69). Joint display tabulation sought examples of convergence, complementary issues or discrepancies between the qualitative and quantitative datasets (70).

RESULTS

Feasibility Outcomes

Eligibility, Screening, and Recruitment

Recruitment of participants took place from the 3rd of September 2019, was paused on the 15th of March 2020, when 20 participants had been recruited, and then ceased on the 2nd of June 2020 due to the COVID-19 pandemic, the shielding of KTRs and the cessation of kidney transplant surgeries in the UK. An amendment to ethics was submitted and approved on the 6th of August 2020. **Figure 1** below depicts the feasibility CONSORT diagram (59).

Whilst there were 51 new KTRs within the trial recruitment period, $n = 13$ were not screened due to acute illness of potential participants, staff leave of the principal investigator completing recruitment, and some participants being identified right before the recruitment was halted due to the outbreak of COVID-19 (see **Figure 1**). Of the 38 new KTRs screened, 32 were eligible for the study with a screening rate of 84.2% (95% CI 68.6 to 94.0%). Twenty consented to the trial, with a consent rate of 62.5% (95% CI 43.7 to 79.0). Reasons for declining participation included multiple hospital appointments ($n = 2$), declining research participation ($n = 2$), caring for family members ($n = 1$), and return to work pressures ($n = 1$). Unfortunately, 3 participants who consented, were unable to complete baseline assessment and randomization (consent fails). Seventeen participants completed baseline assessments and were randomized to UC ($n = 8$) or the DHI IG ($n = 9$) (**Figure 1**).

Participant Characteristics

Of the 17 participants, 10 were male (58.8%), with a median age of 49 (IQR 39.6) years. The median transplant vintage was 62 days (IQR 53.0, 68.0). **Table 2** demonstrates the baseline participant characteristics.

The median eGFR (IQR) was 40 (32 to 60), 43 (40 to 58.5) and 52 (33 to 66) (mL/min/1.73 m^2). Most participants were prescribed triple immunosuppressant regime at baseline (Tacrolimus, Prednisolone and Mycophenolate Mofetil) (**Table 2**). The median total daily dose of mg of Prednisolone was maintained throughout the trial. At baseline,

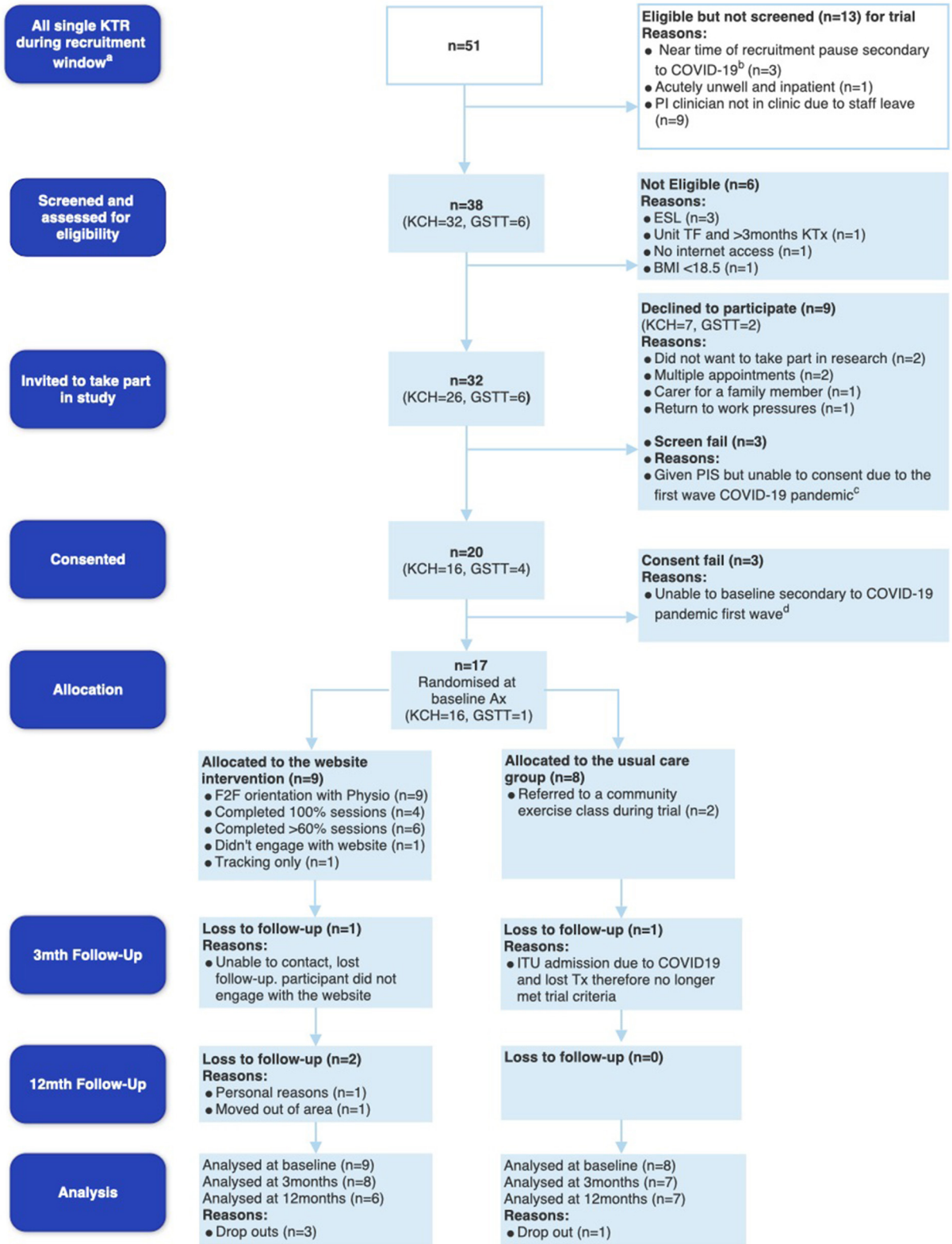

FIGURE 1 | Feasibility CONSORT diagram. [a]Indicates the recruitment window (3rd September 2019–15th March 2020 for KCH and 19th February−15th March 2020 for GSTT), [b]indicates potential participants at KCH who were eligible days before recruitment was put on hold due to Coronavirus disease 2019 (COVID-19) on

(Continued)

FIGURE 1 | the 15th March 2020, [c]demonstrates the 3 potential participants at KCH who were given patient information sheets but unable to consent due to the first wave of COVID-19, and [d]indicates 3 participants who consented at GSTT but unfortunately due to pausing of recruitment, became ineligible and were therefore not baselined or randomized. KTR, kidney transplant recipients; PI, Principal Investigator; KCH, King's College Hospital; GSTT, Guy's and St Thomas' Hospital; ESL, English as a second language; TF, transfer; BMI, body mass index; ITU, Intensive Care Unit.

TABLE 2 | Participant characteristics at baseline.

Variable		Total ($n = 17$)	Intervention group ($n = 9$)	Usual care ($n = 8$)
Age	Years, median (IQR)	49.0 (39.0 to 59.0)	39.0 (33.0 to 44.0)	59.5 (53.5 to 65.0)
Sex	Males, *N* (%)	10 (58.8%)	5 (55.6%)	5 (62.5%)
Ethnicity	White Caucasian, *N* (%)	6 (35.3%)	3 (33.3%)	3 (37.5%)
	Black African and Caribbean, *N* (%)	9 (52.9%)	5 (55.6%)	4 (50%)
	Asian, *N* (%)	2 (11.8%)	1 (11.1%)	1 (12.5%)
Post-transplant time	days	62.0 (53.0 to 68.0)	62.0 (58.0 to 79.0)	59.0 (49.5 to 66.50)
Donor type	Live related, *N* (%)	2 (11.8%)	1 (11.1%)	1 (12.5%)
	Live unrelated, *N* (%)	2 (11.8%)	1 (11.1%)	1 (12.5%)
	Deceased, *N* (%)	13 (76.5%)	7 (77.8%)	6 (75.0%)
Two or more previous KTx	*N* (%)	4 (23.5%)	3 (33.3%)	1 (12.5%)
Episodes of acute rejection	*N* (%)	4 (23.5%)	2 (22.2%)	2 (25.0%)
CKD diagnosis	GN, *N* (%)	7 (41.2%)	5 (55.6%)	2 (25.0%)
	DN, *N* (%)	2 (11.8%)	1 (11.1%)	1 (12.5%)
	HT, *N* (%)	2 (11.8%)		2 (25.0%)
	Other and unknown, *N* (%)	6 (35.3%)	3 (33.3%)	3 (37.5%)
RRT before KTx	Pre-emptive transplant, *N* (%)	1 (5.9%)		1 (12.5%)
	HD, *N* (%)	10 (58.8%)	6 (66.7%)	4 (50%)
	PD, *N* (%)	3 (17.6%)	1 (11.1%)	2 (25%)
	HD and PD, *N* (%)	3 (17.6%)	2 (22.2%)	1 (12.5%)
RRT duration pre KTx	Months, median (IQR)	34.0 (24.0 to 58.0)	37.0 (34.0 to 58.0)	30.0 (22.5 to 52.0)
Baseline body weight	Kilograms, median (IQR)	92.6 (72.0 to 96.1)	94.5 (63.0 to 102.0)	81.3 (73.6 to 94.6)
Baseline BMI	kg/m^2, median (IQR)	27.9 (23.9 to 32.9)	30.0 (23.9 to 33.6)	26.8 (24.6 to 29.8)
Immunosuppression regime (total daily dose)	Tacrolimus, median (IQR)	16.0 (8.0 to 20.0)	16.0 (10.0 to 20.0)	13.0 (6.0 to 24.0)
	Prednisolone, median (IQR)	5.0 (5.0 to 7.5)	5.0 (5.0 to 5.0)	8.8 (5.0 to 10.0)
	Mycophenolate Mofetil, median (IQR)	1,000 (1,000 to 1,000)	1,000 (500 to 1,000)	1,000 (1,000 to 1,000)
Baseline renal function ($mL/min/1.73\ m^2$)	CKD-EPI creatinine eGFR, median (IQR)	40 (32 to 60)	42.0 (29.0 to 64.0)	40.0 (33.0 to 44.0)
Smoking history	Current smoker, *N* (%)	2 (11.8%)	1 (11.1%)	1 (12.5%)
	Ex-smoker, *N* (%)	6 (35.3%)	3 (33.3%)	3 (37.5%)
Anti-hypertensive medications	Taking antihypertensives, *N* (%)	11 (64.7%)	7 (77.8%)	4 (50.0%)
	Number of antihypertensive medications, median (IQR)	1.0 (0.0 to 1.0)	1.0 (0.1 to 1.0)	0.5 (0.0 to 1.0)
Baseline blood pressure (mmHg)	SBP, median (IQR)	138.0 (121.0 to 149.0)	137.0 (121.0 to 148.0)	143.0 (117.5 to 150.0)
	DBP, median (IQR)	83 (73.0 to 88.0)	83.0 (73.0 to 86.0)	85.5 (75.0 to 90.5)
Diabetes diagnosis	Type 1 diabetes, *N* (%)	1 (5.9%)	1 (11.1%)	
	Type 2 diabetes, *N* (%)	2 (11.8%)		2 (25%)
	PTDM, *N* (%)	1 (5.9%)	1 (11.1%)	
Diabetic medication	Insulin only, *N* (%)	3 (17.6%)	2 (22.2%)	1 (12.5%)
Number of comorbidities*	One, *N* (%)	9 (52.9%)	6 (66.7%)	3 (37.5%)
	Two or more, *N* (%)	8 (47.1%)	3 (33.3%)	5 (62.5%)

*Median and IQR ranges (IQR) are presented for continuous data. Proportion percentages and frequency numbers are shown for categorical data. *Indicates comorbidities included a medical history of diabetes, hypertension, cerebrovascular event, osteoarthritis, brain hemorrhage, cardiovascular disease, cancer or respiratory disease. Episodes of acute rejection were classified as yes or no within the first 3 months from medical notes and biopsy reports. CKD, chronic kidney disease; KTx, Kidney Transplant; GN, glomerular nephritis; DN, Diabetic Nephropathy; HT, Hypertension cause; RRT, Renal replacement therapy; HD, hemodialysis; PD, peritoneal dialysis; PTDM, post-transplant diabetes mellitus; BMI, body mass index; eGFR, estimated glomerular filtration rate; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.*

only one IG participant had a diagnosis of post-transplant diabetes mellitus. **Supplementary Material 1** depicts detailed sample characteristics.

Retention

Four out of the 17 participants that were randomized did not complete the trial (IG $n = 3$, UC $n = 1$). The total sample 12-month retention rate was 76.4% (95% CI 50.0 to 93.2). The IG 12-month retention rate was 66.7% (95% CI 29.2 to 92.5). The UC 12-month retention rate was 87.5% (95% CI 47.4 to 99.7%). Withdrawal reasons are depicted in **Figure 1**.

Adherence to the DHI

Adherence with the 12-weekly sessions varied. The median number of total sessions completed by IG participants was 10 (IQR 5 to 12) out of the 12-weekly sessions (**Table 3**). Six out of the nine IG participants (66%, 95% CI 29.9 to 92.5%) met the progression criteria of adhering to 60% or more of the sessions. Four participants completed all 12 sessions. Three participants were partial completers and had individual adherence rates of 75, 42, and 83%, respectively. One IG participant chose to only use the body weight and PA tracking functions of the website, and but did not complete the 12-weekly sessions. Another IG participant chose not to engage with the website and was lost to follow-up (**Figure 1**). "Trigger messages" were activated in 7 participants, with 2 participants re-engaging with and completing the 12-week ExeRTiOn DHI. Three IG participants chose to re-visit the ExeRTiOn DHI after completion of the sessions to review content ($n = 2$) or continue with the physical activity and body weight tracking function ($n = 1$). Six of the nine IG participants (66.7%) chose to view the ExeRTiOn DHI with their smart phones (see **Table 3**). **Table 3** below summarizes IG participants engagement with the ExeRTiOn DHI.

TABLE 3 | Intervention group participants engagement with the ExeRTiOn DHI.

Variable		IG participants ($n = 9$)
Devices used to view the ExeRTiOn DHI, *n* (%)	Smartphones	6 (66.7)
	Tablet	1 (11.1)
	Laptop	1 (11.1)
	PC	1 (11.1%)
Number of logins to the ExeRTiOn DHI, median (IQR)		13 (7 to 22)
Number of sessions completed, median (IQR)		10 (5 to 12)
Minutes to complete session 1, median (IQR)		5 (1 to 10)
Minutes to complete session 2, median (IQR)		9 (6 to 16)
Minutes to complete session 3, median (IQR)		6 (5 to 12)
Minutes to complete session 4, median (IQR)		7 (4 to 8)
Minutes to complete session 5, median (IQR)		9 (8 to 10)
Minutes to complete session 6, median (IQR)		4 (2 to 19)
Minutes to complete session 7, median (IQR)		7 (6 to 7)
Minutes to complete session 8, median (IQR)		4 (1 to 6)
Minutes to complete session 9, median (IQR)		3 (1 to 5)
Minutes to complete session 10, median (IQR)		9.5 (5 to 17)
Minutes to complete session 11, median (IQR)		18 (7 to 20)
Minutes to complete session 12, median (IQR)		12 (8 to 19)
Total per-participant physical activity minutes entered into DHI, median (IQR)		650 (250 to 1736.0)
Number of goals set per participant, median (IQR)		3 (1 to 5)
Type of goals set on the ExeRTiOn DHI, *n* (%)	PA only	2 (22.2%)
	diet only	1 (11.1%)
	both PA and diet	4 (44.4%)
	no goals set	2 (22.2%)

Continuous data summarized using Median and IQR. Categorical data is shown using proportions (n, %). IG, intervention group; DHI, digital health intervention; IQR, interquartile range; PA, physical activity.

Fidelity of the ExeRTiOn DHI

The ExeRTiOn DHI was retrospectively mapped to the behavior change wheel (BCW) (26, 27) and coded to the behavior change technique taxonomy version 1 (BCTTv1) (25). All physiotherapist encounters were anonymized and coded in NVIVO, refer to **Supplementary Material 2**. ExeRTiOn content was read and re-read and coded using a BCTTv1 coding framework (27). Whilst BCT's known to inform PA and healthy eating behaviors (24) were central to the design and development of the ExeRTiOn DHI (16), *post-hoc* coding revealed 11 additional BCT's.

The most frequently represented BCT in the ExeRTiOn DHI was BCT "prompt and cues" (25) that was used 25 times. These in-built prompts occurred throughout each of the 12-weekly sessions and facilitated participant engagement with the ExeRTiOn DHI. The most frequent BCT in the physiotherapist interactions was BCT "social support (unspecified)" (27) which was used 83 times. This included advice, praise, and encouragement throughout the personalized messages. "Social support (unspecified)" was thought to influence each of the three target behaviors of the ExeRTiOn DHI (Increase PA, engagement with the ExeRTiOn DHI, and the use of a balanced diet (including healthy eating and portion control).

Outcome Acceptability

Assessment visits with recruited participants took place from the 27th of September 2019 to the 22nd of March 2021. The median time to complete assessments at baseline, 3- and 12-months was 70 min (IQR 60 to 88) ($n = 17$), 48 min (IQR 30 to 60) ($n = 15$), and 50 min (IQR 48 to 53) ($n = 13$). There were no missing data at baseline. Missing data at 3- and 12-months was due to study dropouts ($n = 4$), and the challenges associated with conducting research in an extremely clinically vulnerable population during the COVID-19 pandemic. At 3-months 8 participants were unable to complete full outcomes due to shielding during the fast wave of the COVID-19 pandemic, with 6 assessments being conducted over the telephone. Therefore, face-to-face outcomes (bioimpedance analysis, pulse wave velocity, augmentation index, waist- and hip-circumference, and 6-minute walk test) were not collected. Clinical data (bloods, body weight, blood pressure and heart rate) were collected from medical records. Questionnaires and qualitative interviews were

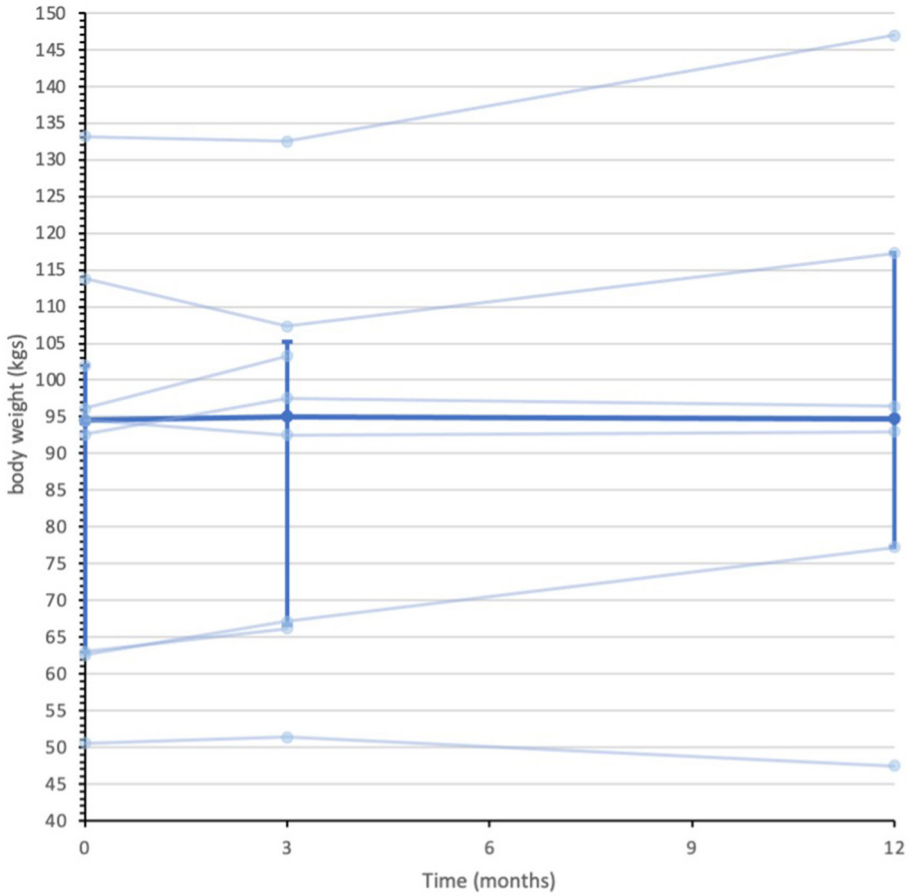

FIGURE 2 | Data series of individual and median body weight values for IG participants ($n = 9$). Individual data series for participants in the intervention group depicted by the pale blue lines. Median depicted by darker blue line, with IQR error bars. Median was calculated from all recorded data at each assessment point. $n = 9$ at baseline, $n = 8$ at 3-months, and $n = 6$ at 12-months.

conducted over the telephone. At 12-months, 12 out of the 13 participants completed face-to-face assessment with COVID-19 safety procedures *in situ*. One participant (UC) requested a virtual follow-up.

Safety and Hospitalizations

There were no associated serious adverse events. Six non-related serious adverse events occurred evenly across the study sample ($n = 3$ IG, $n = 3$ UC group). Reasons included hospitalization for COVID-19 ($n = 1$ UC, $n = 1$ IG), urgent transplant renal artery angioplasty ($n = 1$), elevated blood glucose levels due to post-transplant diabetes mellitus ($n = 1$), an episode of Cytomegalovirus viraemia ($n = 1$) and acute transplant rejection ($n = 1$). Unfortunately, a UC participant lost their transplant during intensive care admission for COVID-19 and were withdrawn from the trial (**Figure 1**). Seven participants had an episode of transplant rejection confirmed via biopsy. Ten (5 from each group) experienced Cytomegalovirus viraemia requiring treatment with valganciclovir.

Secondary Outcomes

Secondary quantitative outcome data are summarized in **Supplementary Material 3**. Median (IQR) IG bodyweight were 94.5 (63.0 to 102.0) kilograms (kgs) at baseline, 95.0 (66.7 to 105.3) kgs at 3-months and 94.7 (77.2 to 117.3) kgs 12-months. In contrast, the UC group median (IQR) body weight measures were 81.3 (73.6 to 94.6) kgs at baseline, 86.2 (75.4 to 96.5) kgs at 3-months and 93.3 (70.3 to 101.9) kgs at 12-months. **Figures 2**, **3** display individual and median body weight values for both groups.

Median 6-minute walk distance (IQR) measurements were 450 (450 to 540) meters (m) at baseline, 525 m (472.5 to 615 m) at 3-months, and 495 m (465 to 615 m) at 12-months in the IG. In the UC group, the median 6-minute walk distance (IQR) were 517.5 m (436 to 570 m) at baseline, 507.5 m (442.5 to 605 m) at 3-months, and 435 m (435 to 555 m) at 12-months. Median BMI, waist- and hip-circumference, pulse wave velocity, augmentation index, and questionnaires appeared comparable across the sample (see **Supplementary Materials 3**, **4**).

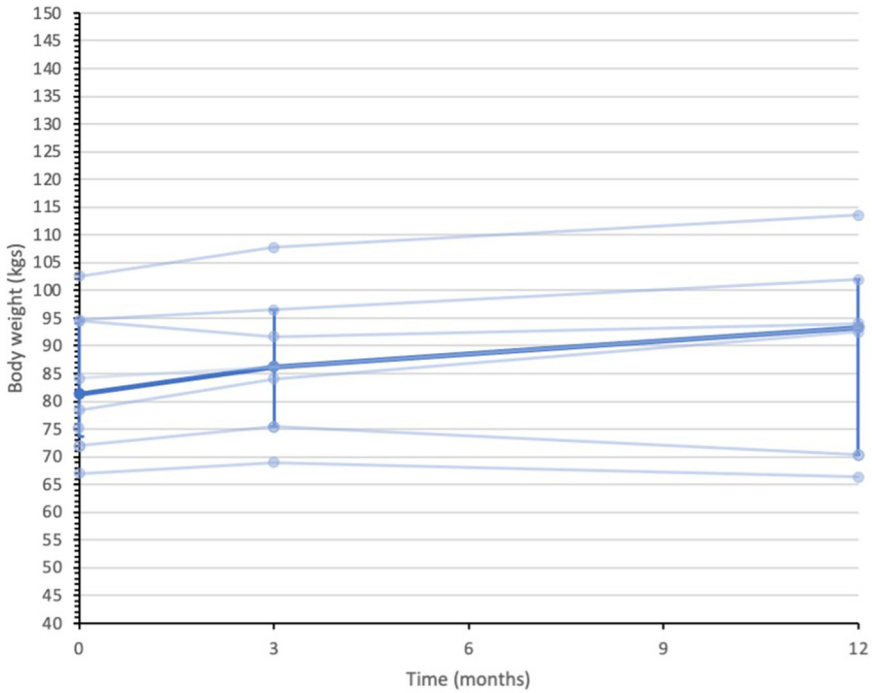

FIGURE 3 | Data series of individual and median body weight values for UC participants ($n = 8$). Individual data series for participants in the usual care group are depicted by the pale blue lines. Median depicted by darker blue line, with IQR error bars. Median was calculated from all recorded data at each assessment point. $n = 8$ at baseline, $n = 7$ at 3-months, and $n = 7$ at 12-months.

Qualitative Results

Thirteen participants were invited to and completed individual semi-structured interviews between the 31st of January 2020 and the 20th of August 2020 (**Supplementary Material 5**). One interview was conducted face-to-face prior to COVID-19. The remaining 12 interviews were conducted over the telephone. Topic guides (see **Supplementary Material 6**) were amended to include questions regarding the impact of COVID-19.

Reflexive thematic analysis (44) revealed four main themes relating to the experience of using the ExeRTiOn DHI, and the experience during trial participation. **Figure 4** below summarizes the final thematic map. Illustrative quotes for each theme and subtheme are depicted in **Table 4**.

Theme 1- Optimizing Participation and Recruitment

Research participation was seen as an important opportunity to "give back" to the community after receiving the "gift" of a kidney transplant. This altruistic view was consistently associated with reports of fostering research participation. Clear written and verbal communication, and rapport with the research staff aligned with a positive recruitment experience. The ability to ask questions and seek answers from a specialist physiotherapist was perceived as an important source of information. Largely, the recruitment within 3-months of transplantation was acceptable. However, one participant felt that recruitment window was too short (**Table 4**).

Theme 2- the Impact of COVID-19

The breath and severity of COVID-19 was consistently reported across the dataset. Shielding measures were viewed to have had a direct impact on physical and mental well-being. Unique barriers were presented by participants who were shielding at home and influenced PA behavior and motivation. "Trigger messages," sent by the trial physiotherapist were identified as a tool to navigate personal barriers such as time, work, challenges arising from COVID-19, and to support participants to re-engage with the DHI. Support (from families and professionals), mental resilience, and a positive mindset were frequently reported as facilitators to navigate the unique challenges experienced by the KTRs that arose from the shielding measures in place during the outbreak of COVID-19 (**Table 4**).

Theme 3- Engagement With the DHI Is a Choice

Engagement with the ExeRTiOn DHI was described as an individual choice, influenced by both personal and technical factors (**Figure 4**). Previous knowledge and experience of PA and healthy eating behaviors, preference for mode of delivery of the weight gain prevention and self-efficacy appeared to be linked with self-efficacy in this dataset. The ExeRTiOn DHI was suggested as a flexible mode to deliver interventions in the acute recovery phase of kidney transplantation (**Table 4**).

The brief one-on-one orientation session at the start of the intervention with the trial physiotherapist was widely reported

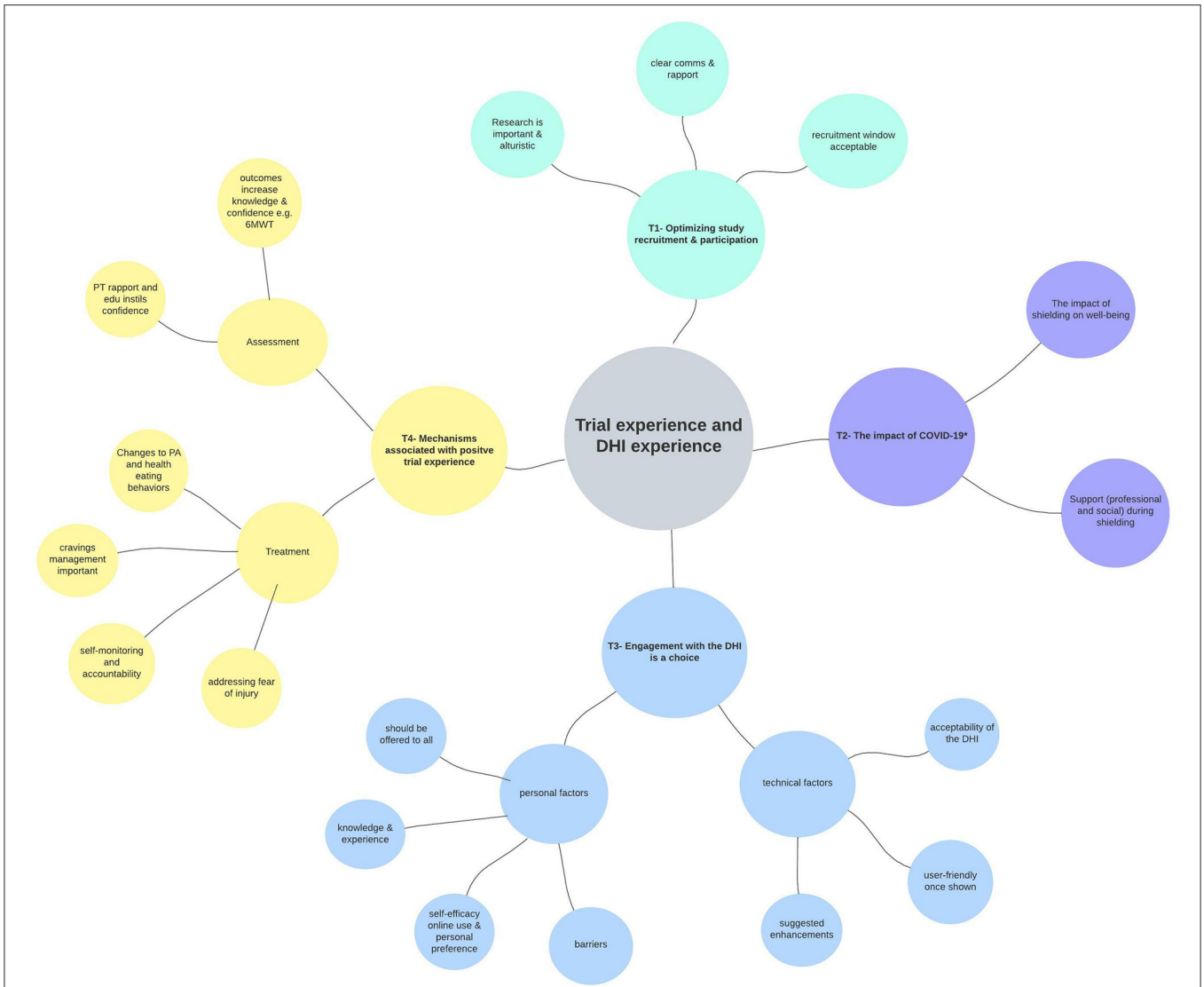

FIGURE 4 | Thematic map from reflexive thematic analysis ($n = 13$). Key themes are represented in different colors. The research question is shown at the center of the diagram, with outward branching themes (T1 to T4), and subthemes from the qualitative analysis. The * in Theme 2 above depicts the first wave of COVID-19 and the shielding enforced to Kidney Transplant Recipients (23rd March 2020 to the 1st of August 2020). Ax, assessment; comms, communication; DHI, digital health intervention; edu, education; PA, physical activity; PT, physiotherapist; tech, technical; 6MWT, six-minute walk test.

as essential. Some participants felt the DHI was easy to use whilst others felt some enhancements could be considered. For example, it was suggested to reduce the length of activities within session 10 (overcoming barriers) and session 11 (problem solving). Participants also suggested the "home exercise diary tab" could be categorized into different functional abilities, and the addition of a virtual group exercise class could facilitate motivation and engagement. One participant reported initial difficulty with the DHI. However, this improved with repeated use. Overall, participants felt that the ExeRTiOn DHI was acceptable, and provided a supportive space for new KTRs to address PA and healthy eating behaviors after kidney transplantation (**Table 4**).

Theme 4- Mechanisms of Action

The face-to-face study visits were viewed as a key mechanism and were consistently aligned with a positive study experience. Participants apportioned value to the opportunity to have an additional "check-up" and "benchmark" their functional abilities. The 6-minute walk test and bioimpedance analysis were the most valued outcomes. The completion of the 6-minute walk test acutely post-transplant with the trial physiotherapist was suggested to enhance confidence in walking ability, irrespective of group allocation.

Participants in the DHI group reported both changes to PA and healthy eating behaviors, with session 2 (management of cravings) being the most valued session. Self-monitoring

TABLE 4 | Themes relating to the acceptability, feasibility, and experience of both trial participation and the ExeRTiOn DHI with illustrative quotes.

Subtheme/divergent quotes if evident from dataset	Illustrative quotes
Theme 1—Optimizing participant recruitment	
Research is important and altruistic	*I am happy to do research, and you know, if it helps the next person down the line. because somebody in front has helped me. (P01, female, UC group)* *When I had one taken out, kidney taken out, I've given it straight to, I've donated it to the cancer research… because if I can help in anyway, by helping someone else, you know- all be it. (P02, male, UC group)*
Clear communication and rapport is essential	*Yeah, it was good there was no pressure, I felt like I could ask questions. Uhm, you know the paperwork I filled out was pretty self-explanatory, uhm it was very detailed you know it was very good. (P03, male, UC group)* *I think initially having that talk with physio did help me um because all you hear is hearsay quite a lot, especially when you're in the kidney clinic and talking to other participants, you're not sure who, who is being honest and who's not [laughter] but it just creates more paranoia and curiosity. (G03, IG)*
Recruitment window acceptable	*It's not an unreasonable time. And I think especially where your target people…their likely to have the time. Um. at that you know- it's not as if they're you know. they're not, especially in the first 3-months, they're not leaping around, um. Worrying about you know a busy schedule. (P10, female, IG)* *When I was recruited, I just wanted to kind of get going and basically see what the website was all about (P07, female, IG)*
Limited contrasting quotes	*I thought it was too soon. Because after the operation, I didn't even feel myself for the past- the last three- six months. (P09, female, UC group)*
Theme 2—The impact of COVID-19	
The impact of shielding on well-being	*It has made exercise a bit more difficult because I look after my son full-time now at home and it's hard to carve out time to exercise and I can't run in the park, I can't go to classes. So, we were doing Joe Wicks every day, but that's not the same as being outside in the fresh air exercising. (P03, male, UC group)* *I just feel like I don't want to do nothing, I can't be bothered, I just want to be left alone (P06, female, IG)*
Support (professional and social) during shielding	*I think, um it will help because I spoke to my physio quite a bit, she used to call me and um, she, she would, I'd tell her sometimes and shed be like you know what you know keep busy, do this and do that and stuff like that she would give me advice. (G03, female, IG)* ***Interviewer:*** *Has there been anything else COVID-19 has made it harder for you to do, in regard to the trial?* ***P07:*** *uhm no, because like I said I can access it on my phone that I have with me, so no. (female, IG)*
Theme 3—Engagement with the ExeRTiOn DHI is a choice	
Personal factors	*We are both [partner] definitely working slightly longer for working from home. And therefore, you feel tired from a different way. (P15, male, IG) She [research fellow] helped me go to the website, and in the beginning, I actually forgot about going to the website because I uh wasn't used to, so she actually reminded me sometimes to go and do my exercise. (P05, male, IG)* *I was just [pause] following the programme through.. Um but that was just my personal thing. Just because I have- you know I have the knowledge and the confidence to do my own thing. (P10, female IG)* *It would be cool to know that is the kind of thing that is presented to people once they have had a transplant. Because there are going to be people who are in worse positions then me… I think it would be good to give them the option. Because it's always nice to have the option to do this (P15, male, IG)*
Technical factors	*The problem-solving thing, um there was steps where it said identify the problems…it was a bit too much, there was a lot of things that you had to write down (GO3, female, IG)* *Maybe under different tabs for example- different link or tab. This is for older people with less strength. And then for I don't know, younger participants? Because I have seen some there was some transplant participants (hospital name), they are younger. They can lift more whilst they recover (P12, male, IG)* *I would say instead of pictures, maybe get videos, uhm but I think there is a video where there is instructing, what sort of exercise would look like (GO3, female, IG)* *Like the website was straight forward and the videos explained anything that if I'd had queries to, the videos would answer it. (P07, female, IG)*
Limited contrasting quotes	*I think if it was something more like [pause], let me see, in a group or more personal thing, think like not on the phone you get into a group to do the exercises it would be more motivating to do it (P06, female, IG)* *If I'm honest I don't think there is much of a change in my opinion. I found there was far much more on that site erm that I even needed.. you know rather than going on the internet, rather than going on you know other websites and stuff I found that this particular website that there was a lot on there to help. (P04, male, IG)* *To navigate around it, I found, I found it a bit difficult at first, I didn't really get it. (P06, female, IG)* *The more you use it, the more you get used to it, so then it is not so bad... I realized that if I just give it go, then I would be able to do it. (P06, female, IG)*
Theme 4—Mechanisms of action associated with a positive study experience	
Assessment factors	*Yeah. I think that one was good. Because [pause] we need these things to check if everything is working well in our life. So yeah. I think it helped. It put my mind- it give me piece of mind. (P09, female, UC group)* ***Interviewer****: what your overall experience of this research trial has been like for you?* ***P01*** *excellent. excellent. It has shown me that I can walk. If I put my mind to it [laughs]... really walk. (P01, female, UC group)* *It was just the conversation you're having whilst you're doing the trial uhm, I think makes you a lot more at ease anyway. Like [physio name]*

(Continued)

TABLE 4 | Continued

Subtheme/divergent quotes if evident from dataset	Illustrative quotes
	[pause] you know talking to her like she was my sister sort of thing not as like a doctor. You know yeah it made you feel very comfortable. (P02, male, UC group) *I was more muscles than my fat because I was very worried about the fat. but when she-she measured the muscles within me and the fat she told me that I was more muscles than the fat I was thinking of. She-she even went ahead to tell me about the percentage of muscles that I had so I was very very uh-u-h I actually felt very good. (P05, male, IG).*
Treatment factors	*If I do exercise, what if I damage my new kidney, that's the only thing that comes to your mind…: but when I saw the exercises on there, it was very much um, you know puts you at ease and you know, you knowing that it's not anything that is going to hurt you physically. (G03, female, IG) When I started, I had pains in my abdomen, but gradually it went away, as I began to exercise. (P05, male, IG)* *What the exercise on the website does is, is quite um almost like a baby step kind of thing, like it is all up to your pace, it's all up to um what pace you can do, and I think the more active you have become, the more you can go faster, the more you can do extra steps or anything like that, so without it I don't think I would have like you know recovered as fast as I did. (G03, female, IG)* *To learn about the-the-the exercise, yes about the exercise, so I go there to remind myself about the exercise and-and-and the cravings. And-and sometimes I-I show the uh food the proportion to my wife and telling her that and I need to eat more vegetables and fruits than the carbohydrate. (P05, male, IG)* *It [DHI] made me do exercise, for someone who doesn't like exercise at all, uhm [laughter] it made me at least do 10 minutes a day, because obviously I have the kids and now that they are not in school so at least taking 10 minutes out of my day, to do that. I've actually started to do that, and it's been a thing I have been doing since so that's helped (P07, female, IG)* *With the tracking your weight and you're exercising, or you know your activities through your day or your week. I found by keeping a track of it kind of motivates you to want to add more to the activity part, and then to the part where you've got the weight, your-I mean for myself as well I look at it and I'm like you know I want to try and bring that weight down down down. (P04, male, IG)* *So, my point there is in terms of being accountable to something. Even though it's not a- a human being, you are being accountable to a system, and you know-you know for these 12 weeks, you need to you know, every week you need to be putting the inputs in [weight and activity tracker]. (P10, female, IG)* *It's helped me to make better choices when I eat, or I was having problems with craving at first. But when I watched that video on how to manage cravings that was helpful. So I'd say that one, that one stood out, I forgot about that one, that one stood out, that video (P06, female, IG)*
Limited contrasting quotes	*I don't want to sort of overwork it and end back up at stage 1 again. (P02, male, UC group)* *It was a big wound. It was really, paining. and it maybe could affect your kidney. Because I don't know how the kidney. I don't to shake the kidney, I don't want anything to go wrong, so I take it easy. So that that was why you know taking it easy. Not to do stress there. Serious exercise, or shaking myself, or doing something worse, just taking it easy. (P09, female, UC group)*

UC refers to usual care group participants, IG, intervention group participants, P and G, to participant numbers.

and monitoring and feedback by the trial physiotherapist were suggested to be associated with accountability and could encourage engagement with the ExeRTiOn DHI. In contrast, participants from the UC group reported little to no difference in PA and healthy eating behaviors.

The fear of injuring the new kidney was widespread in this dataset. IG participants viewed the ExeRTiOn DHI as "baby steps" or "steppingstones" to build up PA after surgery. This gradual approach was described as a potential mechanism for the ExeRTiOn DHI to improve PA behavior and confidence. In contrast, participants in the UC group reported that they didn't want to "push-it" with PA after kidney transplantation. Data describing limited changes in PA activity largely originated in data from UC participants.

The ability to access "expert" advice and social support by the trial physiotherapist through the secure message function was seen to further enhance the positive DHI experience. A Consistent report from all interview participants, irrespective of randomization, was that the DHI should be offered to all new KTRs post-surgery (**Table 4**).

Integrated Mixed Methods Analyses

The integration of qualitative and quantitative results suggests that an RCT using the ExeRTiOn DHI is feasible and acceptable for new KTRs. Further studies should ensure there is clear communication and rapport with researchers and valued patient assessment outcomes (e.g., 6-minute walk test and bioimpedance analysis are included). Craving management, self-monitoring of PA and body weight, monitoring and social support (unspecified) by the trial physiotherapist, and gradual PA were identified as factors that could have contributed to the success of the DHI.

DISCUSSIONS

The primary feasibility outcomes achieved in this study were a screening rate of 84.2% (95% CI 68.8 to 94.0), a consent rate of 62.5% (95% CI 43.7 to 79.0%), 12-month retention rate of 76.4% (95% CI 50.0 to 93.0), adherence rate to baseline assessment of 100% (95% CI 80.5 to 100.0), 3-month assessment of 88.3% (95% CI 63.6 to 98.5), 12-month assessment of 76.4% (95% CI 50.0 to 93.2%), and an adherence rate to the ExeRTiOn DHI of 66.7% (95% CI 28.9 to 92.5). There were no associated adverse events, and 29.4% of participants had a non-related adverse event.

TABLE 5 | Mixed-methods results against feasibility outcomes and progression criteria.

Feasibility measure	Definition	Rates with confidence intervals	Progression criteria	Notes
Screening rate	% Of screened participants that met the inclusion criteria during the study recruitment window	32/38 84.2% (95% CI 68.8 to 94.0)	≥50% deemed eligible approached to do the study	
Total consent rate	% Participants recruited from the total eligible potential participants in the units	20/32 62.5% (95%CI 43.7 to 79.0)	>50% of people approached consent to study who have been screened and deemed eligible to take part in the trial	Target sample of $n = 50$ not met due to changes in recruitment criteria due to COVID-19 pandemic
Trial retention at 12 months	% Participants completed trial from total sample	13/17 76.4% (95% CI 50.0 to 93.2)	Retain ≥60% of the sample at 12 months follow up	Progression criteria for retention met despite COVID-19 pandemic
Adherence to data collection at baseline Ax	% Participants who attended the baseline study visit AND completed all secondary outcomes	17/17 100% (95% CI 80.5 to 100.0)		Full outcomes include Body weight, BMI, BIA, PWV, AI, 6MWT, EQ-5D-5L, CFS, GPPAQ and self-efficacy for physical exercise and nutrition
Adherence to 3-month Ax	% Of participants who attended a 3-month assessment	15/17 88.3% (95%CI 63.6% to 98.5%)		Two participants dropped out at 3-months (one in each group)
Adherence to data collection at 3-month Ax	% Participants completing full outcome data collection at 3-months assessment from total trial sample	9/17 52.9% (95% CI 27.8 to 77.0%)		Eight participants unable to complete full assessment due to shielding during the first wave of the COVID-19 pandemic BIA, PWV, AI, waist, and hip circumference and 6MWT data were not captured
Adherence to 12-month Ax	% Of participants who attended a 12-month assessment	13/17 76.4% (95% CI 50.0 to 93.2)		Two further dropouts occurred at 12-months
Adherence to data collection at 12-month Ax	% Participants completing full outcome assessment at 12 months from total trial sample	13/17 76.4% (95% CI 50.0 to 93.2)		Participants were assessed around routine clinic visits due to COVID-19 pandemic
Adherence to the online intervention (IG only)	% Treatment group participants completing 60% (≥7/12) sessions	6/9 66.67% (95% CI 29.93 to 92.51)		6/9 participants adhered to 60% or more of the sessions Qualitative data further explored engagement
Safety and hospitalization (adverse events)	% Of participants who had a NRAE. NRAE defined as a non-elective hospital admission, of >24 h, not related to the study	5/17 29.4 (95% CI 7.8 to 51.1)	Capture and report	One participant had two NRAE's There were no related AE's
Expected and unexpected harms	Expected harms could include musculoskeletal injuries from performing exercises or slips and trips	No slips, trips or musculoskeletal injures reported	Capture and report	

Definitions, raw numbers, proportions, and 95% confidence intervals are shown for each of the feasibility outcomes above. Willingness to be randomized is reported in the qualitative results. Ax refers to assessment, BMI, body mass index; BIA, bioimpedance analysis; PWV, pulse wave velocity; AI, augmentation index; 6MWT, six-minute walk test; CFS, Chalder fatigue scale; NRAE, non-related adverse events.

Despite the outbreak of COVID-19 during this study, all *a priori* progression criteria were achieved. **Table 5** below demonstrates the mixed-methods results against the feasibility outcomes and progression criteria. The 12-month retention rate of 76.4% from this study exceeded the progression criteria (60%) and was comparable to previous face-to-face exercise interventions in people living with chronic kidney disease (71). Adherence rates to study visits were satisfactory despite the COVID-19 pandemic occurring during data collection.

The few existing trials utilizing exit surveys and semi-structured interviews have reported participation with other online interventions are positive and could improve accountability in KTRs (72, 73). The nested qualitative analysis in this study builds on these findings. Our interview participants postulate factors associated with a positive study experience (see **Table 4** and **Figure 4**). The rapport with the trial physiotherapist, the education provided, and the assessment outcomes themselves such as the 6-minute walk test appeared to contribute to the acceptability of this feasibility RCT and the ExeRTiOn DHI.

The progression criteria for adherence to the ExeRTiOn DHI were satisfied, with 66% of the IG participants completing 60% or more of the 12-weekly sessions. This shows promise, given that dropout rates tend to be higher with DHI when compared with face-to-face interventions (74). Whilst other research utilizing DHI's in KTRs have reported good adherence rates (73, 75), these DHI were supported by either live video

calls (73) or face-to-face sessions (75). In comparison, whilst demonstrating lower adherence rates, the ExeRTiOn DHI was completed independently, with minimal remote monitoring by the trial physiotherapist. Further studies would benefit from cost-effectiveness evaluations DHI's with minimal remote monitoring such as the ExeRTiOn DHI.

A key strength of this feasibility RCT was the involvement of KTRs throughout the design, development, and evaluation of the ExeRTiOn DHI. Prior research (10) informed iterative refinements to the ExeRTiOn product prior to this feasibility RCT. This combined intervention design approach (30), with the person-based approach (31) at the center, was thought to contribute to the acceptability of the ExeRTiOn DHI.

To our knowledge, this is the first theory-informed weight-gain prevention DHI in KTRs to be mapped to the behavior change wheel (26, 27) and coded to the behavior change technique taxonomy (version 1) (25). Online weight management interventions that include brief human interaction and personalized feedback have been shown to be clinically and statistically effective in the general population, and people living with excess weight (76–78). Qualitative data revealed the behavior change techniques social support (unspecified), goal setting behavior, self-monitoring of behavior, and outcome of behavior, were valued by our participants. Self-monitoring and goal setting are suggested behavior change techniques to promote PA and healthy eating behaviors (24).

The need for support to engage with online interventions is echoed in the few studies that explore PA and dietary combined interventions in new KTRs (15). Exit survey data from Serper et al. (72) reported participants would have valued technical support and contact with the research team. The brief personalized orientation session with the trial physiotherapist was seen as essential in this feasibility RCT, and in our previous study (10) to enhance DHI engagement. As this is a feasibility study, it was not designed to evaluate effectiveness, or the mechanisms responsible for the treatment effect. Future study design would benefit from the evaluation of what the most effective "active ingredients" and unpicking which behavior change techniques potentially mediate the treatment effect.

The management of cravings and the gradual build-up of PA to reduce fear avoidance, self-monitoring and remote monitoring by the physiotherapist were identified as valued content of the ExeRTiOn DHI. The addition of group exercise videos was suggested to improve the ExeRTiOn DHI. Similarly, Gibson et al. (73) reported KTRs participants would value the opportunity to play-back the videos to increase flexibility. Further studies would benefit from exploring delivery of educational videos to include both live and on-demand content such as kidney beam (22).

This feasibility study, by design, was not powered to detect clinically meaningful differences between groups (59). However, descriptive data on clinical outcomes such as body weight can help inform the design of future definitive studies. A reduction in 5% body weight from baseline measures is widely considered to be clinically meaningful to reduce glycaemia and cardiovascular disease risk factors (79–81). In this small sample the median body weight in the ExeRTiOn IG group from baseline to 12-months was <5% of the baseline median weight. The usual care group appeared to increase their body weight by 12 kg by the end of this 12-month feasibility study. However, adequately powered studies are required to further explore this.

The 6-minute walk test was valued by our participants to provide confidence in their functional ability in the acute post-transplant period. Booth and Adams (82) reported similar findings in a sample of advanced cancer participants completing the incremental shuttle walk test. Their participants, and family members reported increased confidence in participants functional abilities (70). The 6-minute walk test has been shown to predict mortality in other solid organ recipients (83) and be reproducible and low cost to use in children and adolescent KTRs (84).

There is no suggested minimally clinically important difference for the 6-minute walk test in KTRs. The minimally clinically important difference for the 6WMT in other populations is variably reported; 54 to 80 m in respiratory disease (85), 32 to 43, 1 m in heart failure (86, 87), and 32 m in people with multiple medical issues (88). A study in haemodialysis participants revealed that for every 100 m increase in 6-minute walk distance, there was a 5% increase in survival (89). In this current study, the IG appeared to increase their median 6-minute walk distance by 75 m from baseline to 3-months, and 45 m from baseline to 12-months. In contrast, the UC groups reduced median 6-minute walk distance by 10 m from baseline to 3-months, and by 82.5 m from baseline to 12-months. Our data suggest that the 6-minute walk test is an outcome that warrants further exploration and could provide meaningful information to KTRs and clinicians to build confidence post transplantation.

There were six non-related serious adverse events recorded in the study (3 from each group). There were no slips, trips or injuries associated with completing the ExeRTiOn DHI independently. Other studies have raised concerns for recruiting participants within the first 6 months of transplantation (73). However, this feasibility study suggests that it is possible to complete assessments and intervene safely in a sample of KTRs recruited within 3-months of transplantation.

The impact of the outbreak of COVID-19 reduced the intended sample size from 50 to 17 for this feasibility RCT, which could have influenced the validity and results. Study recruitment was prematurely halted due to the COVID-19 pandemic, which halted all non-COVID research in the UK. Due to this, and unknown timelines for when kidney transplant surgeries would resume in the UK, the Trial Management Group advised to close recruitment.

Information regarding the conduction of this trial during the COVID-19 pandemic has been transparently reported (90), and the authors accept the limitations and challenges COVID-19 had on sample size, and data collection. The reduced sample size could have explained the higher median body weight (94.5 kg vs. 81.3 kg) and age (59 years vs. 39 years) in the UC group compared to the DHI group at baseline. Moreover, it is possible that this may have influenced our findings relating to the acceptability of the DHI. Secondary outcome results warrant further exploration

in a powered RCT. However, the qualitative results, and mixed-methods analysis revealed engagement with the ExeRTiOn DHI is influenced by personal factors and choice, and participants irrespective of randomization welcomed an individualized DHI to address weight gain prevention in new KTRs.

Missing outcome data was due to shielding practices resultant from COVID-19, not due to issues with the outcomes themselves. Lack of blinding could have influenced the results. Due to the nature of the study design, exercise and behavioral studies are often unable to achieve double blinding. Future follow-up studies should include blinding of the outcome assessor to improve validity. Despite these limitations, this study provides insights into future trial design. Research questions regarding the cost-effectiveness and the clinical value of the ExeRTiOn DHI across multiple sites remain unanswered. However, this was beyond the score of this feasibility RCT. Therefore, a mixed methods multi-center RCT evaluating the clinical value and cost effectiveness of the ExeRTiOn DHI is planned.

CONCLUSIONS

This mixed-methods feasibility RCT revealed a personalized DHI for weight gain prevention after kidney transplantation was found to be feasible and acceptable to new KTRs. Despite the limitations, and the challenges faced whilst conducting research with KTRs during COVID-19, all pre-set feasibility criteria were met. Mixed-methods results provides insight into future trial design. A follow-up multi-center RCT is planned to further evaluate the clinical value and cost-effectiveness of the ExeRTiOn DHI.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the London Dulwich Research Ethics Committee (19/LO/1138). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EC, SG, and JC conceived and designed the study. EC was involved in data acquisition. EC, JG, SG, KB, RP, and JC were involved in the statistical analyses. SG, JC, KB, and JG supervised and mentored the study. EC and SG take responsibility that this study has been reported honestly, accurately, transparently, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned (and, if relevant, registered) have been explained. All authors assisted in the interpretation of data, contributed important intellectual content during manuscript drafting or revision, accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved, and approved the final submitted manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.887580/full#supplementary-material

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The Impact of Zinc Supplementation on Critically Ill Patients With Acute Kidney Injury: A Propensity Score Matching Analysis

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Background: Zinc is an essential trace element involved in multiple metabolic processes. Acute kidney injury (AKI) is associated with low plasma zinc, but outcomes with zinc supplementation in critically ill patients with AKI remain unknown. Our objective was to investigate the effectiveness of zinc supplementation in this patient population.

Methods: Critically ill patients with AKI were identified from the Medical Informative Mart for Intensive Care IV database. Prosperity score matching (PSM) was applied to match patients receiving zinc treatment to those without zinc treatment. The association between zinc sulfate use and in-hospital mortality and 30-day mortality, need for renal replacement therapy (RRT), and length of stay was determined by logistic regression and Cox proportional hazards modeling.

Results: A total of 9,811 AKI patients were included in the study. PSM yielded 222 pairs of patients who received zinc treatment and those who did not. Zinc supplementation was associated with reduced in-hospital mortality (HR = 0.48 (95% CI: 0.28, 0.83) $P = 0.009$) and 30-day mortality (HR = 0.51 (95% CI, 0.30, 0.86) $P = 0.012$). In the subgroup analysis, zinc use was associated with reduced in-hospital mortality in patients with stage 1 AKI and those with sepsis.

Conclusions: Zinc supplementation was associated with improved survival in critically ill patients with AKI. The supplementation was especially effective in those with stage 1 AKI and sepsis. These results need to be verified in randomized controlled trials.

Keywords: critical ill, acute kidney injury, zinc supplementation, sepsis, intensive care unit

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INTRODUCTION

Acute kidney injury (AKI) is common in critically ill patients admitted to the intensive care unit (ICU), and its prevalence has risen as high as 50% in recent decades (1). To date, no specific treatment strategies have definitively improved outcomes in these patients. Current therapeutic approaches for critically ill patients with AKI revolve around volume status control (2), hemodynamic management (3, 4), renal replacement therapy (RRT) (5) and avoiding nephrotoxic drugs. However, whether this population benefits from these treatment

options remains controversial (6). Fluid and electrolyte management, metabolic stabilization, and nutritional supplements including diuretics, sodium bicarbonate, ondansetron, and thiamine also have been investigated in critically ill patients with AKI (7–9).

Zinc is an essential micronutrient involved in numerous metabolic processes. Its deficiency results in immune dysfunction and infection, and zinc is viewed as fundamentally important in critical illness (10–12). Low plasma zinc has been observed in patients with AKI regardless of RRT treatment (13), and zinc has shown potential antioxidant activity in renal ischemia reperfusion injury (14). The benefit of zinc supplementation for the immune response has been demonstrated in both adults and critically ill children (15, 16). However, little evidence is available regarding the impact of zinc treatment on critically ill patients with AKI. In this propensity score matching (PSM) study, our objective was to investigate the efficacy of zinc supplementation in critically ill patients with AKI. We hypothesized that zinc supplementation as adjunctive therapy would be associated with improved survival in this patient population.

METHODS

Database

The study data were extracted from the publicly available Multiparameter Intelligent Monitoring in Intensive Care IV (MIMIC IV, version 1.0) database. These data cover more than 50,000 critically ill patients admitted to the Beth Israel Deaconess Medical Center during 2008 to 2019 (17). Access to the database was approved by the institutional review boards of the Massachusetts Institute of Technology (No. 0403000206) and the Beth Israel Deaconess Medical Center (2001-P-001699/14). One author (LX) had access to this database (Certification Number 37851920) and retrieved the data on October 15, 2021. Given that all data were anonymous, informed consent was waived. All procedures were performed in compliance with relevant guidelines and regulations.

Population Selection Criteria

Adults (≥18 years) who were admitted to ICU for more than 48 h with confirmed AKI according to the Kidney Disease: Improving Global Outcomes (KDIGO) criteria were eligible for inclusion. The KDIGO criteria (18) were as follows: an increase in serum creatinine by 50% from baseline within the previous 7 days, increase in serum creatinine by 0.3 mg/dl within the last 48 h, or oliguria (urine output <0.5 ml/kg/h) for 6 h or more. Baseline serum creatinine level was considered to be the minimum serum creatinine within 7 days prior to admission. The first serum creatinine measured at ICU admission was used as the baseline value when preadmission serum creatinine was not available. Serum creatinine during the first 48 h after ICU admission was used to define AKI stage. Patients were excluded if they were discharged or died within 48 h after ICU admission. For patients with multiple ICU admissions, only the first admission was included.

Endpoints

The objective of the current study was to investigate the efficiency of zinc sulfate as adjunctive therapy in critically ill patients with AKI. The primary endpoint was in-hospital mortality, defined as survival status at hospital discharge. Secondary outcomes were 30-day mortality, need for RRT, ICU length of stay (LOS), and hospital LOS.

Data Collection and Definitions

Data for each patient within 24 h of ICU admission were collected from the MIMIC IV database and managed using Structured Query Language (i.e., SQL) with Navicat Premium (version 9.6). The extracted information included age, sex, ethnicity, admission type, platelets, red blood cell (RBC) count, hemoglobin, white blood cell count, serum creatinine, anion gap, international normalized ratio (INR), activated partial thromboplastin time (APTT), glucose, simplified acute physiology score II (SAPS II), estimated glomerular filtration rate (eGFR), AKI stage, mean arterial pressure (MAP), RRT, mechanical ventilation, and use of vasopressors, antibiotics, or anticoagulants. In addition, the following comorbidities were included, all collected and defined according to the Implementation of the International Statistical Classification of Disease and Related Health Problems, 10th Revision: chronic kidney disease (CKD), hypertension, diabetes, heart failure, cancer, coronary artery disease, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), stroke chronic liver disease, and sepsis (19). Zinc supplementation was defined as an oral intake of 50 mg/d zinc element provided by zinc sulfate tablets. Sepsis was defined as a recorded or suspected infection plus a Sequential Organ Failure Assessment score ≥2 based on the diagnostic criteria of the International Consensus Definitions for Sepsis and Septic Shock (20). CKD was defined as structural or functional injury for more than 3 months. Missing data variables in the MIMIC IV database were common, and in the present study, all variables had <5% missing values (**Supplementary Table S1**). Components were removed from the study if the proportion of missing values reached 20%, and removed factors included C-reactive protein, albumin, triglycerides, cholesterol, and serum lactate.

Statistical Analysis

Continuous variables in the current study are expressed as mean ± standard deviation or median with interquartile range and were compared using the student's *t*-test or the Mann–Whitney *U* test as appropriate. Categorical variables are presented as numbers and percentages and were compared using the Chi-square or Fisher's exact test.

PSM was performed to match patients who received zinc sulfate supplementation over the recommended dietary allowance to those who did not. Patients were matched in a 1:1 greedy nearest neighbor algorithm with a caliper width of 0.2. We generated the propensity score according to the following variables: age, sex, ethnicity, admission type, platelet, RBC count, hemoglobin, white blood cell count, serum creatinine, anion gap, INR, APTT, glucose, MAP, eGFR, vasopressor use, antibiotic use, anticoagulant use, AKI stage, SAPS II at ICU admission, CKD, diabetes, hypertension, heart failure, COPD,

ARDS, cancer, chronic liver disease, coronary artery disease, stroke, and sepsis. Standardized mean difference was applied before and after matching to evaluate the efficiency of PSM in reducing differences between the two groups. Finally, 222 matched pairs were established for further analysis.

Multivariable Cox proportional hazards regression analysis was used to estimate the relationship between zinc sulfate administration and mortality, with adjustments for confounding variables based on $P < 0.05$ in univariate analysis and potential confounders judged by clinical expertise. Linear regression was performed to evaluate the association between use of zinc sulfate and LOS. Data are given as hazard ratios (HRs) with 95% confidence intervals (CIs).

Stratification analysis was performed to explore whether the association between zinc sulfate administration and in-hospital mortality differed across various subgroups classified by AKI stage, CKD, diabetes, hypertension, heart failure, chronic liver disease, ARDS, cancer, coronary artery disease, and sepsis in the population after PSM matching.

All statistical analyses were performed using SPSS 21.0 (SPSS Inc., IBM, USA) and R 3.5.3. A $P < 0.05$ was considered to be statistically significant.

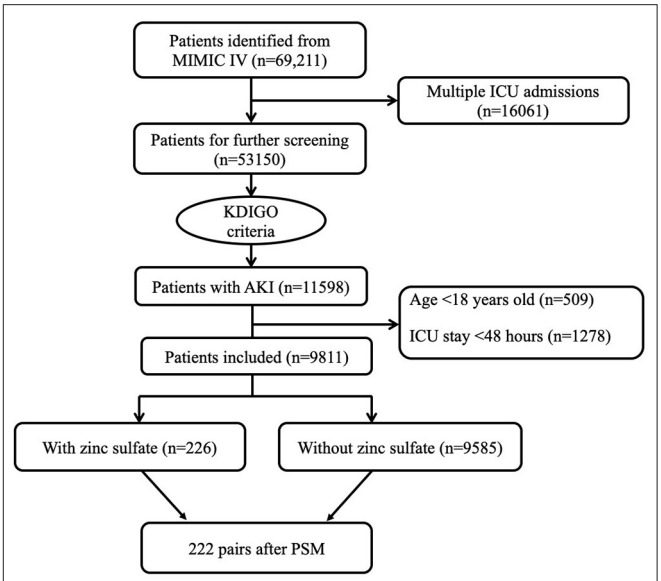

FIGURE 1 | Flowchart of patient selection. ICU, intensive care unit; MIMIC IV, Multiparameter Intelligent Monitoring in Intensive Care Database IV; PSM, propensity score matching.

RESULTS

Study Population

A total of 115,985 critically ill patients with AKI were admitted to the ICU during the study period. According to the exclusion criteria, 9,811 eligible patients were fully enrolled. Of these, 226 patients were exposed to zinc sulfate once daily within 48 h after ICU admission, whereas 9,585 patients did not receive zinc sulfate therapy (**Figure 1**).

Before PSM, there were significant differences in ethnicity and admission type between the two groups. The baseline RBC count, hemoglobin, and eGFR were higher in the non-zinc group when compared to the zinc group. Conversely, patients in the zinc group had a higher INR. Vasopressor use and anticoagulant use were more common in the zinc group. Patients with CKD were more likely to be given zinc sulfate (**Table 1**).

Association Between Zinc Sulfate and Clinical Outcomes

A multivariable Cox proportional hazard model was run for clinical outcomes between the two groups. In the pre-matched cohort, zinc sulfate use was associated with reduced in-hospital mortality [HR = 0.59 (95% CI: 0.40, 0.89) $P = 0.011$] and 30-day mortality [HR = 0.56 (95% CI: 0.32, 0.85) $P = 0.009$] after adjustment for confounding factors associated with mortality (**Supplementary Table S2**). A logistic regression model was used to estimate the impact of zinc sulfate on RRT, showing that zinc sulfate was associated with increased need for RRT [HR = 1.57 (95% CI: 1.07, 2.32) $P = 0.023$]. Furthermore, zinc sulfate use was associated with longer ICU LOS [HR = 1.80 (95% CI: 1.34, 2.42) $P < 0.001$] and hospital LOS [HR = 1.35 (95% CI: 1.21, 2.32) $P < 0.001$] (**Table 2**).

After PSM, 222 patients who received zinc sulfate were matched to 222 patients who did not. The baseline characteristics and comorbidities were well balanced between the two groups, and the standardized differences of the means were provided (**Supplementary Table S3**). Among the 222 propensity-matched pairs, zinc sulfate use was associated with reduced in-hospital mortality [HR = 0.48 (95% CI: 0.28, 0.83) $P = 0.009$] and 30-day mortality [HR = 0.51 (95% CI: 0.30, 0.86) $P = 0.012$]. However, zinc sulfate use was not associated with the application of RRT [HR = 1.31 (95% CI: 0.72, 2.39) $P = 0.371$], ICU LOS [HR = 0.86 (95% CI: 0.58, 1.27) $P = 0.444$], or hospital LOS [HR = 0.97 (95% CI: 0.65, 1.45) $P = 0.895$] (**Table 2**).

Subgroup Analysis

According to the KDIGO criteria, zinc sulfate use was associated with reduced in-hospital mortality in patients with stage 1 AKI but not in those with stage 2 or 3 AKI. Of interest, the improved in-hospital outcome was observed in patients with sepsis in the ICU [HR = 0.25 (95% CI: 0.11, 0.57) $P = 0.001$]. When the analysis was restricted to patients with diabetes, heart failure, or hypertension, zinc sulfate administration was not associated with hospital outcome. Similar analyses in other groups were not significant (**Table 3**).

DISCUSSION

The present study demonstrated an association of zinc supplementation with reduced in-hospital mortality and 30-day mortality in critically ill patients with AKI, even after adjustment for major covariates. Results of the subgroup analysis suggested that zinc treatment might have a beneficial impact on patients with stage 1 AKI according to KDIGO criteria.

There are several possible mechanisms by which zinc treatment could exert beneficial effects on critically ill patients

TABLE 1 | Baseline characteristics of groups before propensity score matching analysis.

Variables	Non-zinc group (n = 9,585)	Zinc group (n = 226)	*P*	SMD
Age	67 ± 15	65 ± 14	0.090	0.136
Sex, male, *n* (%)	5,743 (59.9)	125 (55.3)	0.163	0.075
Ethnicity, *n* (%)			0.001	0.178
White	6,185 (64.5)	156 (69.0)		
Black	870 (9.1)	32 (14.2)		
Other	2,530 (26.4)	38 (16.8)		
Admission type, *n* (%)			0.004	0.177
Observation	999 (10.4)	37 (16.4)		
Elective	494 (5.2)	7 (3.1)		
Emergency	5,694 (59.4)	140 (61.9)		
Urgent	2,398 (25.0)	42 (18.6)		
Laboratory parameters				
Platelets, 10^9/L	195.4 ± 110.9	200.0 ± 126.5	0.542	0.036
RBC count, 10^9/L	3.4 ± 0.8	3.2 ± 0.7	<0.001	0.291
Hemoglobin, g/dl	10.3 ± 2.3	9.6 ± 1.9	<0.001	0.384
WBC count, 10^9/L	13.2 ± 9.7	13.4 ± 7.3	0.762	0.013
Serum creatinine,	1.9 ± 2.1	2.2 ± 2.2	0.051	0.145
Anion gap, mmol/L	15.7 ± 5.1	15.8 ± 5.0	0.699	0.035
APTT, seconds	41.6 ± 27.1	42.3 ± 27.0	0.692	0.029
INR	1.6 ± 1.0	1.7 ± 1.1	0.024	0.134
Glucose, mg/dl	153.9 ± 86.4	148.6 ± 66.7	0.318	0.078
Co-morbidities, *n* (%)				
CKD	1,791 (18.7)	62 (27.4)	0.001	0.199
Diabetes	2,042 (21.3)	58 (25.7)	0.114	0.093
Heart failure	2,041 (21.3)	55 (24.3)	0.270	0.070
Hypertension	2,398 (25.0)	44 (19.5)	0.057	0.155
Chronic liver disease	558 (5.8)	17 (7.5)	0.282	0.067
COPD	170 (1.8)	3 (1.3)	0.801	0.091
ARDS	2,003 (20.9)	45 (19.9)	0.719	0.039
Cancer	1,042 (10.9)	19 (8.42)	0.238	0.083
Coronary artery disease	2,085 (21.8)	42 (18.6)	0.253	0.100
Stroke	275 (2.9)	5 (2.2)	0.558	0.043
Sepsis	1,271 (13.3)	40 (17.7)	0.053	0.122
MAP, mmHg	82.4 ± 19.2	80.4 ± 18.3	0.116	0.106
eGFR, ml/min/1.73 m^2	58.1 ± 34.6	52.6 ± 36.0	0.018	0.160
Mechanical ventilation, *n* (%)	6,171 (64.4)	150 (66.4)	0.537	0.040
Vasopressor use, *n* (%)	3,663 (38.2)	102 (45.1)	0.035	0.147
Anticoagulant use, *n* (%)	785 (8.2)	32 (14.2)	0.001	0.174
Antibiotic use, *n* (%)	7,432 (77.5)	186 (82.3)	0.089	0.118
AKI stage, *n* (%)			0.117	0.053
1	7,848 (81.9)	183 (81.0)		
2	432 (4.5)	5 (2.2)		
3	1,305 (13.6)	38 (16.8)		
Scoring systems				
SAPSII score	43.2 ± 14.9	44.9 ± 15.3	0.105	0.177

AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; APTT, activated partial thromboplastin time; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; INR, international normalized ratio; MAP, mean arterial pressure; RBC, red blood cell; SAPSII, Simplified Acute Physiology Score II; SMD, standardized mean difference; WBC, white blood cell.

with AKI. Zinc is required for both the innate and adaptive immune systems. Zinc deficiency induces a cumulative loss of B cell and T cell maturation, which subsequently results in lymphopenia and impaired natural killer (NK) cell function (10, 21). It has been proposed that zinc administration could restore lymphocyte production and NK cell activity (22). Zinc

TABLE 2 | Association of zinc use with clinical outcome in critically ill patients with AKI.

	Non-zinc group	Zinc group	*P*	Unadjusted HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
Pre-matched cohort	*n* = 9,585	*n* = 226					
Primary outcome							
In-hospital mortality, *n* (%)#	2,156 (22.5)	36 (15.9)	0.019	0.49 (0.36, 0.69)	<0.001	0.59 (0.40, 0.89)	0.011
Secondary outcomes							
RRT$	1,657 (17.3)	60 (26.5)	<0.001	1.73 (1.28, 2.33)	<0.001	1.57 (1.07, 2.32)	0.023
30-day mortality, *n* (%)#	2,317 (24.2)	39 (17.3)	0.016	0.65 (0.46, 0.93)	0.017	0.56 (0.32, 0.85)	0.009
Length of ICU stay*	5.9 ± 5.2	7.8 ± 6.4	<0.001	1.79 (1.36, 2.35)	<0.001	1.80 (1.34, 2.42)	<0.001
Length of hospital stay*	11.7 ± 6.9	15.8 ± 7.2	<0.001	3.17 (2.33, 4.31)	<0.001	1.35 (1.21, 2.31)	<0.001
Post-matched cohort	*n* = 222	*n* = 222					
Primary outcome							
In-hospital mortality, *n* (%)#	55 (24.8)	36 (16.2)	0.022	0.43 (0.28, 0.66)	<0.001	0.48 (0.28, 0.83)	0.009
Secondary outcomes							
RRT$	59 (26.6)	60 (26.4)	0.915	1.02 (0.67, 1.56)	0.915	1.31 (0.72, 2.39)	0.371
30-day mortality, *n* (%)#	58 (26.1)	39 (17.6)	0.029	0.60 (0.38, 0.95)	0.030	0.51 (0.30, 0.86)	0.012
Length of ICU stay*	6.4 ± 5.7	7.8 ± 6.4	0.018	0.81 (0.56, 1.17)	0.255	0.86 (0.58, 1.27)	0.444
Length of hospital stay*	12.2 ± 6.9	15.9 ± 7.2	<0.001	1.02 (0.70, 1.48)	0.924	0.97 (0.65, 1.45)	0.895

AKI, acute kidney injury; CI, confidence interval; HR, hazard ratio; ICU, intensive care unit; RRT, renal replacement therapy.

#Cox regression was used for estimating the impact of zinc sulfate use on mortality outcomes, with adjustment for confounding variables selected based on P < 0.05 in univariate analysis.

$Logistic regression analysis was used to evaluate the association of zinc sulfate and RRT.

**A generalized linear model was used to calculate beta coefficients (estimates) and P-values.*

also has been suggested to regulate metallothionein, which has a role in free radical scavenging and the inflammatory response (23). Additionally, abnormal elevation of blood glucose has been recognized as an indicator of poor prognosis in critically ill patients (24, 25), and the presence of zinc is essential for insulin secretion and glucose homeostasis (26, 27). Of interest, in animal models of bowel anastomosis, zinc supplementation has enhanced wound healing in cutaneous and gastrointestinal wounds (28). Thus, zinc use could be an indispensable medical option in patients with surgical and burn trauma.

Mild to moderate zinc deficiency is often found during the early stages of patient care after admission to the ICU because of increased metabolic rate, inadequate nutritional intake, and ongoing feeding difficulties. In most cases, plasma zinc concentration seems to reflect poor nutrition. However, in patients with an inflammatory response, rapid declines in zinc are partially the result of its redistribution into the cellular compartment (29, 30). Consequently, measurement of plasma zinc, especially in critically ill patients with AKI, can be uninformative and may be misleading. Of note, the decision to use zinc supplementation for patients, particularly in the ICU, solely depends on physician clinical judgment according to indirect clues, such as clinical characteristics (i.e., impaired wound healing, acrodermatitis enteropathica), poor nutrition, and related lab values (i.e., serum alkaline phosphatase, a zinc-dependent metalloenzyme) (31).

In our study, we found an association in the unmatched analysis between zinc supplementation and longer ICU and hospital LOS. There were differences in mortality rates between the two groups, and some patients who died early in the non-zinc group would have had shorter ICU or hospital LOS. We also found, however, that zinc use was not independently associated with ICU or hospital stay in the PSM analysis. A systematic review of four randomized controlled trials (RCTs) of zinc supplementation in non-critically ill patients showed no effect on ICU or hospital LOS or duration of mechanical ventilation (32). Most such studies have included patients with mild and moderate disease, and further investigations are needed to confirm any benefits of zinc use, whether for all critically ill patients with AKI or exclusively for those with zinc deficiency.

Subgroup analysis of various AKI stages indicated that the beneficial effect of zinc supplementation on mortality was especially observed in patients with stage 1 AKI. However, stage 2-3 AKI patients represented more severe clinical cases, and it is possible that this population may not benefit from zinc supplementation. A recent study of critically ill patients in the ICU with COVID-19, however, showed an association of oral zinc supplementation with reduced AKI incidence (33). An exact mechanism for a renal protective effect of zinc has not been identified, but zinc may mitigate AKI risk through antioxidant action (14). The potential of zinc in renal protection in critically ill patients warrants further study.

Our results additionally show that zinc supplementation was independently associated with reduced mortality in patients with sepsis. This finding is consistent with previous studies showing that zinc supplementation improved survival rates in murine models of sepsis (34, 35). However, data conflict on the association of zinc use with clinical outcomes in the context of sepsis in humans. Some relevant RCTs have shown a beneficial effect of zinc use in reducing mortality

TABLE 3 | The association between zinc sulfate therapy and in-hospital mortality in subgroups.

Subgroup	*N*	HR	95% CI	*P*	*P* for interaction
AKI stages					0.006
1	358	0.39	0.24, 0.64	<0.001	
2–3	86	0.75	0.34, 1.65	0.795	
CKD					0.083
No	321	0.45	0.28, 0.73	0.001	
Yes	123	0.16	0.06, 0.45	<0.001	
Diabetes					0.010
No	321	0.45	0.28, 0.73	0.001	
Yes	123	0.16	0.06, 0.45	<0.001	
Heart failure					0.001
No	331	0.44	0.27, 0.70	0.001	
Yes	113	0.28	0.10, 0.78	0.015	
Hypertension					0.019
No	354	0.47	0.30, 0.75	0.002	
Yes	90	0.05	0.01, 0.24	<0.001	
Chronic liver disease					0.292
No	410	0.49	0.31, 0.77	0.002	
Yes	34	0.33	0.07, 1.68	0.182	
ARDS					0.098
No	356	0.52	0.31, 0.87	0.012	
Yes	88	0.23	0.09, 0.57	0.001	
Cancer					0.340
No	401	0.52	0.33, 0.83	0.006	
Yes	43	0.71	0.21, 1.85	0.058	
Coronary artery disease					0.454
No	359	0.40	0.25, 0.64	<0.001	
Yes	85	0.67	0.14, 1.81	0.449	
Sepsis					0.018
No	355	0.63	0.37, 1.06	0.081	
Yes	89	0.25	0.11, 0.57	0.001	

AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; CI, confidence interval; CKD, chronic kidney disease; HR, hazard ratio.

rate and improving neurological development in neonates (36, 37). In contrast, Newton et al. found that the use of zinc had no notable impact on survival rate and hospital stay in neonatal sepsis (38). Of note, in another study, parenteral zinc administration was associated with an exaggerated acute phase response (39) and potentially interfered with nutritional status. A recent meta-analysis comparing outcomes for hospitalized patients receiving zinc with a control intervention (15) showed that zinc supplementation significantly reduced mortality in sepsis, in agreement with our current finding of survival benefit. Most of these previous studies involved neonatal sepsis.

Here, using PSM, we provide initial results supporting zinc supplementation in critically ill patients with AKI. Currently, evidence is limited for zinc as an adjunctive treatment in this patient population. Some studies have evaluated zinc supplementation in critically ill patients with COVID-19, and an ongoing RCT is assessing intravenous zinc in this patient population (40).

Several potential limitations in the current study should be acknowledged. First, because of the single-center retrospective design, we cannot rule out unknown confounder effects. A multi-center reearch could provide a more robust and representative evidence. Second, zinc was administered by the enteral route in our study, and many factors could lead to poor absorption of zinc in critically ill patients, such as malabsorption syndrome and inflammatory diseases of the bowel. Although plasma zinc is not routinely measured, it was thought to be useful as a diagnostic marker for evaluating the severity of sepsis. A prospective cohort trial monitoring zinc levels after zinc therapy *via* parenteral route would enable us to better investigate the association between zinc supplementation and prognosis in these population. Third, in the absence of evidence-based medical guidelines, zinc administration decisions were left to the discretion of the clinicians, which might be a source of bias. More evidence was warranted to further clarify the beneficial effect of zinc supplementation in ICU patients. Forth, the number of patients in the stage 2-3 AKI group was relatively small,

which may have resulted in selection bias and affected statistical significance. A longitudinal study with longer follow-up as well as a large sample size should be considered and studied in the future. Finally, given the ongoing pandemic, it cannot be overstated that using supplements in critical cases risks side effects for ICU patients. Further prospective studies should be considered. Thus, these findings should not be used to guide clinical practice and rather can serve to highlight the need for further investigation into the potential benefits of zinc supplementation in critically ill patients with AKI.

CONCLUSION

Zinc supplementation is associated with improved outcomes in critically ill patients with stage 1 AKI and sepsis. Well-designed prospective studies are needed to confirm these findings.

DATA AVAILABILITY STATEMENT

The clinical data used to support the findings of this study was supplied by Monitoring in Intensive Care Database III version 1.4 (MIMIC-III v.1.4). Although the database is publicly and freely available, researchers must complete the National Institutes of Health's web-based course known as Protecting Human Research Participants to apply for permission to access the database. The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by MIMIC III database used in the present study, the Institutional Review Boards (IRB) of the Massachusetts Institute of Technology and does not contain protected health information. The Ethics Committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

WX designed the study and drafted the manuscript. CL and LX analyzed the data and extracted database. DZ contributed to scientific discussion and data interpretations. MK contributed to scientific discussion. XY prepared the tables. HH supervised the study and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.894572/full#supplementary-material

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Interaction of Hydration Status and Physical Activity Level on Early Renal Damage in Children: A Longitudinal Study

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Background: Optimal water intake positively affects various aspects of human physiology, especially renal function. Physical activity (PA) may have an impact on hydration status and renal health, but the interaction of hydration status and PA level on renal function is not well-studied in children.

Methods: We conducted four waves of urine assays in our child cohort (*PROC*) study from October 2018 to November 2019 in Beijing, China. We measured urinary specific gravity, β_2-microglobulin (β_2-MG), and microalbumin (MA) excretion to assess hydration status and renal damage in the context of PA level and other covariates among 1,914 primary school children. We determined the associations of renal damage with the interaction of hydration status and PA level using generalized linear mixed-effects models.

Results: The prevalence of dehydration was 35.0%, 62.1%, 63.9%, and 63.3%, and the prevalence of insufficient PA was 86.2%, 44.9%, 90.4%, and 90.2% from wave 1 to wave 4 among 1,914 primary school children. From wave 1 to wave 4, the prevalence of renal tubular damage had a significant increasing trend of 8.8%, 15.9%, 25.7%, and 29.0% ($Z = 16.9$, $P < 0.001$), while the prevalence of glomerular damage revealed a declining trend of 5.6%, 5.5%, 4.4%, and 4.1% ($Z = -2.4$, $P = 0.016$). There were stable longitudinal associations of renal tubular and glomerular damage with hydration status (euhydration: OR = 0.50 and 0.33, respectively) but not with PA level. In multivariate analysis, significant interactions of hydration status and PA level were noted with renal tubular damage ($\beta = 0.43$, $P = 0.014$) and glomerular damage ($\beta = 0.60$, $P = 0.047$). Children with euhydration and insufficient PA were less likely to have renal tubular damage (OR = 0.46, 95% CI: 0.39, 0.53) or glomerular damage (OR = 0.28, 95% CI: 0.20, 0.39); children with euhydration and sufficient PA were also less likely to have renal tubular damage (OR = 0.57, 95% CI: 0.44, 0.75) or glomerular damage (OR = 0.47, 95% CI: 0.30, 0.74), adjusting for age, sex, BMI z-score, standardized SBP, sleep duration, computer/cell phone screen time, and fruit and vegetable intake.

Conclusion: Children with euhydration and either sufficient or insufficient PA were less likely to have early renal damage. Adequate daily water intake for children is important, especially after PA.

Keywords: water intake, hydration status, dehydration, physical activity, renal damage, children, longitudinal study, China

INTRODUCTION

Water has been described as the "most essential" nutrient, the major constituent of the human body (1). Water intake directly affects health, and optimal water intake plays a vital role in various aspects of human physiology, especially in renal function (2–4). Global data suggest that children's water intake fails to meet recommended guidelines with the high prevalence of dehydration as a frequent consequence (5, 6). In China, high academic pressures often make students have a short inter-curriculum break; children may not drink enough fluids to reduce the micturition frequency. Due to the above behavioral habits and physiological reasons, dehydration has an adverse impact on the growth and development of children and can lead to target organ damage including cardiovascular (7) and renal (2, 8) disorders.

Healthy lifestyles promote the renal health of children, reducing long-term renal damage in adulthood (9, 10). Physical activity (PA) is a well-recognized feature of health in childhood (11, 12). However, the lack of PA among children is increasingly prevalent globally (11, 13) due to academic pressures and modern lifestyles of video games, computer and cell phone access, and urban living. PA patterns are established and modifiable early in childhood and can impact the eventual development of hypertension (14) and renal disease (15, 16).

Healthy children may have higher risk of dehydration due to higher levels of PA. Without adequate hydration, childhood renal function can be compromised, given their dynamic metabolic status (17, 18). Associations between PA and hydration status and their interactions with renal disease have been inconsistent across studies and populations (17–20). We have reported previously that dehydration status aggravated renal impairment over the school week days, notably tubular abnormalities (21). To our knowledge, there are no studies investigating the interaction of hydration status and PA level on renal damage in schoolchildren. Hence, we examined longitudinal associations of renal damage with euhydration and sufficient PA to investigate the potential interaction between hydration status and PA level on renal damage in children.

METHODS

Study Design and Participants

The PROC study (www.chictr.org.cn/enIndex.aspx, No. ChiCTR2100044027, official website as https://www.procstudy.com) enrolled 1,914 children aged 6–8 years newly in six non-boarding primary schools in Beijing in 2018 [detailed elsewhere (22)]. All participants were recruited from the PROC cohort and were followed for four waves of repeated urine assays from October 2018 to November 2019 [detailed elsewhere (23)]. In brief, the wave 1 of urine assay was conducted at baseline and waves 2–4 of urine assays were conducted within a 1-week span periodically during the 1-year follow-up visit (**Figure 1**).

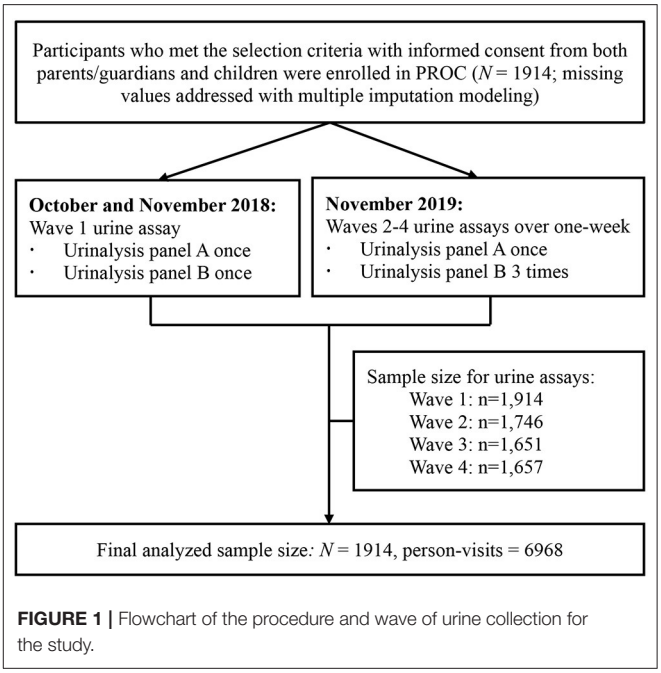

FIGURE 1 | Flowchart of the procedure and wave of urine collection for the study.

Urine Measurements and Outcome Indicators

Urine collection and test procedures were detailed elsewhere (23). In brief, fasting urine assays were conducted at baseline in wave 1, 24-h urine assays were conducted from Sunday to Monday in wave 2, and fasting urine assays were conducted on Wednesday in wave 3 and Friday in wave 4. Specific gravity (SG), β_2-microglobulin (β_2-MG), and microalbumin (MA) were measured *via* urinalysis panels A and B. Urinalysis panel A includes urobilinogen, bilirubin, ketone, occult blood, protein, nitrite, white blood cells, sugar, SG, pH, color, and turbidity; urinalysis panel B includes β_2-MG, microalbumin, potassium, sodium, uric acid, and creatinine. Dehydration status of participants was defined as SG $\geq$ 1.020, with euhydration status defined as SG $<$ 1.020 (4, 21). Renal tubular damage was defined as elevated β_2-MG $>$ 0.2 mg/L (24), and glomerular damage was defined as elevated MA $\geq$20 mg/L (25).

Data Collection of Covariates

Anthropometric measurements were conducted by trained staff and included height, weight, BMI, and blood pressure [detailed elsewhere (23)]. Z-scores of height, weight, and body mass index (BMI) were calculated using 2007 WHO standards and standardized SBP was calculated by age and sex group. Lifestyle information were reported by parents using self-administrated questionnaires, including sleep duration (Children's Sleep Habits Questionnaire [CSHQ]) (26, 27), fruit and vegetable intake (FVI; 16-item Mediterranean Diet Quality Index in children and adolescents [KIDMED]) (23, 28), and computer/cell phone screen time and PA time (self-administrated questionnaire, including 17 activities indoor and outdoor lasting at least 15 min, based on Children's Leisure Activities Study Survey Chinese version [CLASS-C]) (29). Short sleep for children was defined as sleep duration <10 h/day. Insufficient FVI was defined as <4/day. Long screen time was defined as computer/cell phone screen time ≥2 h/day. PA level as the main lifestyle variate was reported daily by parents for 1 week and was calculated both as a weekly average (total PA time/7 days) and as weekly patterns (week-day PA time/5 days and weekend PA time/2 days). PA levels were linked to the date of the urinalysis. Insufficient PA was defined as <1 h/day with weekend and weekday urinalysis: Monday tests linked to the PA level over the weekend, and Tuesday–Friday weekday tests linked to the PA level during that weekday school period.

Statistical Analysis

Descriptive statistics are presented by study wave. Categorical variables such as sex or lifestyle covariates are presented as counts and percentages. Continuous variables such as height z-score, weight z-score, or BMI z-score are described as the mean ± standard deviation (SD). Multiple imputations were performed for variables with missing values and 50 complete datasets were obtained for analysis. Trend χ^2 tests were performed to determine the prevalence trend by the study waves. Generalized linear mixed-effects models were generated to determine the associations and odds ratio (OR) with 95% confidence interval (95% CI) of renal damage with the direct association and interaction of hydration status and PA level, while the week-day and intra-wave of the urinalysis were included as random effects. The values in **Table 1** were calculated based on the first imputed dataset, while statistical inferences of the parameters in **Tables 2–4** were performed on 50 datasets using PROC MIANALYZE. A two-tailed *P*-value of 0.05 was used to define statistical significance. All data were analyzed using Statistical Analysis System V.9.4 (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Demographic Characteristics

From the PROC cohort, 1,914 participants aged 6.6 ± 0.3 years were enrolled, half boys and half girls, with 87% retention through wave 4 (**Table 1**). The height *z*-score, weight *z*-score, and BMI *z*-score suggested appropriate nutritional status as would be expected in a general pediatric population. The SBP and DBP was 101 ± 8 and 56 ± 6 mmHg, respectively. The prevalence of short sleep, long screen time, and insufficient FVI was 75.3%, 5.0%, and 48.6%, respectively. The prevalence of dehydration was 35.0%, 62.1%, 63.9%, and 63.3%, and the prevalence of insufficient PA for weekly patterns was 86.2%, 44.9%, 90.4% and 90.2% from waves 1–4. An increased trend for tubular damage was noted for waves 1–4 (8.8%, 15.9%, 25.7%, and 29.0%; $Z = 16.9$, $P < 0.001$), while glomerular damage showed a decreasing trend (5.6%, 5.5%, 4.4%, and 4.1%; $Z = -2.4$, $P = 0.016$; **Table 1**).

Binary Associations of Renal Damage With Hydration Status and Physical Activity

Stable longitudinal associations of renal tubular damage with hydration status were observed, but no association was seen with

TABLE 1 | Demographic characteristics among 6–9-year-old children, Beijing, China (*N* = 1914).

Characteristics	Wave 1 (*n* = 1,914)	Wave 2 (*n* = 1,746)	Wave 3 (*n* = 1,651)	Wave 4 (*n* = 1,657)
Age (year)[a]	6.6 ± 0.3	7.6 ± 0.3	7.6 ± 0.3	7.6 ± 0.3
Sex, boys[b]	956 (50.0)	875 (50.1)	820 (49.7)	826 (49.8)
Height z-score[a]	0.67 ± 0.96	0.79 ± 0.97	0.77 ± 0.97	0.77 ± 0.97
Weight z-score[a]	0.70 ± 1.41	0.99 ± 1.48	0.98 ± 1.48	0.98 ± 1.46
Body mass index (BMI) z-score[a]	0.40 ± 1.54	0.70 ± 1.59	0.69 ± 1.59	0.70 ± 1.57
Systolic blood pressure (SBP in mmHg)[a]	101 ± 8	101 ± 8	101 ± 9	101 ± 8
Diastolic blood pressure (DBP in mmHg)[a]	56 ± 6	56 ± 6	56 ± 6	56 ± 6
Short sleep (<10 h/d)[b]	1,441 (75.3)	1,327 (76.0)	1,246 (75.5)	1,249 (75.4)
Long screen time (≥2 h/d)[b]	95 (5.0)	86 (4.9)	79 (4.8)	80 (4.8)
Insufficient fruit/vegetable intake (FVI <4 /d)[b]	931 (48.6)	864 (49.5)	813 (49.2)	818 (49.4)
Insufficient physical activity (PA weekly average <1 h/d)[b]	1,451 (75.8)	1,323 (75.8)	1,259 (76.3)	1,267 (76.5)
Insufficient PA (weekly patterns <1 h/d)[b]	1,649 (86.2)	784 (44.9)	1,493 (90.4)	1,495 (90.2)
Dehydration status (specific gravity ≥1.02)[b]	670 (35.0)	1,085 (62.1)	1,055 (63.9)	1,049 (63.3)
Renal tubular damage (β_2-MG >0.2 mg/L)[b]	168 (8.8)	277 (15.9)	425 (25.7)	481 (29.0)
Renal glomerular damage (MA ≥20 mg/L)[b]	107 (5.6)	96 (5.5)	72 (4.4)	68 (4.1)

β_2-MG, β_2-microglobulin; MA, microalbumin; [a]Mean and standard deviation; [b]n (%).

TABLE 2 | Bivariate associations of renal damage with hydration status and physical activity level using generalized linear mixed-effects models among 6–9-year-old children in Beijing ($N = 1914$).

Dependent variables	Independent variables	Model 1		Model 2		Model 3	
		cOR (95%CI)	*P*	aOR (95%CI)	*P*	aOR (95%CI)	*P*
Tubular damage	Dehydration	ref.		ref.		ref.	
	Euhydration	0.52 (0.46, 0.59)	<0.001	0.50 (0.44, 0.57)	<0.001	0.50 (0.44, 0.57)	<0.001
Tubular damage	Insufficient PA	ref.		ref.		ref.	
	Sufficient PA	0.94 (0.78, 1.13)	0.51	0.94 (0.78, 1.13)	0.50	0.93 (0.77, 1.12)	0.47
Glomerular damage	Dehydration	ref.		ref.		ref.	
	Euhydration	0.36 (0.28, 0.47)	<0.001	0.32 (0.25, 0.42)	<0.001	0.33 (0.25, 0.43)	<0.001
Glomerular damage	Insufficient PA	ref.		ref.		ref.	
	Sufficient PA	1.12 (0.84, 1.50)	0.44	1.14 (0.84, 1.53)	0.40	1.15 (0.85, 1.55)	0.35

PA, physical activity; cOR, crude odds ratio; aOR, adjusted odds ratio; CI, confidence interval; ref., reference group; Model 1, unadjusted; model 2, adjusting for age, sex, and BMI z-score; model 3, adjusting for age, sex, BMI z-score, standardized SBP, sleep duration, screen time, and fruit and vegetable intake. All models included two random effects, namely, the week-day and intra-wave of the urinalysis.

TABLE 3 | Multivariable associations of renal damage with hydration status and physical activity level using generalized linear mixed-effects models among 6–9-year-old children in Beijing ($N = 1914$).

Dependent variables	Independent variables	Model 1		Model 2		Model 3	
		Estimate (95%CI)	*P*	Estimate (95%CI)	*P*	Estimate (95%CI)	*P*
Tubular damage	Intercept	−1.16 (−1.72, −0.61)	<0.001	−2.31(−3.89, −0.73)	0.004	−2.15 (−3.74, −0.56)	0.008
	Euhydration	−0.75 (−0.90, −0.59)	<0.001	−0.78 (−0.93, −0.63)	<0.001	−0.78 (−0.93, −0.63)	<0.001
	Sufficient PA	−0.21 (−0.43, 0.01)	0.066	−0.20 (−0.42, 0.02)	0.074	−0.21 (−0.43, 0.02)	0.069
	Interaction	0.45 (0.11, 0.79)	0.010	0.44 (0.09, 0.78)	0.012	0.43 (0.09, 0.77)	0.014
Glomerular damage	Intercept	−2.61 (−2.91, −2.30)	<0.001	−3.21 (−5.80, −0.63)	0.015	−2.98 (−5.56, −0.40)	0.024
	Euhydration	−1.18 (−1.49, −0.87)	<0.001	−1.28 (−1.60, −0.96)	<0.001	−1.27 (−1.59, −0.95)	<0.001
	Sufficient PA	−0.08 (−0.44, 0.28)	0.67	−0.06 (−0.43, 0.31)	0.76	−0.05 (−0.42, 0.32)	0.80
	Interaction	0.60 (0.01, 1.19)	0.047	0.57 (−0.03, 1.17)	0.060	0.57 (−0.04, 1.17)	0.065

PA, physical activity; CI, confidence interval; Model 1, unadjusted; model 2, adjusting for age, sex, and BMI z–score; model 3, adjusting for age, sex, BMI z–score, standardized SBP, sleep duration, screen time, and fruit and vegetable intake. All models included two random effects, namely, the week–day and intra–wave of the urinalysis.

PA level (weekly patterns) in unadjusted model 1 and model 2 adjusting for age, sex, and BMI. Children with euhydration were less likely to have renal tubular damage (OR = 0.50, 95% CI: 0.44, 0.57) adjusting for age, sex, BMI *z*-score, standardized SBP, sleep duration, screen time, and FVI (model 3; **Table 2**). Stable longitudinal associations of renal glomerular damage with hydration status were observed in unadjusted model 1 and adjusted model 2. Children with euhydration were less likely to have renal glomerular damage (OR = 0.33, 95% CI: 0.25, 0.43) adjusting for age, sex, BMI z-score, standardized SBP, sleep duration, screen time, and FVI (model 3; **Table 2**).

Multivariable Associations of Renal Damage With Hydration Status and Physical Activity

More extensive multivariable analyses showed consistent results with binary analysis that children with euhydration were less likely to have renal tubular damage ($\beta = -0.78$, 95% CI: −0.93, −0.63; $P < 0.001$) and renal glomerular damage ($\beta = -1.27$, 95% CI: −1.59, −0.95; $P < 0.001$) adjusting for age, sex, BMI z-score, standardized SBP, sleep duration, screen time, and FVI (model 3; **Table 3**). Moreover, we observed significant interaction of hydration status and PA level (weekly patterns) on renal tubular damage in adjusted model 3 ($\beta = 0.43$, $P = 0.014$) and on renal glomerular damage in unadjusted model 1 ($\beta = 0.60$, $P = 0.047$; **Table 3**).

Interaction of Hydration Status and Physical Activity Level on Renal Damage

Taking children with dehydration and insufficient PA (weekly patterns) as reference, renal tubular damage was less likely to happen among those with euhydration and insufficient PA (OR = 0.46, 95% CI: 0.39, 0.53) or with euhydration and sufficient PA (OR = 0.57, 95% CI: 0.44, 0.75), adjusting for age, sex, BMI z-score, standardized SBP, sleep duration, screen time, and FVI (model 3; **Table 4**). Renal glomerular damage was less likely to happen among those with euhydration and insufficient PA (OR = 0.28, 95% CI: 0.20, 0.39) or with euhydration and sufficient PA (OR = 0.47, 95% CI: 0.30, 0.74), adjusting for age, sex, BMI z-score, standardized SBP, sleep duration, screen time, and FVI (model 3; **Table 4**).

TABLE 4 | Interaction of hydration status and physical activity level on renal damage using generalized linear mixed–effects models among 6–9–year–old children in Beijing ($N = 1914$).

Dependent variables	Independent variables	Model 1		Model 2		Model 3	
		cOR (95%CI)	*P*	aOR (95%CI)	*P*	aOR (95%CI)	*P*
Tubular damage	Dehydration + Insufficient PA	ref.					
	Dehydration + Sufficient PA	0.81 (0.65, 1.01)	0.066	0.82 (0.66, 1.02)	0.074	0.81 (0.65, 1.02)	0.069
	Euhydration + Insufficient PA	0.47 (0.41, 0.55)	<0.001	0.46 (0.39, 0.53)	<0.001	0.46 (0.39, 0.53)	<0.001
	Euhydration + Sufficient PA	0.60 (0.46, 0.79)	<0.001	0.58 (0.44, 0.76)	<0.001	0.57 (0.44, 0.75)	<0.001
Glomerular damage	Dehydration + Insufficient PA	ref.					
	Dehydration + Sufficient PA	0.93 (0.65, 1.33)	0.67	0.94 (0.65, 1.37)	0.76	0.95 (0.66, 1.38)	0.80
	Euhydration + Insufficient PA	0.31 (0.22, 0.42)	<0.001	0.28 (0.20, 0.38)	<0.001	0.28 (0.20, 0.39)	<0.001
	Euhydration + Sufficient PA	0.52 (0.33, 0.80)	0.004	0.47 (0.30, 0.73)	0.001	0.47 (0.30, 0.74)	0.001

PA, physical activity; cOR, crude odds ratio; aOR, adjusted odds ratio; CI, confidence interval; ref., reference group; Model 1, unadjusted; model 2, adjusting for age, sex, and BMI z–score; model 3, adjusting for age, sex, BMI z–score, standardized SBP, sleep duration, screen time, and fruit and vegetable intake. All models included two random effects: the week–day and intra–wave of the urinalysis.

DISCUSSION

Our study used longitudinal data from 1,914 children aged 6–9 years to assess the association between hydration status, PA level, and early renal damage in a general pediatric population in China. Overall prevalence of dehydration was 35% in children newly enrolled in elementary school and 63% when they experienced 1 year of schooling. We found that children with euhydration and sufficient PA were less likely to have early renal damage, controlling for key covariates, including age, sex, BMI, SBP, sleep duration, screen time, and FVI. A novel finding is the significant interaction of hydration status and PA level in terms of both tubular and glomerular renal damage. Children with euhydration and sufficient PA were 43% less likely to have tubular damage and 53% less likely to have glomerular damage. However, this was similar in children with euhydration and insufficient PA who were 54% less likely to have tubular damage and 72% less likely to have glomerular damage, presenting a slightly lower risk than in children with sufficient PA with the same euhydration status. These findings underscore the primary necessity of adequate water intake during PA and daily life to prevent early renal damage in schoolchildren.

The dramatic increased prevalence of dehydration (determined by urine SG) among children aged 6.6 ± 0.3 years at baseline from 35% to 63% at 1 year follow-up is notable. Similar with our follow-up prevalence, another study suggested a similar two-thirds prevalence of dehydration among Chinese children and adolescents (4). A systematic review focusing on water intake and hydration state in children reported that 60% ± 24% of children from 19 countries failed to meet the guidelines of water/ fluid intake recommended by the U.S. Institute of Medicine (IOM), European Food Safety Authority (EFSA), and Chinese and Indonesian health authorities (5). The difference in hydration between those newly enrolled and 1-year later may be due to unfavorable school environment with inadequate water access and limited time for drinking during and between classes (6).

Water intake is associated with cognition of children (30). Children's subjective feeling of thirst is not well correlated with fluid intake and this can lead to dehydration (31). A cross-sectional study of 141 adolescents aged 15–17 years reported that 90% of were dehydrated during school as determined by urine SG (32). Dehydration and inadequate water intake can affect the school performance including alertness, concentration, and fatigue (32), can impair renal function (21), and can even lead to chronic kidney disease (2). We observed stable and consistent longitudinal associations between hydration status and renal stress; children with euhydration were less likely to have tubular or glomerular damage. This finding is consistent with our previous study (21). Moreover, a decreasing temporal trend over the school week of MA only in the children with euhydration (21). Almost all available evidence support that we should promote adequate water intake, especially among students in elementary schools.

The prevalence of insufficient PA among our participants was about 76% *via* the estimate of weekly average <1 h/day, similar with the result of the National Survey of Children's Health 2017–2018 reported that about 22.6% Chinese children and adolescent had 60 min of physical activity every day during the past week (33). The prevalence of insufficient PA estimated *via* weekly patterns was about 45% at weekend and 90% at week-day, and similar trend of weekend PA level was more than week-day was reported in a cross-sectional study among 15,203 children aged 6–12 years in China (14). Different from other studies (16, 34–37), we did not observe longitudinal associations between PA level (weekly patterns) and renal damage, similar with an interventional study among obese boys in Portugal (38).

We observed a significant interaction of hydration status and PA level on both tubular damage and glomerular damage. Our generalized linear mixed-effects models including this interaction term showed that children with euhydration and sufficient PA were 43% less likely to have tubular damage and 53% less likely to have glomerular damage. Children with euhydration and insufficient PA were 54% less likely to have tubular damage

and 72% less likely to have glomerular damage, presenting a higher risk than seen with sufficient PA in the same euhydration status. Few studies focus on the interaction of hydration status and PA level on renal damage in adult populations (39–41), and we have found no prior longitudinal study conducted in children. One study on healthy male adults reported that the renal function (estimated glomerular filtration rate, eGFR) did not change after acute exercise, whereas it significantly decreased after prolonged exercise, suggested that prolonged physical activity without proper hydration could be a risk factor of renal function impairment (39). We hypothesize that insufficient PA may be renal-protective compared to sufficient PA in euhydration status. A cross-sectional study among 242 Spanish school children aged 8.9 ± 1.2 years reported that PA level (practice ≥1 h/day) was associated with a higher risk of dehydration status (OR = 1.75), adjusting for sex and other lifestyle factors (17), suggest that increased PA may lead to dehydration. A study examined renal circadian rhythm in obese adolescents, after conducting dietary restriction, increased PA, and psychological support among 34 adolescents (mean age 15.7 years), the investigators observed lower diurnal free water clearance compared with nocturnal values (42), suggesting transient renal stress from diurnal PA. A trial found that the risk for acute kidney injury (AKI) is higher in participants with greater hyperthermia and dehydration during physical work; alleviating hyperthermia and/or limiting dehydration equally reduced AKI risk (40). One trial enrolled 14 men to study hypohydration caused by physical work and found that increased renal injury happened at the proximal tubules (41). This is consistent with our study using β_2-MG excretion to estimate proximal tubular function. We further observed a combined effect of euhydration and PA level in terms of glomerular damage. We conclude that to prevent renal damage and potential functional impairment in children, optimized daily water intake, especially after PA, is needed.

The major strength of this study was the use of longitudinal urinalysis data of a general healthy pediatric population in China with a large sample size. Our use of imputation methods for missing data can reduce bias (43). Furthermore, we used linear mixed-effects models and chose key covariates to adjusted for associations of hydration status and PA level with renal damage, especially SBP and lifestyle factors such as sleep duration, screen time, and FVI. However, our study was limited by not considering other renal function indicators or biomarkers. Urine β_2-MG, MA were tested *via* different machines due to the limited capacity of individual testing sites within allowed condition for sample processing. Hydration status was assessed using the SG of morning urine, which may overestimate the prevalence of dehydration. Moreover, hydration status and renal damage may be transient (44), and the result may only represent the situation at the time being of test and survey. We sought to minimize bias from these effects with random effects modeling with longitudinal data.

CONCLUSION

We have found longitudinal interactions of hydration status and PA level on early renal damage and have found increased dehydration among the children over time in China. We found that children can be protected from early renal damage by euhydration, either with sufficient or insufficient PA. Our findings underscore the necessity of advocating adequate water intake, especially after PA, to prevent potential function impairment in healthy children and possible utilization among those with compromised renal function, especially with CKD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Capital Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YH, DH, and ML conceptualized and designed this study. ML, WS, HX, and JZ carried out the survey. DH read and reported the clinical significance of the assay. ML performed statistical analysis of the data. NA checked data analysis process. ML and WS drafted the manuscript. SV, DH, and YH edited, helped interpret, and revised the manuscript. All authors were involved in writing the study and had final approval of the submitted and published versions.

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Low-Density Lipoprotein Cholesterol and Mortality in Peritoneal Dialysis

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Background: In dialysis patients, lowering low-density lipoprotein cholesterol (LDL-C) did not provide benefits, which seemed implausible in clinical practice. We hypothesized a U-shaped association between LDL-C and mortality in dialysis patients.

Methods: In this multi-center retrospective real-world cohort study, 3,565 incident Chinese peritoneal dialysis (PD) patients between January 1, 2005, and May 31, 2020, were included. The associations between baseline LDL-C and mortality were examined using cause-specific hazard models.

Results: Of 3,565 patients, 820 died, including 415 cardiovascular deaths. As compared with the reference range (2.26-2.60 mmol/L), both higher levels of LDL-C (> 2.60 mmol/L) and lower levels of LDL-C (< 2.26 mmol/L) were associated with increased risks of all-cause mortality (hazard ratio [HR],1.35, 95% confidence index [CI], 1.09-1.66; HR 1.36, 95%CI, 1.13-1.64) and cardiovascular mortality (HR, 1.31, 95% CI, 1.10-1.72; HR, 1.64; 95% CI, 1.22-2.19). Malnutrition (albumin < 36.0 g/L) modified the association between LDL-C and cardiovascular mortality (P for interaction = 0.01). A significantly increased risk of cardiovascular mortality was observed among patients with malnutrition and lower levels of LDL-C (HR 2.96, 95%CI 1.43-6.12) or higher levels of LDL-C (HR 2.81, 95%CI 1.38-5.72).

Conclusion: Low and high levels of LDL-C at the start of PD procedure were associated with increased all-cause and cardiovascular mortality risks. Malnutrition may modify the association of LDL-C with cardiovascular mortality.

Keywords: peritoneal dialysis, mortality, low-density lipoprotein cholesterol (LDL-C), nutrition–clinical, cardiovascular mortality

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INTRODUCTION

Low-density lipoprotein cholesterol (LDL-C) is a well-established causal risk factor for the development of cardiovascular disease (1). Many prospective randomized controlled trials of lipid-lowering drug therapy clearly show that lowering LDL-C levels reduces the risk of future cardiovascular events (2–4). Interestingly, a prospective cohort study from Denmark reported that

in the general population, the association between levels of LDL-C and the risk of all-cause mortality was U-shaped, with low and high levels associated with an increased risk of all-cause mortality (5). A recent study of young Koreans not taking lipid-lowering drugs showed a U-shaped association between LDL-C levels and mortality (6). Apparently, a U-shaped association seems more plausible in clinical practice.

In dialysis patients, lowering LDL-C did not provide benefits. Two large, well-designed trials examined the effect of statin therapy on the combined endpoint of death from cardiovascular causes, non-fatal myocardial infarction, and stroke in dialysis patients (7, 8). Despite a significant decrease in serum LDL-C levels, both trials found that the initiation of statin therapy provided no cardiovascular benefit in these populations. Another multi-center, prospective cohort study of 630 incident Korean peritoneal dialysis (PD) patients reported that total cholesterol, high-density lipoprotein cholesterol (HDL-C), and LDL-C were also not associated with mortality (9). Another study showed that a combination of statin and ezetimibe benefits the prognosis in non-dialysis chronic kidney disease and dialysis patients. The beneficial effect on atherosclerotic events was not statistically significant in the dialysis subgroup (10). Furthermore, the KDIGO guidelines recommend that statin therapy not be routinely initiated in dialysis patients (11). It is worth noting that these findings above may be implausible in clinical practice. We hypothesized that a U-shaped association between LDL-C and mortality, and there may be an optimal range of LDL-C, which was associated with the lowest mortality risk in dialysis patients. The two large well-designed trials above may lower levels of LDL-C less than the optimal range, which may contribute to a high risk of mortality. Thus, we conducted a real-world study to examine the association between LDL-C and mortality in continuous ambulatory peritoneal dialysis (CAPD) patients.

MATERIALS AND METHODS

Study Design and Participants

We conducted a retrospective real-world cohort study that included 3,565 incident Chinese CAPD patients from five PD centers in China between January 1, 2005, and May 31, 2020. No patients were excluded in the primary analysis to evaluate the association between LDL-C one week before the start of PD and mortality in the real-world setting. The data were anonymous, and the need for informed consent was waived. The study protocol complied with the Declaration of Helsinki and had full approval from each Clinical Research Ethics Committee.

Data Collection and Follow up

Two well-trained nurses collected demographic data, comorbidities, and laboratory data one week (5.3 $\pm$ 1.2 days) before the start of PD in each facility, including age at study entry, sex, body mass index, current smoker, current alcohol use, systolic blood pressure, comorbidities (diabetes mellitus, prior cardiovascular disease, and hypertension), medication use (beta-blockers, angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers [ACEI/ARBs], diuretics, and statins), and laboratory measurements (serum albumin, estimated glomerular filtration rate [eGFR], total cholesterol, HDL-C, and LDL-C).

The primary outcome was all-cause and cardiovascular mortality. Details for the CAPD follow-up were previously described elsewhere (12). The follow-up period was from the start of PD to the date of death, transfer to hemodialysis, receiving renal transplantation, transfer to other dialysis centers, loss of follow-up, or May 31, 2020. Patients who were lost to follow-up were censored at the date of the last examination (**Supplementary Materials**).

Statistical Analysis

Continuous variables were presented as means with standard deviations (SDs) for normally distributed data or medians with interquartile range (IQR) for skewed data. The normality of the parameters was examined using the Shapiro–Wilk test. Categorical variables were expressed as the number of patients. We used restricted-cubic-spline plots to explore the shape of the association between LDL-C and mortality, fitting a restricted-cubic-spline function with four knots (at the 25th, 50th, 75th, and 95th percentiles) (13). All parameters were compared among groups based on restricted cubic spline plots for the primary analysis.

To explore the association of LDL-C with mortality, we primarily used cause-specific hazard models. We then constructed sub-distribution hazard models to confirm the association observed in the primary analysis. Transfer to hemodialysis, receiving renal transplantation, transfer to other centers, and loss of follow-up before death were considered competing risks. The main difference between the two hazard models is that subjects experiencing a competing risk event remain in the risk set in the subdistribution hazard model but are removed in the cause-specific hazard model (14, 15). These models were constructed after the adjustment of the following variables. The univariate model represented unadjusted hazard ratios (HRs). The multivariate model was adjusted for age, sex, body mass index, current smoker, current alcohol use, comorbidities, medication use, and laboratory measurements (excluded LDL-C). The results from multivariable hazard models were presented as HRs and 95% confidence intervals (95% CIs). Cumulative primary outcomes were derived using the cumulative incidence function for a competing risk, and the difference among curves was analyzed using the Gray test. To evaluate the modification effects of subgroups on the association between LDL-C and mortality, we tested for interactions of age, sex, diabetes mellitus, prior cardiovascular disease, hypertension, and malnutrition (defined as serum albumin levels $<$ 36.0 g/L) (16).

To minimize the potential for reverse causation, we conducted analyses that excluded patients with prior cardiovascular disease or those deaths in the first two years of follow-up. In addition, as for those patients with a short-term follow-up period, the interesting outcomes may not be wholly observed with under-reporting of the incidence of mortality. We further analyzed the association in those patients with at least 24 months of follow-up for fully observing outcomes. We also examined

the association in patients with age ≥ 18 years, those with a follow-up period ≥ three months, and those without statin use. Missing data for low-density lipoprotein (n = 39) or any other explanatory variables (n = 147) at the start of PD were replaced by the most recent available values by checking patients' medical records of receiving the first PD procedure. All analyses were conducted with Stata 15.1. statistical software (StataCorp, College Station, TX).

RESULTS

Baseline Characteristics

Of 3,565 patients, the mean age was 49.2 ± 15.1 years (range, 3 to 101 years), 52.1% of patients were male sex. The mean LDL-C levels were 2.56 ± 0.89 mmol/L (range 0.4 to 9.4 mmol/L). We chose the reference group based on the results of restricted-cubic-spline analysis, in which hazard ratios were 1.0 compared with the median LDL-C (2.43 mmol/L). Based on restricted cubic spline plots for the primary outcome, we selected a level of 2.26 to 2.60 mmol/L (87 to 101 mg/dL) as the reference category for LDL-C (**Figure 1**). There were 1,225 (34.4%) patients with LDL levels < 2.26 mmol/L and 1,439 (40.3%) with LDL levels > 2.60 mmol/L. Thus, 2664 (74.7%) patients were at higher risk of mortality. **Table 1** presented the characteristics of patients by categories of baseline LDL-C. Baseline variables were markedly different among the low, moderate, and high groups. The high group had higher systolic blood pressure and total cholesterol levels, frequency of diabetes mellitus, prior cardiovascular disease, and hypertension. In contrast, the low group had lower systolic blood pressure levels, a lower frequency of diabetes mellitus, and prior cardiovascular disease.

LDL-C and Outcomes

During the 14131.6 person-years of follow-up, 820 (23.0%) patients died, 481 (13.5%) patients transferred to hemodialysis, 241 (6.8%) patients received renal transplantation, 459 (12.9%) patients transferred to other dialysis centers, and 61 (1.7%) patients had been the loss of follow-up. Of 820 deaths, 415 (50.6%) deaths were due to cardiovascular disease, 142 (17.3%) deaths due to infectious disease, 76 (9.3%) deaths due to gastrointestinal bleeding, 15 (1.8%) deaths due to malignancy, 73 (8.9%) deaths due to other reasons, and 99 (12.1%) deaths due to unknown reasons. Deaths occurred in 292 (63.8/1000 person-years), 181 (46.5/1000 person-years), and 347 (61.3/1000 person-years) patients in those < 2.26, 2.26-2.60, and > 2.60 mmol/L patients, respectively (**Table 2**). Cumulative all-cause mortality and cardiovascular mortality were significantly lowest in the moderate group (P < 0.001, **Figure 2**). The adjusted cumulative incidence function showed a similar pattern (**Supplementary Figure 1**).

In the multivariate cause-specific hazard model, the adjusted HRs of all-cause mortality were 1.35 (95% CI, 1.09 to 1.66) and 1.36 (95%CI, 1.13 to 1.64) for the low and high groups, respectively, compared with the moderate group, after adjustments for demographic factors, comorbidities, medication use and laboratory variables (the multivariate model in **Table 3**). Similarly, in the multivariate cause-specific hazard model, the adjusted HRs of cardiovascular mortality were 1.64 (95% CI, 1.22 to 2.19) and 1.31 (95%CI, 1.10 to 1.72) for the low and high groups, respectively, compared with the moderate group (the multivariate model in **Table 3**).

We confirmed this association using a subdistribution hazard model. As compared with the reference range, higher levels of LDL-C (> 2.60 mmol/L) were associated with increased risks of all-cause mortality (HR, 1.37; 95% CI, 1.14 to 1.65) and cardiovascular mortality (HR, 1.32; 95% CI, 1.08 to 1.73). Lower levels of l LDL-C (< 2.26 mmol/L) were also associated with increased risks of all-cause mortality (HR, 1.36; 95% CI, 1.10 to 1.67) and cardiovascular mortality (HR, 1.66; 95% CI, 1.24 to 2.23) compared with the reference range (multivariate model in **Supplementary Tables 1**, **2**).

Sensitivity Analysis

We performed sensitivity analyses in patients without prior cardiovascular disease, without deaths during the first 2 years of follow-up, the follow-up period ≥ 24 months, or age ≥ 18 years, respectively. Similar results were observed in patients without prior cardiovascular disease, those with age ≥ 18 years, and those without statin use (**Tables 3**, **4** and **Supplementary Tables 1**, **2**). Notably, higher adjusted HRs of all-cause and cardiovascular mortality were observed in patients without deaths during the first 2 years of follow-up and those with a follow-up period ≥ 24 months (**Tables 3**, **4** and **Supplementary Tables 1**, **2**).

Subgroup Analyses

Associations of low-density lipoprotein cholesterol with all-cause and cardiovascular mortality were shown in **Supplementary Tables 3**, **4**. We found that malnutrition (defined as serum albumin < 36.0 g/L) modified the association between LDL-C and cardiovascular mortality (*P* for interaction = 0.010, **Supplementary Table 4**). In further analysis, a significantly increased risk of cardiovascular mortality was observed among patients with malnutrition and lower levels of LDL-C (HR 2.96, 95%CI 1.43-6.12) or higher levels of LDL-C (HR 2.81, 95%CI 1.38-5.72). In contrast, there was no significant association among those without malnutrition. There were no other significant subgroup interactions.

DISCUSSION

In this real-world study of 3,565 incident Chinese CAPD patients, we found a U-shaped association between levels of LDL-C and the risk of all-cause and cardiovascular mortality, with low and high levels associated with an increased risk. The optimal range of LDL-C associated with the lowest risk of all-cause and cardiovascular mortality was 2.26 to 2.60 mmol/L (87 to 101 mg/dL). Our findings were robust because we showed consistent results across different hazard models and several sensitivity analyses. These new results in PD patients were likely to have implications for the interpretation of levels of LDL-C in clinical practice. The cut-off of the

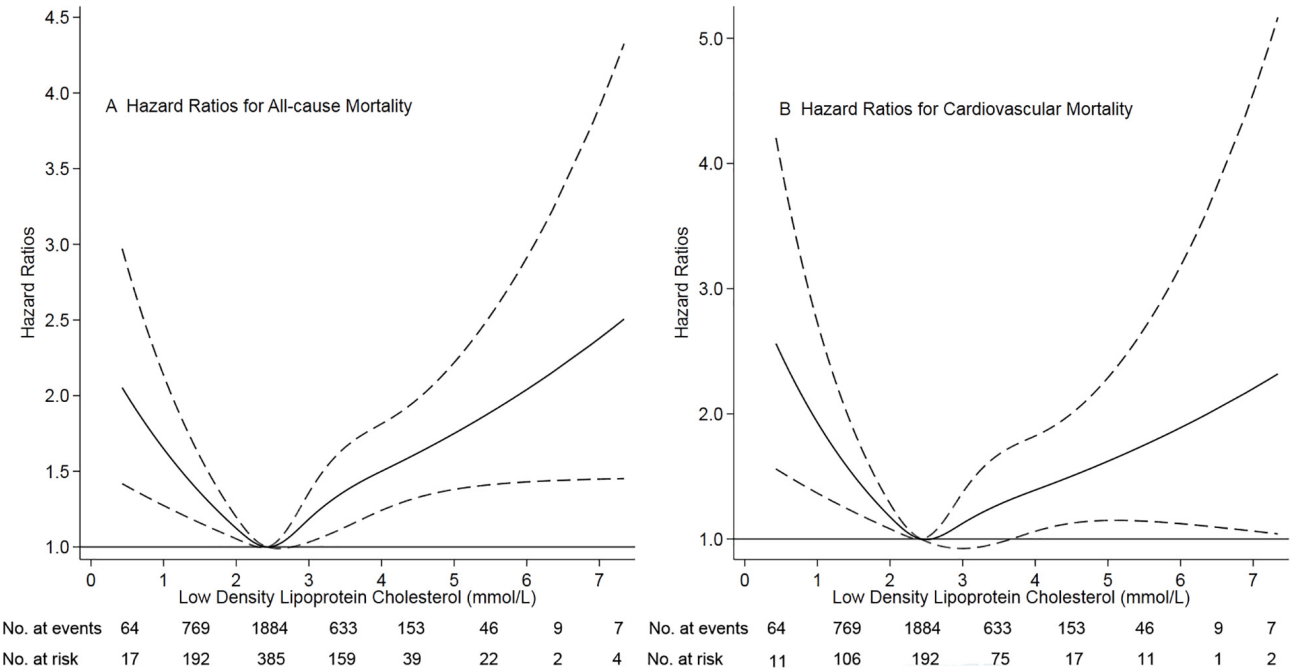

FIGURE 1 | Association of low-density lipoprotein cholesterol with risk of mortality. Panel **(A)** showed a restricted-cubic-spline plot of the association between LDL-C and all-cause mortality. Panel **(B)** showed a restricted cubic spline plot of the association between LDL-C and cardiovascular mortality. All plots were adjusted for age, sex, body mass index, current smoker, current alcohol use, systolic blood pressure, comorbidities, medication use, and laboratory variables. Dashed lines indicate 95% confidence intervals. The median LDL-C (2.43 mmol/L, [89 mg/dL]) was the reference standard, indicated by the grayline. LDL-C, low-density lipoprotein cholesterol.

TABLE 1 | Baseline patient characteristics by categories of low-density lipoprotein cholesterol.

		Low-density lipoprotein cholesterol			
	Overall	**Low (< 2.26 mmol/L)**	**Moderate (2.26-2.60 mmol/L)**	**High (> 2.60 mmol/L)**	**P-value**
Number of patients, *n*	3565	1225	901	1439	
LDL-C (mmol/L)	2.56 ± 0.89	2.41 ± 0.09	1.73 ± 0.40	3.36 ± 0.76	
Age (years)	49.2 ± 15.1	49.1 ± 15.2	48.9 ± 15.2	49.4 ± 15.0	0.729
Male sex, n (%)	1856 (52.1%)	657 (53.6%)	495 (54.9%)	704 (48.9%)	0.007
Body mass index (kg/m^2)	22.3 ± 3.3	22.1 ± 3.2	22.0 ± 3.0	22.6 ± 3.5	< 0.001
Systolic blood pressure (mmHg)	137.3 ± 22.8	133.3 ± 21.6	137.6 ± 22.1	140.6 ± 23.7	< 0.001
Current smoker, *n* (%)	354 (9.9%)	128 (10.4%)	63 (7.0%)	163 (11.3%)	0.002
Current alcohol use, *n* (%)	129 (3.6%)	45 (3.7%)	21 (2.3%)	63 (4.4%)	0.036
Comorbidities, *n* (%)					
Diabetes mellitus	674 (18.9%)	169 (13.8%)	140 (15.5%)	365 (25.4%)	< 0.001
Prior cardiovascular disease	379 (10.6%)	82 (6.7%)	85 (9.4%)	212 (14.7%)	< 0.001
Hypertension	2469 (69.3%)	791 (64.6%)	573 (63.6%)	1105 (76.8%)	< 0.001
Medication use, *n* (%)					
Beta-blocker	1338 (37.5%)	394 (32.2%)	304 (33.7%)	640 (44.5%)	< 0.001
Diuretics	557 (15.6%)	145 (11.8%)	121 (13.4%)	291 (20.2%)	< 0.001
Statin	524 (14.7%)	156 (12.7%)	100 (11.1%)	268 (18.6%)	< 0.001
Laboratory measurements					
Albumin (g/L)	34.5 ± 5.3	34.5 ± 5.0	35.0 ± 5.3	34.1 ± 5.5	< 0.001
eGFR (mL/min*1.73m^2)	7.19 ± 3.83	7.20 ± 3.45	6.82 ± 3.73	7.50 ± 4.11	< 0.001
Total Cholesterol (mmol/L)	4.38 ± 1.19	4.72 ± 0.32	3.38 ± 0.58	5.01 ± 1.35	< 0.001
HDL (mmol/L)	1.14 ± 0.40	1.18 ± 0.36	1.03 ± 0.32	1.20 ± 0.45	< 0.001

LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol.

TABLE 2 | Incidence rate of death according to low-density lipoprotein cholesterol.

Outcomes	Low-density lipoprotein			
	All levels	Low (< 2.26 mmol/L)	Moderate (2.26-2.60 mmol/L)	High (> 2.60 mmol/L)
All-cause mortality				
Deaths, n	820	292	181	347
Deaths, per 1,000 person-years	58.0	63.8	46.5	61.3
Cardiovascular mortality				
Deaths, n	415	163	88	164
Deaths, per 1,000 person-years	29.4	35.6	22.6	29.0

The incidence rate was calculated by dividing the proportion of events by the total effective observation time in the risk, which is converted to the number of episodes per 1,000 years.

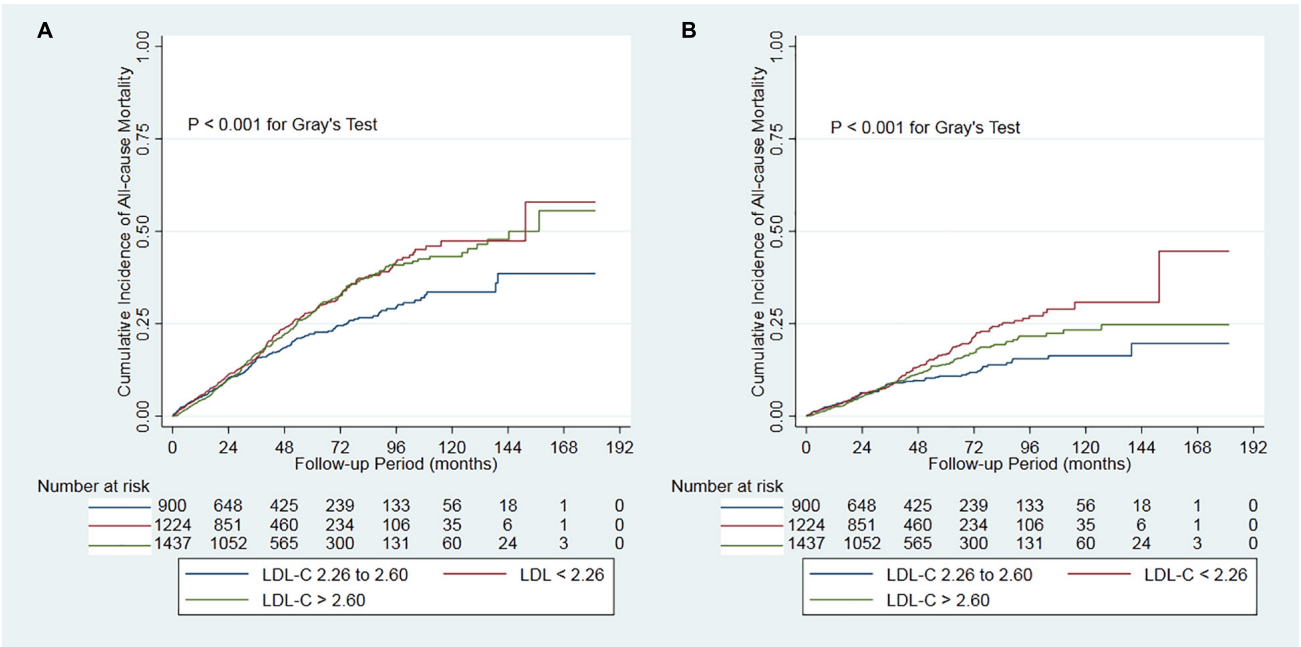

FIGURE 2 | Cumulative mortality by categories of low-density lipoprotein cholesterol. Panel **(A)** showed cumulative all-cause mortality by categories of LDL-C. Panel **(B)** showed cumulative cardiovascular mortality by categories of LDL-C. LDL-C, low-density lipoprotein cholesterol.

range of LDL-C 2.26-2.60 mmol/L deserved a pathogenetic hypothesis, especially focusing on the potential role of low LDL-C levels on cardiovascular mortality. As we know, high levels of LDL-C are associated with increased risks of all-cause and cardiovascular mortality. The association between low levels of LDL-C and increased risks of all-cause and cardiovascular mortality could be explained by reverse causation. Comorbidities have been reported to cause a decrease in levels of LDL-C (17) and are prevalent in dialysis patients. Thus, lower levels of LDL-C may be associated with high risks of all-cause and cardiovascular mortality. Based on our findings, keeping the appropriate range of LDL-C over a long time may improve the prognosis of CAPD patients. In addition, Early publications estimated that 40 to 66 percent of PD patients in the United States are malnourished (18–23). When evaluating the association of LDL-C with cardiovascular mortality, we should simultaneously pay attention to the nutrition status of dialysis patients, especially potential causes of hypoalbuminemia such as very low protein intake and decompensated cirrhosis.

Continuous ambulatory peritoneal dialysis patients tend to show elevated levels of total cholesterol and LDL-C and decreased levels of HDL-C (24). Contrary to the general population, lowering levels of LDL-C did not provide benefits for dialysis patients. In the 4-D study, 1255 hemodialysis patients (80 percent were not treated with a statin) with type 2 diabetes and elevated serum LDL cholesterol levels were randomly assigned to the placebo or atorvastatin group (7). After four weeks, atorvastatin successfully lowered LDL cholesterol (3.10 to 1.90 mmol/L [121 to 72 mg/dL]) versus no change with placebo (3.20 to 3.10 mmol/L [125 to 120 mg/dL]). At a median follow-up of four years, however, there was no difference in the incidence of the combined endpoint of death between both groups (HR, 0.92, 95% CI 0.77-1.10). In the AURORA

TABLE 3 | Association between low-density lipoprotein cholesterol and all-cause mortality*.

	HR (95% CI) by low-density lipoprotein		
	Low (< 2.26 mmol/L)	**Moderate (2.26-2.60 mmol/L)**	**High (> 2.60 mmol/L)**
Univariate model	1.36 (1.13 to 1.64)	1.0	1.31 (1.09 to 1.57)
Multivariable model	1.35 (1.09 to 1.66)	1.0	1.36 (1.13 to 1.64)
Patients without prior cardiovascular disease	1.38 (1.11 to 1.72)	1.0	1.36 (1.12 to 1.66)
Patients without deaths during the first 2 year of follow-up	1.59 (1.20 to 2.10)	1.0	1.67 (1.30 to 2.15)
Patients with follow-up period ≥ 24 months	1.59 (1.20 to 2.10)	1.0	1.67 (1.30 to 2.15)
Patients with age ≥ 18 years	1.34 (1.09 to 1.66)	1.0	1.37 (1.13 to 1.65)
Patients without statin use	1.28 (1.02 to 1.60)	1.0	1.34 (1.10 to 1.63)

**Unless stated, model adjusted for age, sex, body mass index, systolic blood pressure, current smoker, current alcohol use, comorbidities, medication use, and laboratory variables. HR, hazards ratio.*

TABLE 4 | Association between low-density lipoprotein cholesterol and cardiovascular mortality*.

	HR (95% CI) by low-density lipoprotein		
	Low (< 2.26 mmol/L)	**Moderate (2.26-2.60 mmol/L)**	**High (> 2.60 mmol/L)**
Univariate model	1.55 (1.20 to 2.01)	1.0	1.27 (1.08 to 1.64)
Multivariable model	1.64 (1.22 to 2.19)	1.0	1.31 (1.10 to 1.72)
Patients without prior cardiovascular disease	1.69 (1.24 to 2.29)	1.0	1.30 (1.08 to 1.72)
Patients without deaths during the first 2 year of follow-up	2.57 (1.70 to 3.89)	1.0	1.89 (1.28 to 2.79)
Patients with follow-up period ≥ 24 months	2.57 (1.70 to 3.89)	1.0	1.89 (1.28 to 2.79)
Patients with age ≥ 18 years	1.64 (1.22 to 2.21)	1.0	1.32 (1.11 to 1.74)
Patients without statin use	1.59 (1.12 to 2.18)	1.0	1.33 (1.10 to 1.78)

**Unless stated, model adjusted for age, sex, body mass index, systolic blood pressure, current smoker, current alcohol use, comorbidities, medication use, and laboratory variables. HR, hazards ratio.*

trial, 2776 statin-naive hemodialysis patients were randomly assigned to the rosuvastatin (10 mg/day) or placebo group (8). At three months, mean serum LDL cholesterol levels were lowered significantly with rosuvastatin (2.60 to 1.50 mmol/L [100 to 58 mg/dL]) versus no change with placebo (2.56 to 2.53 mmol/L [99 to 98 mg/dL]). At a median follow-up period of 3.8 years, the incidence of the primary composite endpoint (death from cardiovascular causes, non-fatal myocardial infarction, or non-fatal stroke) was similar in the two groups (9.2 versus 9.5 events per 100 patient-years; HR 0.96, 95% CI 0.84-1.11). The individual components of the primary composite endpoint and all-cause mortality were also not significantly different between the two groups. Active therapy did not provide benefits for any prespecified subgroups. A meta-analysis had moderate- to high-quality evidence that, among patients on dialysis, statin treatment had little or no effect on all-cause mortality (HR 0.96, 95% CI 0.88-1.04), cardiovascular mortality (HR 0.94, 95% CI 0.82-1.07), and major cardiovascular events (HR 0.95, 95% CI 0.87-1.03) (25). Based on the findings above, the 2013 KDIGO guidelines recommend that statin therapy not be routinely initiated in dialysis patients (11). In the present study, we found a U-shaped association between levels of LDL-C and the risk of mortality in CAPD patients. Our findings are consistent with those in the general population from Denmark's well-designed prospective cohort study (5). They reported that in the general population, the association between levels of LDL-C and the risk of all-cause mortality was U-shaped, with low and high levels associated with an increased risk of all-cause mortality. They also found that the lowest risk of all-cause mortality was at an LDL-C level of 3.60 mmol/L (140 mg/dL). In our study, the lowest risk of mortality was found at a level of LDL-C of 2.43 mmol/L (94mg/dL). Notably, the 4-D study and AURORA study lowered levels of LDL-C to 1.90 mmol/L and 1.50 mmol/L, respectively, which both were significantly lower than 2.43 mmol/L. Based on our findings, too lower levels of LDL-C were associated with increased risk of mortality. Despite focusing on different dialysis population, this may be why lowering LDL-C did not provide benefits in these two well-designed studies due to too strictly managing of LDL-C levels. Nonetheless, due to markedly difference in baseline variables and the feature of retrospective study, our findings need to be confirmed by large well-designed prospective cohort study.

Our previous study found that hyperlipidemia may harm long-term survival in diabetes mellitus patients on CAPD (26). In this study, among diabetes mellitus patients, hyperlipidemia was as a high risk of mortality as non-hyperlipidemia (HR 1.02, 95%CI 0.73 to 1.43) during the overall follow-up period, but from 48-month follow-up onwards, hyperlipidemia patients had 3.60 (95%CI 1.62 to 8.01)-fold higher risk of all-cause mortality than those non-hyperlipidemia. In the present study, sensitivity analyses found that higher adjusted HRs of all-cause and cardiovascular mortality were observed in patients without deaths during the first 2 years of follow-up and those

with a follow-up period ≥ 24 months. These findings also suggested that lower or higher LDL-C levels may have a long-term adverse effect on mortality in CAPD patients. In 4-D and AURORA studies, survival plots showed that lowering LDL-C had a better long-term prognosis in hemodialysis patients (7, 8). However, the authors did not report and further analyze these findings. Further studies regarding the association between the management of serum LDL-D and prognosis in dialysis patients should have enough long follow-up period.

The strengths of our study included a large number of patients, high completeness of real-world data, and rigorous different multivariate hazard models. Nevertheless, some limitations should be mentioned. First, as a retrospective observational cohort study, this study cannot necessarily prove causation between LDL-C and mortality. A potential limitation was the possibility of residual confounding from unmeasured variables. Second, the lack of LDL-C during the follow-up period was a significant limitation, which may underestimate the association between LDL-C levels and mortality due to regression dilution bias (27). However, regression dilution bias may lead to over-adjustment (28). Third, missing values were replaced by the most recent available deals, not using multiple imputations. Although multiple imputations can randomly fill these missing values, the most recent available values may more appropriately present a patient's clinical status. Fourth, malnutrition was only defined by serum albumin, not including prealbumin, serum cholesterol, body mass, or muscle. Lastly, all eligible patients were from China, suggesting our findings may lack generalization to other ethnic populations.

In conclusion, low and high levels of LDL-C at the start of PD procedures were associated with increased all-cause and cardiovascular mortality risks. Plus, the appropriate range of LDL-C of 2.26 to 2.60 mmol/L (87 to 101 mg/dL) was associated with the lowest mortality risk. Simultaneously, we should pay attention to the nutrition status because it may modify the association of LDL-C with cardiovascular mortality. Our findings suggested that maintaining the appropriate range of LDL-C via lipid management may improve the prognosis in CAPD patients. Nonetheless, if confirmed in more studies, our findings will have significant clinical and public health implications.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China. The Ethics Committee of The First Affiliated Hospital of Nanchang University, Nanchang, China. The Ethics Committee of Jiujiang No. 1 People's Hospital, Jiujiang, China. The Ethics Committee of Zhujiang Hospital of Southern Medical University, Guangzhou, China. The Ethics Committee of The Second Affiliated Hospital of Guangzhou Medical University, Guangzhou, China. Written informed consent for participation was not provided by the participants' legal guardians/next of kin because: The data were anonymous, and the need for informed consent was waived.

AUTHOR CONTRIBUTIONS

XWu: conceptualization. XWu, JW, and LZ: methodology. XWu and LZ: software. JW: validation. XWa and XZ: formal analysis and investigation. XWa, XZ, FP, YW, and XF: resources. NW: data curation. LZ: writing—original draft preparation. XWu and JW: writing—review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.910348/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Lifestyle and chronic kidney disease: A machine learning modeling study

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Background: Individual lifestyle varies in the real world, and the comparative efficacy of lifestyles to preserve renal function remains indeterminate. We aimed to systematically compare the effects of lifestyles on chronic kidney disease (CKD) incidence, and establish a lifestyle scoring system for CKD risk identification.

Methods: Using the data of the UK Biobank cohort, we included 470,778 participants who were free of CKD at the baseline. We harnessed the light gradient boosting machine algorithm to rank the importance of 37 lifestyle factors (such as dietary patterns, physical activity (PA), sleep, psychological health, smoking, and alcohol) on the risk of CKD. The lifestyle score was calculated by a combination of machine learning and the Cox proportional-hazards model. A CKD event was defined as an estimated glomerular filtration rate <60 ml/min/1.73 m^2, mortality and hospitalization due to chronic renal failure, and self-reported chronic renal failure, initiated renal replacement therapy.

Results: During a median of the 11-year follow-up, 13,555 participants developed the CKD event. Bread, walking time, moderate activity, and vigorous activity ranked as the top four risk factors of CKD. A healthy lifestyle mainly consisted of whole grain bread, walking, moderate physical activity, oat cereal, and muesli, which have scored 12, 12, 10, 7, and 7, respectively. An unhealthy lifestyle mainly included white bread, tea >4 cups/day, biscuit cereal, low drink temperature, and processed meat, which have scored −12, −9, −7, −4, and −3, respectively. In restricted cubic spline regression analysis, a higher lifestyle score was associated with a lower risk of CKD event (p for linear relation < 0.001). Compared to participants with the lifestyle score < 0, participants scoring 0–20, 20–40, 40–60, and >60 exhibited 25, 42, 55, and 70% lower risk of CKD event, respectively. The C-statistic of the age-adjusted lifestyle score for predicting CKD events was 0.710 (0.703–0.718).

Conclusion: A lifestyle scoring system for CKD prevention was established. Based on the system, individuals could flexibly choose healthy lifestyles and avoid unhealthy lifestyles to prevent CKD.

KEYWORDS

lifestyle, chronic kidney disease, machine learning, scoring system, cohort study

Introduction

Chronic kidney disease (CKD) is a progressive condition, which affects approximately 10% of adults worldwide (1). As a growing public health problem, CKD not only increases the risk of end-stage kidney disease (ESRD) and cardiovascular disease (1) but also exerts a major effect on global mortality (2). A healthy lifestyle is important for the preservation of kidney function, while the quality of evidence-based lifestyle recommendations for CKD remains weak.

On the basis of cardiovascular risk, a healthy lifestyle was defined as eating a high-quality diet, moderate- to vigorous-intensity physical activity (PA), modest body mass index, never smoking, and drinking alcohol in moderation (3–8). Although CKD and cardiovascular disease are closely connected by similar epidemiological risk factors and mechanisms, a straightforward extrapolation of cardiovascular healthy lifestyle to CKD prevention needs to be cautiously handled (9). Recently, the relationship between CKD incidence and certain lifestyle, such as dietary patterns (10–12), physical activity (13, 14), alcohol consumption (15–17), cigarette smoking (18, 19), sleep (20), and psychological health (21, 22), has been investigated in some studies with a limited sample size, and the results were equivocal. Furthermore, individual lifestyle varies greatly in the real world. Currently, a comprehensive comparison of the relationship between individual lifestyle and CKD in a large-scale population is lacking.

Traditional approaches are difficult to analyze large datasets with multidimensional variables and non-linear relationships among risk factors and outcomes (23, 24). As a common form of artificial intelligence, machine learning successfully predicted long-term outcomes and selected suitable interventions, mainly depending on its high efficiency in handling tremendous variables and capturing high-dimensional relationships (24–28). Harnessing the cohort of UK biobank and supercomputer platform, we aimed to establish a machine learning-based CKD lifestyle-recommendation system, and test its performance *via* predicting the incidence of CKD.

Methods

This study was an analysis of the UK Biobank cohort, which received approval from the National Information Governance Board for Health and Social Care and the National Health Service North West Multicenter Research Ethics Committee. All participants provided informed consent through electronic signature at the baseline assessment (29). The current analysis was approved by the UK Biobank with an ID of 66,536.

Study population

The UK Biobank cohort recruited more than 500,000 community dwelling participants, aged 40–69 years, from across the United Kingdom. At baseline, we excluded participants with estimated glomerular filtration rate (eGFR) $\leq$ 60 ml/min/1.73 m^2, chronic renal failure [International Classification of Diseases-10 (ICD-10) code: N18; or ICD-9 code: 5,859], self-reported chronic renal failure (code: 1,192), initiated renal replacement therapy (30), and subjects with <5% of whole lifestyle data.

Assessment of lifestyle behaviors

We included diet, physical activity, smoking, alcohol drinking, sleep, and psychological health as the main six lifestyle behaviors (3, 31). For the assessment of dietary intakes, participants completed a questionnaire which included diet items with the frequency of consumption. Fruit, vegetable, bread, cereal, and muesli were recorded in slices per week, table spoons per day, pieces per day, and bowls per week (integer units). Intakes of meat, fish, and cheese responses were recorded as "less than once a week," "once a week," "two to four times a week," "five or six times a week," "once or more daily," or "never" (polytomous variables). Tea or water intake was recorded in integer units of cups/glasses per day. We further categorized bread as white, brown, and whole-grain bread; cereal as bran, biscuit, oat cereal, or muesli; vegetable as raw and cooked vegetable; meat as pork, beef, and lamb; fish as oily fish and non-oily fish. The frequency of alcohol intake was recorded as "daily or almost daily," "three or four times a week," "one or two times a week," "special occasions only or never" (polytomous variables). Smoking status and alcohol drinking status were categorized into current and non-current (such as never and former) at the time of assessment. An adequate sleep was defined as having a sleep duration of 7–9 h/day. We defined psychological health as

participants who did not suffer from illness, injury, bereavement, and stress in the past 2 years.

For physical activity, participants were asked with "in the last 4 weeks did you spend any time doing the following: walking for pleasure, other sports (swimming/cycling/keeping fit), strenuous sports (requires sweat or hard breathing), light do it yourself (DIY) (e.g., pruning and watering the lawn) and heavy DIY (e.g., weeding, lawn mowing, carpentry, and digging)." In addition, we included variables of "frequency of stair climbing in the last 4 weeks (ranged from none to $\geq$20 times a day)." Time spent in vigorous, moderate activity, and walking was weighted by the energy expended for these categories of activity, to produce metabolic equivalent of task (MET) min/week of physical activity (calculation procedure of MET were provided in supplements). Vigorous activities included running, cycling uphill, carrying heavy furniture upstairs, martial arts, competitive sports, or intensive exercise. Moderate activities included walking upstairs, going the gym, jogging, energetic dancing aerobics, general sports, using heavy power tools, and other physically demanding DIY and gardening. For non-physical activities time, participants were asked with "in a typical day, how many hours do you spend on driving," "how many hours do you spend using the computer," and "how many hours do you spend watching the television." Details on other covariates are described in the supplement.

Outcomes

We used the equation of the Modification of Diet in Renal Disease study (MDRD) to calculate the eGFR. We defined CKD event incidence as one of the following conditions: eGFR $\leq$ 60 ml/min/1·73 m^2 during the follow-up, mortality and hospitalization due to chronic renal failure and self-reported chronic renal failure (N18 of ICD-10 or 5,859 of ICD-9; or 1,192 of self-report code), initiation of renal replacement therapy (Z99.2,Z94.0 and Z49 of ICD-10) (30). Secondary outcomes included cardiovascular diseases and all-cause mortality.

Statistical analysis

We implemented the model training and parameter tuning in machine learning with Python 3.8.3. Cox proportional hazards models and other statistical analyses were conducted using R 4.0.3. We conducted all the statistical analyses on the supercomputer platform (inspur M5).

Machine learning

Participants who developed CKD events were grouped as incident CKD. We classified those who were free of CKD events as non-CKD. We used the baseline lifestyle factors in both groups to perform the procedure of machine learning. Taking the accuracy of varied machine learning methods, speed and memory consumption into account, we chose the light gradient boosting machine (LightGBM) algorithm for machine learning. LightGBM is a gradient boosting framework that uses tree-based learning algorithms. We conducted the whole procedure with LightGBM Python-package (https://lightgbm.readthedocs.io/en/latest/Python-Intro.html), and parameters setting were provided in the supplement. In ranking the importance of individual variables, we adopted the conditional importance to avoid bias generated from multiple variables with different scales and variable collinearity in our dataset. The mean decrease impurity (MDI) of LightGBM was used as a measure of feature importance.

Cox proportional hazards models

Cox proportional hazards models were used to validate the association of individual lifestyle factors with incident CKD events. The duration of follow-up was calculated as the time between the baseline and the first occurrence of main outcome for participants who developed CKD events, or the end of follow-up for the current data release for those who were free of CKD events. Age and gender were adjusted as confounders. R 4.0.3 was used for the Cox proportional hazards regression (package "survival") and proportional subdistribution hazards regression (package "cmprsk"). We used the ggsurvplot function in the survminer package to plot the cumulative hazard function.

Lifestyle score

The lifestyle scoring system was established based on the combination of machine learning and the Cox proportional-hazards model. The hazard ratios (*HR*s) were used to identify a healthy or unhealthy lifestyle factor, and the modified MDI (MDI/1,000) was adopted as the lifestyle score for every factor. The total lifestyle score equals to the scores of healthy lifestyle factors minus the scores of unhealthy lifestyle factors. We used restricted cubic spline regression analysis to explore the relationship between total lifestyle score and the incident CKD event. Receiver operator characteristic (ROC) curves and C-statistics were adopted to evaluate the performance of the lifestyle scoring system.

Results

Complete data were available for 470,778 participants who were free of CKD in the UK Biobank study. After a median of 11-year follow-up, there were 13,555 participants who developed CKD events and 457,223 participants who stayed free of CKD. The baseline characteristics of these two groups are summarized in Table 1.

TABLE 1 Baseline characteristics of participants who kept free of CKD and during follow-up.

	Keep free of CKD events	Develop CKD events
No. of Participants	457,223	13,555
Male (%)	53.91	50.87
Age (years)	57 (8)	62 (6)
Ethnic background		
White (%)	94.57	94.04
Mixed (%)	0.60	0.44
Asian (%)	1.96	2.33
Black (%)	1.63	2.32
Chinese (%)	0.33	0.18
History of diabetes (%)	8.05	28.85
History of hypertension (%)	27.74	69.92
Body mass index (kg/m^2)	27.29 (4.73)	29.39 (5.38)
Systolic blood pressure (mmHg)	137.52 (18.44)	144.01 (19.88)
Diastolic blood pressure (mmHg)	82.25 (10.05)	83.06 (10.49)
Mean arterial blood pressure (mmHg)	100.67 (11.86)	103.38 (12.26)
Fasting blood glucose (mmol/L)	5.10 (1.18)	5.62 (2.13)
Glycated hemoglobin (%)	5.43 (2.75)	5.44 (3.17)
Estimated GFR (ml/min/1.73 m^2)	85.63 (14.97)	76.24 (14.48)
Urea nitrogen (mmol/L)	5.28 (1.22)	5.86 (1.39)
Uric acid (μmol/L)	305.11 (78.21)	335.34 (82.02)
Total cholesterol (mmol/L)	5.71 (1.13)	5.44 (1.26)
Triglycerides (mmol/L)	1.73 (1.02)	1.98 (1.12)
HDL cholesterol (mmol/L)	1.46 (0.38)	1.35 (0.37)
LDL cholesterol (mmol/L)	3.57 (0.86)	3.39 (0.94)
Current Smoking (%)	10.74	11.60
Current Alcohol Consumption (%)	92.22	87.78

Data are represented as median (interquartile range). The glomerular filtration rate (GFR) was estimated with the use of the Modification of Diet in Renal Disease equation. CKD, chronic kidney disease; GFR, glomerular filtration rate; HDL, high density lipoprotein; LDL, low density lipoprotein.

The importance of 37 lifestyle factors on incident CKD event was ordinally presented in Supplementary Figure 1. Bread, MET minutes of walking, MET minutes of moderate physical activity, tea, MET minutes of vigorous physical activity, water, cereal, raw vegetable, cooked vegetable, and fresh fruit ranked the top ten lifestyle factors associated with CKD.

In the age and gender adjusted Cox proportional hazards models, higher intakes of following dietary factors were associated with the lower risk of CKD event: whole grain bread (*HR* 0.77, 95% confidence interval (*CI*) 0.74–0.79), oat cereal (0.92, 0.89–0.96), muesli (0.74, 0.71–0.77), raw vegetable (0.99, 0.98–1.00), cooked vegetable (0.98, 0.97–0.99), fresh fruit (0.98, 0.97–0.99), dried fruit (0.68, 0.62–0.74), cheese (0.56, 0.49–0.65 for one time or more daily), oily fish (0.72, 0.68–0.77 for two times or more in a week), and non-oily fish (0.87, 0.79–0.95 for two times or more in a week); while higher intakes of following dietary factors were associated with the higher risk of CKD event: white bread (1.36, 1.31–1.41), biscuit cereal (1.17, 1.12–1.22), processed meat (1.40, 1.31–1.50 for two times or more in a week), salt added to food (1.17, 1.08–1.26 for always), pork (1.20, 1.12–1.30 for two times or more in a week), poultry (1.29, 1.18–1.42 for two times or more in a week), beef (1.46, 1.34–1.60 for two times or more in a week), and lamb (1.23, 1.12–1.35 for two times more in a week). For physical activity, walking MET >2,000 min/week (0.94, 0.90–0.99), moderate PA MET >800 min/week (0.96, 0.93–1.00), climbing stair (0.62, 0.57–0.68 for more than 20 times per day), usual walking pace (0.33, 0.32–0.35 at brisk pace), walk for pleasure (0.69, 0.67–0.72), swimming/cycling/keeping fit (0.72, 0.70–0.75), light DIY (0.65, 0.60–0.70), heavy DIY (0.86, 0.83–0.89), and strenuous sports (0.78, 0.75–0.81) were associated with the lower risk of CKD event. In addition, the longer time of mild to moderate physical activity did the subjects reported, the lower the risk of CKD they had. Subjects with current smoking (1.28, 1.21–1.35), tea >5 cups/day (1.12, 1.07–1.18), lower drinking temperature (1.06, 1.00–1.13), variation in diet (1.33, 1.25–1.41), and psychological stress (1.27, 1.23–1.31) showed the higher risk of CKD event, while current alcohol drinking (0.66, 0.62–0.69) and adequate sleep (0.81, 0.78–0.84) were associated with a lower risk (Supplementary Figure 2).

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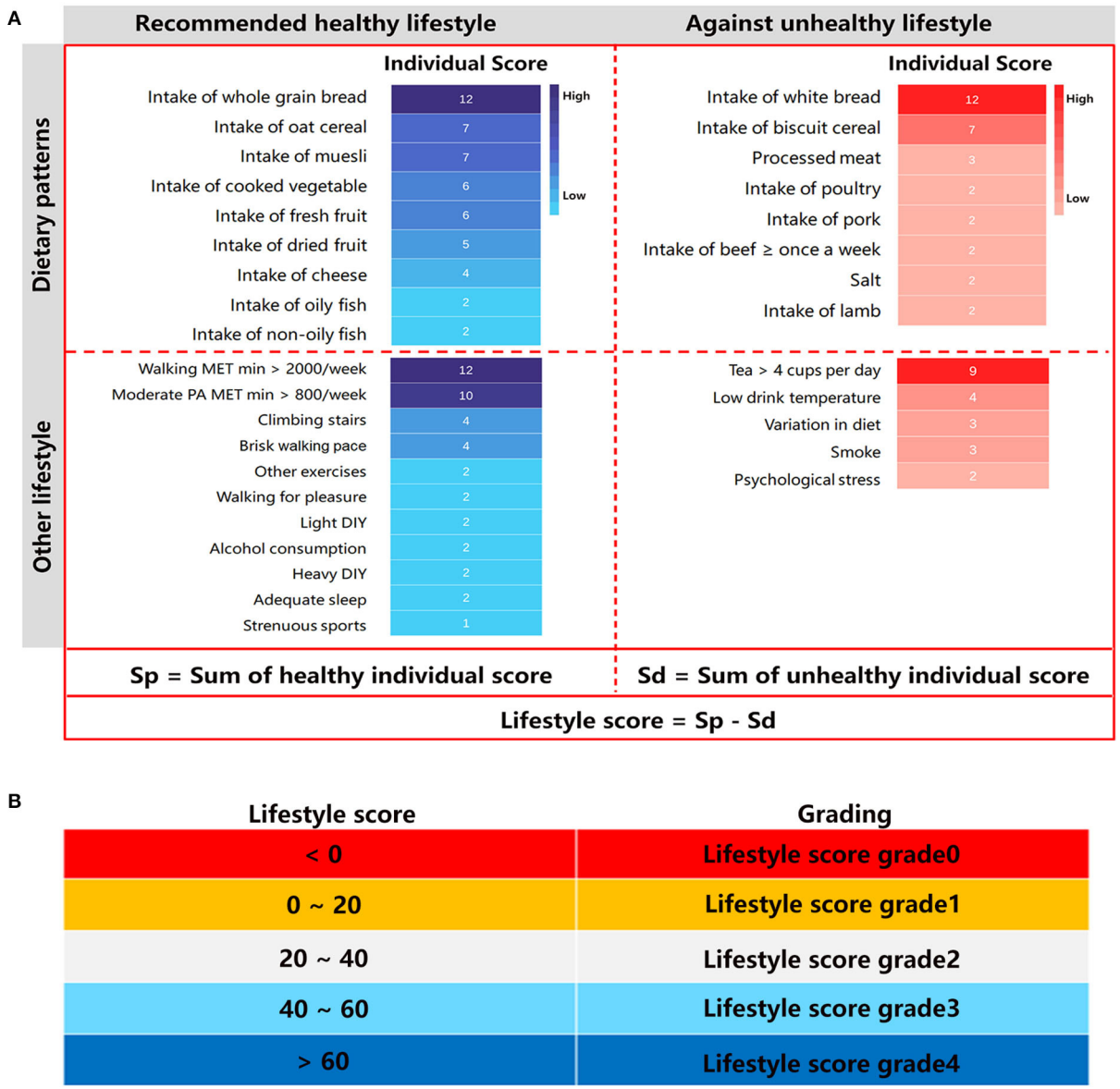

FIGURE 1
Score of lifestyle factors. **(A)** Healthy and unhealthy lifestyles are categorized according to the hazard ratios (*HR*s) in Supplementary Figure 2. The mean decrease impurity (MDI)/1,000 was adopted as the lifestyle score for every factor. Moderate PA included walking upstairs, going the gym, jogging, energetic dancing aerobics, most sports, using heavy power tools, and other physically demanding DIY and gardening. Light DIY included pruning, watering the lawn; other exercises included swimming, cycling, keeping fit, and bowling; Heavy DIY included weeding, lawn mowing, carpentry, and digging. PA, physical activity; DIY, do-it-yourself; MET, Metabolic Equivalent Task. **(B)** The lifestyle score was categorized as <0, 0–20, 20–40, 40–60, corresponding to grade 0, grade 1, grade 2, grade 3 and grade 4 respectively.

Adopting the modified MDI (MDI/1,000) as the individual lifestyle score for healthy and unhealthy lifestyle factors, we summarized the lifestyle score in Figure 1. As the restricted cubic spline regression analysis showed, the relationship between the lifestyle score and the risk of CKD event was manifested as linear (p for linear <0.001) (Figure 2A). A higher individual lifestyle score was significantly associated with a lower risk of cardiovascular diseases (Figure 2B) or all-cause mortality (Figure 2C). Compared with participants with lifestyle score < 0, participants scoring 0–20, 20–40, 40–60, and >60 exhibited 25% (20%, 29%), 42% (39%, 46%), 55% (52%, 58%) and 70% (64%, 74%) lower risk of CKD event, respectively (Figure 2E). For predicting CKD events by the age-adjusted lifestyle score, the area under the curve (AUC) of ROC was 0.710 (0.703, 0.718) and C-statistics 0.706 (0.704, 0.708) (Figure 2D).

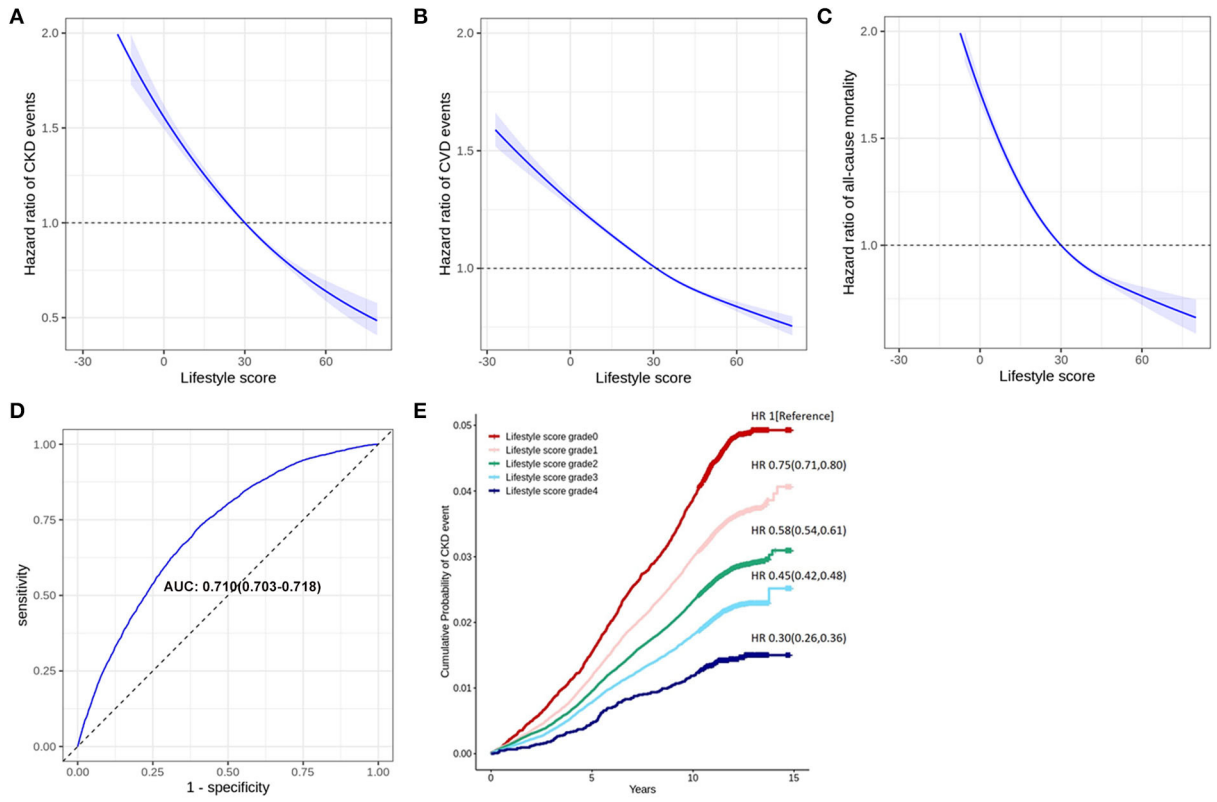

FIGURE 2
Validation of the lifestyle score in long-term outcomes. Panel **(A)** shows a restricted cubic spline regression analysis, which indicates a linear relationship between the total lifestyle score (equals to the scores of healthy lifestyle factors minus the scores of unhealthy lifestyle factors) and risk of CKD events. Panel **(B)** shows the categorization for risk of CKD event according to the total lifestyle score. Panel **(C)** shows the receiver operator characteristic curves (ROC) of the age-adjusted lifestyle score. Panel **(D)** or **(E)** is a restricted cubic spline regression analysis, which indicates a linear relationship between the total lifestyle score (equals to the scores of healthy lifestyle factors minus the scores of unhealthy lifestyle factors) and the risk of CVD events or all-cause mortality.

Discussion

With a systematic comparison of 37 lifestyle factors effects on CKD risk by machine learning, we established a lifestyle scoring system for CKD risk identification. The relationship between the lifestyle score and the risk of CKD event was linear, and an ROC analysis proved that the system was effective in predicting the CKD incidence. Our study not only confirmed that the majority of CVD-based healthy lifestyle factors were applicable to the prediction of CKD, but also, for the first time, provided unified hierarchies of evidence for preventing CKD by lifestyle.

Recent studies involved in the relationship between diet and CKD have exhibited inconsistent results. In some cohort studies, healthy dietary patterns such as Dietary Approaches to Stop Hypertension (DASH) and multicomponent diet were shown to be associated with the lower risk of CKD incidence (32, 33). However, other cohort studies indicated that a healthy dietary pattern (DASH or American Heart Association recommended dietary pattern) was not associated with the incident CKD (11, 12). Using a large-scale cohort of UK Biobank, we confirmed that whole-grain bread, oat cereal, muesli, cheese, fruit, vegetable, and fish were associated with the lower risk of CKD incidence, supporting that healthy dietary patterns are beneficial in CKD prevention. Previous studies suggested that both low and high intake of water were not beneficial in CKD (34), while no significant association was observed between tea and the risk of CKD (35, 36). In our dataset, an excessive intake of tea (>5 cups per day) or water (>4 glasses per day) was associated with the higher risk of developing CKD, which argued for an appropriate consumption of tea or water.

Previous studies suggested that a higher level of total physical activity was associated with a lower risk of developing CKD or ESRD (13, 37), which were also validated in our study. Beyond total physical activity, our analysis of individual physical activity showed that spending any time for walking, swimming/cycling/keeping fit, stair climbing, and light/heavy DIY were associated with the lower risk of CKD event, indicating that occasional light physical activity was also helpful in the prevention of CKD. In addition, a meta-analysis showed that

both short- and long-sleep durations were associated with a higher risk of CKD (20), and psychological distress was also shown to be positively associated with the CKD risk (38). Both sleep and psychological health were validated to be the predictors of CKD event in our analysis.

Previous studies on the relationship between alcohol intake and the risk of CKD were inconsistent. A cohort study showed that regular and occasional binge alcohol drinking were associated with a higher risk of CKD progression when compared with non-drinking (15). In contrast, a meta-analysis indicated that compared to participants with a minimum alcohol consumption, those who had light to heavy (>12 g/day) intake of alcohol were associated with a lower risk of CKD (16). In our study, although the importance of alcohol consumption on CKD was not as high as diet or physical activity, a higher intake of alcohol was associated with a lower risk of CKD, arguing a protective effect of alcohol consumption on the CKD progression. It was suggested that a higher level of high-density lipoprotein cholesterol (HDL-C) and a lower level of plasminogen activator inhibitor-1 might explain the protective role of alcohol (16). Apart from alcohol, our results also demonstrated the detrimental effect of smoking on CKD, which is similar to previous findings (18).

The main strengths of this study included a large-scale population with more than 450,000 participants, long follow-up time, and a comprehensive lifestyle evaluation. More importantly, we used supercomputer to analyze multidimensional variables and non-linear relationships by machine learning, which might be more efficient and accurate than traditional methods. Our study also had some limitations. On one hand, most lifestyle factors were evaluated only at baseline. Although the large-scale population diminished the impact of one-time evaluation bias, repeat assessments often leads to more convincing results. On the other hand, most participants adopted the western lifestyle, which is different from Asia, Africa, and other areas. Whether the lifestyle scoring works well in different populations needs to be further verified. In addition, more than 90% of our participants were Whites. There are significant ethnic disparities in CKD progression (39), and the influence of lifestyle factors on CKD was shown to be varied among different ethnicities (40). Although the homogeneity of race is helpful for controlling confounders, our findings need to be replicated in other populations, such as Asians and blacks.

Conclusion

In conclusion, a lifestyle scoring system for CKD prevention established by integrating dietary patterns, physical activity, sleep, psychological health, smoking, and alcohol was associated with an increased risk of CKD. On the basis of the lifestyle scoring system for CKD risk, people could flexibly choose healthy lifestyles and avoid unhealthy lifestyles. Further study is needed to verify whether the system will improve clinical care and patient outcomes.

Practical application

Harnessing the cohort of UK biobank and supercomputer platform, we established a lifestyle scoring system for CKD prevention. Based on the system, individuals could flexibly choose healthy lifestyles and avoid unhealthy lifestyles to prevent CKD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by National Information Governance Board for Health and Social Care and the National Health Service North West Multicenter Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JH, SY, ZW, and QL designed the study, oversaw the data analysis, and interpreted the data. JH and WL conducted the data analysis and wrote the manuscript. XC contributed to the data analysis and writing of the manuscript. RG and ZL contributed to the study conception and design. BP provided statistical expertise. YW, TL, and YY assisted with the data analysis. LM, MM, and LG contributed to the writing and editing of the manuscript. SY, ZW, and JH are the guarantors of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.918576/full#supplementary-material

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Sleep Quality After Intradialytic Oral Nutrition: A New Benefit of This Anabolic Strategy? A Pilot Study

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Background: Since disturbances of appetite and sleep are closely related and both affect metabolic disorders, it would be expected that a renal specific oral nutritional supplement (RS-ONS) that covers the energy the patient does not consume on the HD day, could contribute to improve the nutritional status and body composition, as well as sleep quality. There is still scarce information related to this topic.

Aim: To evaluate the effect of the use of intra-dialytic RS-ONS vs. RS-ONS at home on sleep quality, nutritional status, and body composition in patients on HD.

Methods: Adult patients < 65 years, with ≥3 months on HD were invited to participate in an open randomized pilot study (ISRCTN 33897). Patients were randomized to a dialysis-specific high-protein supplement provided during the HD session (Intradialytic oral nutrition [ION]) or at home (control), during non-HD days (thrice weekly, for both) 12 weeks. The primary outcome was sleep quality defined by the Pittsburgh Sleep Quality Index (PSQI) score. Nutritional assessment included Malnutrition Inflammation Score (MIS), bioelectrical impedance analysis, anthropometry, 3-day food records, and routine blood chemistries.

Results: A total of 23 patients completed the study. Age was median 35 (range 24–48 years), 42% were women. At baseline, the PSQI score was median 4 (range 2–7), and MIS showed a median of 6 (range 5–8); there were no baseline differences between groups. After intervention, both groups improved their MIS scores and similarly when we analyzed the whole cohort (pre- vs. post-intervention $P < 0.01$). Patients in the ION group improved the overall PSQI score to median 3 (2–5), and assessment of sleep duration and sleep disturbances (pre- vs. post-intervention $P < 0.05$), with a trend toward an effect difference compared to patients consuming the supplement at home (P for treatment-effect across arms 0.07 for PSQI score and 0.05 for sleep latency).

Conclusion: Oral supplementation improved nutritional status in the whole cohort, but only ION improved the PSQI score. More studies are needed to explore the nutritional strategies that influence the relationship between sleep and nutritional status in HD patients.

Keywords: intradialytic oral supplementation, sleep quality, hemodialysis, nutrition, anabolic

INTRODUCTION

Patients with Chronic Kidney Disease (CKD) undergoing hemodialysis (HD) frequently experience loss of appetite and poor sleep quality, conditions that are closely interrelated. It has been reported that having a good nutritional status and good sleep quality are essential to maintaining quality of life. Previous studies have reported that around 30–80% of patients with advanced CKD had sleep disorders, with a higher prevalence and severity in HD patients (1–3). Furthermore, poor sleep quality has been associated with several health consequences such as metabolic abnormalities, disability, pain, restless leg syndrome, fatigue, sleep apnea, depression, and malnutrition (4).

A decreased nutrient and protein intake due to loss of appetite, a hypercatabolic state, metabolic acidosis, comorbidities, and dialysis itself may all contribute to malnutrition and inflammation also known as protein-energy wasting (PEW) (5), identified as a common problem in patients with CKD, with a current prevalence of 24–54% (6). Treatment of PEW with nutritional strategies such as nutritional support have proven effective and has been associated with adequate tolerance to the supplement and better compliance to the HD treatment (7–9). Interventional and observational studies suggest that nutritional supplementation may have the following benefits: improved quality of life (9), increased body weight and maintenance of lean body mass (10), improved response to erythropoietin (11), improved serum albumin or prealbumin levels (9, 12) better nutritional status (subjective global assessment) (13), increased energy and protein intake (14) and lower mortality (15).

Nutritional guidelines establish the need of nutritional supplementation in order to maintain a minimum energy intake of 25–35 kcal/kg/body weight/day, for those patients with chronically inadequate intake and whose protein and energy requirements cannot be attained by dietary counseling; they suggest a minimum of a 3-month of oral nutritional supplements (16). The use of oral supplementation is considered a therapeutic alternative that can provide 7–10 kcal/kg/day and 0.3–0.4 g/kg/day of protein intake, which helps to meet recommended goals and also may supply a great variety of macro and micro nutrients, as well as covering the skipped meal time, during the day the patient attends a HD session (17).

PEW impacts negatively on CKD patient outcomes, including quality of life, rate of hospitalizations, presence and severity of infections, cardiovascular events, survival (5, 7, 8), and sleep quality (9). It has been demonstrated that PEW can be treated *via* oral supplementation (16). It has been known that HD therapy has been associated with net protein loss from the whole body, however, this catabolic process can be reversed by oral nutrition during HD session (18). In addition, the anabolic effects of intradialytic oral nutrition (ION) seem not to be limited to the administration period like parenteral nutrition (18). However, despite the evidence, there is no clear indication of the best time to use oral supplementation (16). The use of a nutritional supplement would allow, in the first instance, to cover both energy and protein intake in this population and, in turn, reduce protein catabolism caused by dialysis treatment, so that its use during the HD session may be even more effective (18). On the other hand, sleep disturbances are increasingly being studied and associated with the intake of both macro and micro nutrients, mainly in the general population, where hours of sleep have been associated with different nutritional outcomes (19). Hemodialysis patients often have frequent sleep disturbances, as well as poor appetite, malnutrition, and body composition (20, 21).

According to this, the purpose of this study was to evaluate the effect of the use of renal specific ION supplementation (RS-ONS) vs. RS-ONS at home on sleep quality, nutritional status, and body composition in patients on HD.

MATERIALS AND METHODS

Study Population

We performed a single center, open randomized pilot study for prevalent patients undergoing HD in our unit. The study was approved by the institutional ethics committee (registration number 2229), in addition to having the registration of International Standard of Randomized Controlled Trials Number (ISRCTN 33897). Eligible participants were adults (>18 years), both sexes, under maintenance HD (at least 3 months on therapy), thrice weekly, and Kt/V >1.2 or URR >65%. Patients with (1) amputation of any extremity, (2) planned renal transplant within the next 3 months, (3) acute kidney injury, hospitalization 1 month prior to the initiation of the study, and (4) those who had ultrafiltration volumes of more than 3 liters per session or with sleep disorders (diagnosed by sleep clinic experts) were excluded. All patients were informed about the nature of the study and signed an informed consent. Simple randomization to one of two groups was performed by an external collaborator without contact with the research team in charge of enrollment and intervention using a website (randomizer.org).

Dietary Assessment

All patients were given personalized nutritional counseling, according to current guidelines (22, 23). Dietary intake was evaluated by 3-day food records, asking the patient to record food intake on a HD day, a non-HD mid-weekday, and a weekend

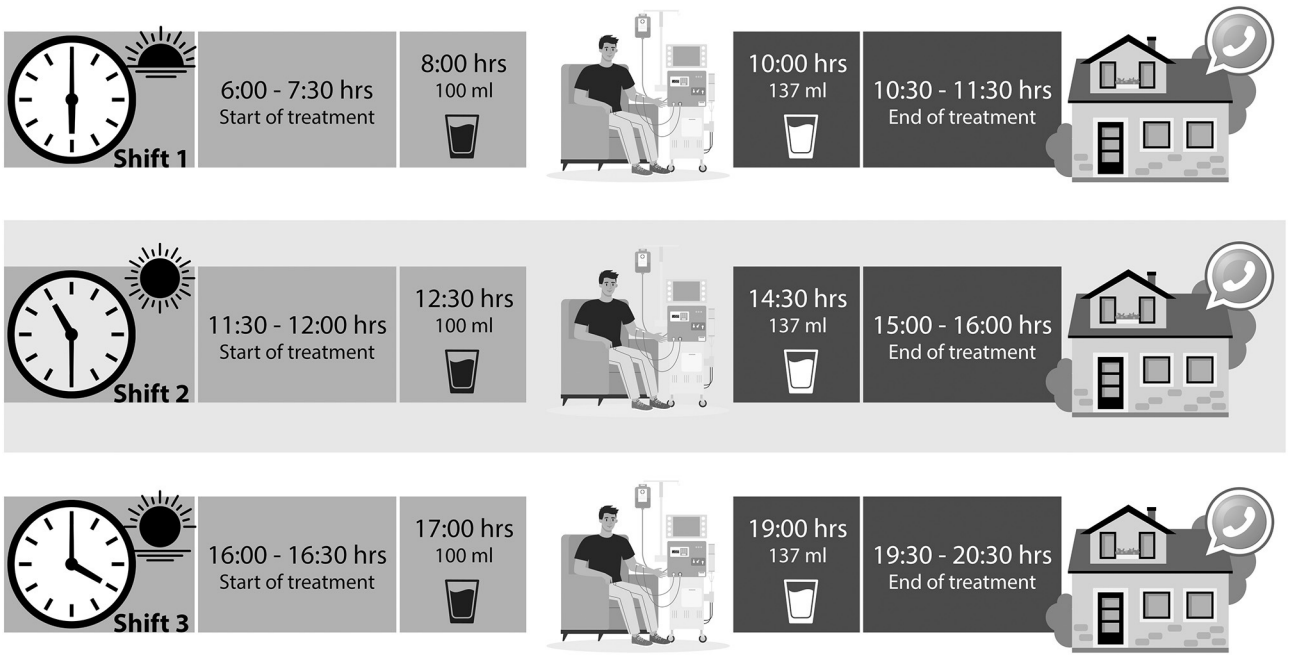

FIGURE 1 | Study protocol. Supplement intake according to the treatment schedule, for patients who took it at home a message was sent to remind them to take it at the time they come to HD.

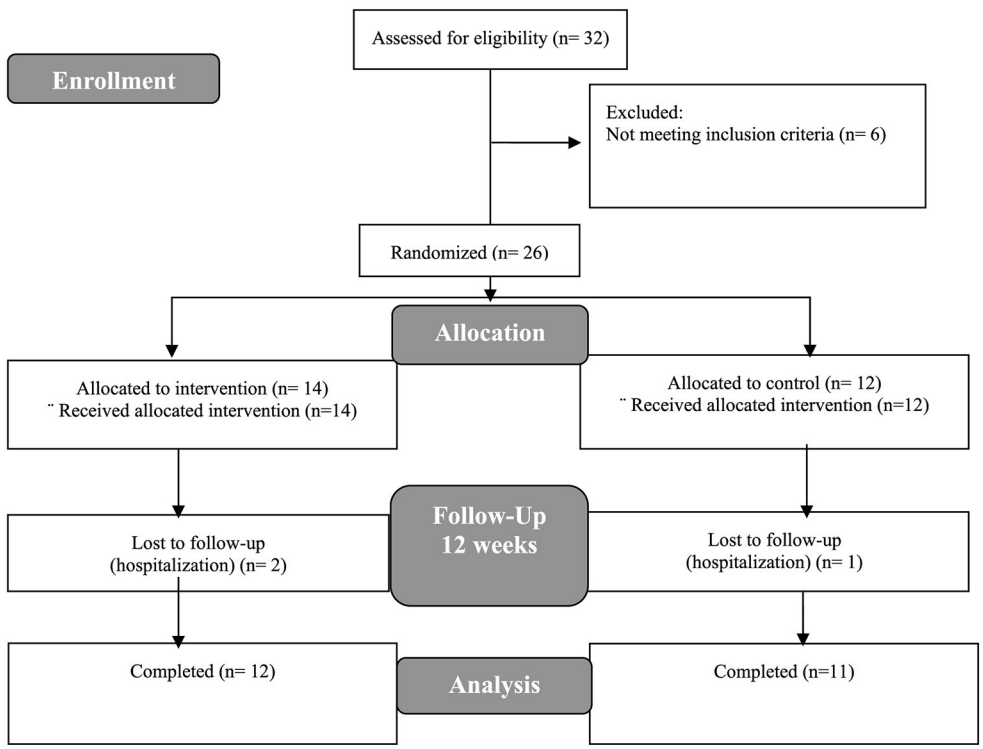

FIGURE 2 | Flowchart of study design.

TABLE 1 | General characteristics at baseline.

Characteristic	All ($n = 26$)	At home ($n = 12$)	ION (14)	*P*-Value
PSQI total score	4 (2–7)	3 (2–6)	6.5 (3–11)	0.06
Age (years)	35 (24–48)	33.5 (24–44)	36 (30–49)	0.59
Dialysis vintage (months)	16 (8–36)	12 (7–33)	21.5 (8–40)	0.66
Gender, women *n* (%)	11 (42)	6 (50)	5 (36)	0.46
Civil status single, *n* (%)	13 (50)	7 (58)	6 (43)	0.36
Diabetes, *n* (%)	12 (46)	5 (42)	7 (50)	0.67
Hypertension, *n* (%)	22 (85)	11 (92)	11(79)	0.36
Biochemical measurements				
Albumin (g/L)	3.6 (3.3–3.7)	3.95 (3.5–4.3)	3.45 (3.2–3.7)	0.05
Serum Iron (μg/dL)	56 (44–74)	54 (42.5–57)	62 (46–82)	0.22
Unsaturated iron binding capacity (μg/dL)	214 (186–256)	230.5 (204–257)	187 (181–248)	0.11
Total iron binding capacity (μg/dL)	256 (237–315)	273.5 (252.5–302.5)	250.5 (227–330)	0.32
Saturation index %	23 (16–34)	20 (14.5–28.5)	25 (19–34)	0.27
Ferritin mg/dL	146 (50.5–330.4)	145.6 (35.4–262.2)	145.6 (89.4–348.5)	0.66
Serum creatinine (mg/dL)	11.4 (10–12.9)	11.4 (10.3–13.1)	11.2 (10–12.9)	0.96
Sodium (mmol/L)	139 ± 2.5	139 ± 2.6	139 ± 2.4	0.79
Serum potassium (mmol/L)	5.2 (5.1–5.6)	5.2 (5.1–5.4)	5.3 (5.1–5.7)	0.69
Serum phosphorus(μg/L)	4.4 (3.4–5.6)	4.1 (3.5–5.3)	4.6 (3.4–7.3)	0.59
Total KT/V	1.9 ± 0.5	1.8 ± 0.3	1.99 ± 0.7	0.44
Dietary intake and food groups (by 1,000 kcal) per day				
Energy, kcal	1,397 (1,087–1,850)	1,302 (1,087–1,628)	1,427 (1,240–1,850)	0.30
Dietary energy intake, kcal/kg/day	22.8 (16.5–30.5)	20.6 (16.5–28.2)	23.7 (20.5–30.5)	0.26
Protein g/kg/day	0.96 (0.64–1.14)	0.83 (0.64–1.12)	0.97 (0.91–1.14)	0.28
Fiber g/day	8.9 (6.3–10.7)	8.4 (6.3–10.7)	8.9 (7.6–9.2)	0.92
Fruits (servings)	0.8 (0.6–1.8)	0.7 (0.6–1.8)	0.8 (0.6–0.96)	0.94
Vegetables (servings)	1.4 (0.9–1.9)	1.4 (1.1–1.7)	1.5 (0.9–1.9)	0.84
Legumes (servings)	0.07 (0–0.5)	0.03 (0–0.16)	0.08 (0.03–0.22)	0.32
Cereals (servings)	5.6 (4.5–6.1)	5.6 (5.0–6.1)	5.6 (4.5–5.9)	0.88
Fat (servings)	3.5 (2.3–5.3)	3.5 (2.3–4.8)	3.6 (2.9–5.3)	0.84
Dairy (servings)	0.1 (0–0.5)	0.14 (0–0.5)	0.13 (0–0.4)	0.98
Meat and eggs (servings)	3.6 (2.6–4.7)	3.5 (2.8–4.2)	3.7 (2.6–4.7)	0.96

Data are expressed as mean ± SD, median (25th, 75th centile), or number (%), as appropriate.

day. For this purpose, patients received training on how to record their food consumption by a trained dietitian. Records were reviewed with the patient and corrected with the help of standardized tridimensional and flat food replicas. Food records were introduced into the software Nutrikcal VO® v.1, which determines the energy and macronutrients provided by each food group, according to Mexican guidelines and food composition of typical Mexican foods (24).

Intradialytic Oral Nutrition

The patients in this group received two portions of the RS-ONS in plastic recipients with a lid and straw; the first portion was 100 ml and was received at minute 60, and the second portion was 137 ml and was received 45 min before ending their sessions. The RS-ONS was Nepro? (Abbott Laboratories) with the following nutritional content in 237 ml: 434 kcal, 37.9 g of carbohydrates, 22.8 g of lipids, 19.2 g of protein, and 3 g of fiber in 237 ml (1.83 kcal/ml). Cans were washed with 2.0% wide spectrum chlorhexidine, following appropriate guidelines. The intervention was provided over 36 consecutive HD sessions (12 weeks, 3 weekly). If a patient missed a session, the respective supplement was labeled with a code and saved until the end of the study (**Figure 1**).

Supplementation at Home

The participants in this group received the same RS-ONS on a non-dialysis day at home (thrice weekly for 12 weeks). They were encouraged to consume their can in two portions, following the suggested schedule (as if they had attended the session, at the same time as the intradialytic group) and wash the canned product before consuming it. The patients were asked to return the empty can on the following HD day so they could receive the next can (**Figure 1**).

TABLE 2 | Effects of ION on sleep quality index components, nutritional status, and body composition.

Characteristics	At home		ION	
	Baseline	Final	Baseline	Final
	n = 11	*n* = 11	*n* = 12	*n* = 12
Pittsburg sleep quality index components				
Subjective sleep quality	1 (0–1)	0 (0–1)	1 (1–2)	1 (0–1)
Sleep latency	0.5 (0–1)	0 (0–1)	1.5 (0–3)	1 (0.5–1)
Sleep duration	0 (0–2)	0 (0–1)	1 (0–2)	0 (0–1)*
Habitual sleep efficiency	0 (0–1)	1 (0–1)	0.5 (0–2)	0 (0–1)
Sleep disturbances	1 (0–1)	1 (0–1)	1 (1–2)	1(0.5–1)*
Daytime dysfunction	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Use of medication for sleep	0 (0–1)	1 (0–0)	0.5 (0–1)	0 (0–0)*
PSQI total score	3 (2–6)	3 (1–6)	6.5 (3–11)	3 (2–5)*
Poor sleeper, *n* (%)	4 (33)	2 (18)	8 (57)	1 (8)
Sleep hours	7.5 (5–8)	8 (6.5–8)	7 (5–8)	8 (7–9)*
Nutritional status and body composition				
Body mass index (kg/m^2)	24 (20–29)	23 (20–30)*	22 (20–27)	23 (21–28)*
nPNA (g/kg/day)	0.89 (0.83–1.13)	1.06 (0.95- 1.12)*	0.9 (0.79–1.01)	1.0 (0.9–1.13)
Resistance/height*	354.5 (310–422)	359 (302–454)	355.4 (329-468)	324 (291 - 429)*
Reactance/height*	42 (32–49)	38 (34–44)	41 (35–50)	40 (33.6–45.5)
Phase angle (degres)**	5.9 ± 1.2	6.1 ± 0.9	6.15 ± 1.13	6.4 ± 1.1
Malnutrition inflammation score components				
Medical history	3 (0–6)	1 (0–4)	2.5 (1–6)	2 (1–3)*
Physical exam	2 (0–4)	0 (0–2)*	1.5 (0–3)	0 (0–1)*
Body mass index	0 (0–2)	0 (0–2)	0 (0–2)	0 (0–2)
Laboratory parameters	1 (0–2)	1 (0–2)	2 (0–4)*	1 (0–4)
Total MIS score	6 (2–11)	2.5 (1–8)*	6.5 (2–10)	4 (2–6)*

Wilcoxon rank-sum (Mann-Whitney test). Data are expressed as mean ± SD, median (25th, 75th centile), or number (%), as appropriate. MIS, Malnutrition Inflammation Score.
**Pre- vs. post-intervention $p < 0.05$.*
***At 50kHz.*
Medical history represents the sum of score: change in dry weight, dietary intake, gastrointestinal symptoms, functional capacity and co-morbidity.
Physical exam represents the sum of score: decreased fat stores or loss of subcutaneous fat and signs of muscle wasting.
Laboratory parameters represents the sum of score: albumin and total iron binding capacity.

Study Outcomes

Sleep Quality

The assessment of sleep pattern and quality was performed by a sleep specialist before a HD session with the Pittsburgh Sleep Quality Scale (PSQI), which is a self-report tool of 19 items regarding sleep quality and degree of difficulty sleeping during the last month. It is made up of 7 components: sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction. The 7 components are added to produce an overall score with a range from 0 to 12; higher scores indicate poorer sleep quality. An overall score >5 has a diagnostic sensitivity of between 89.6 and 98.7%, and specificity of 84.4–86.5%, differentiating between bad and good sleepers (25, 26).

Nutritional Status

The Malnutrition Inflammation Score (MIS) calculation method includes (A) medical history: Changes in dry weight after dialysis (3–6 months), dietary intake, gastrointestinal symptoms, functional capacity, and comorbidity according to time on HD. (B) Physical exam, loss of fat stores or subcutaneous fat below eyes, triceps, biceps, chest, and signs of loss of lean mass in temple, clavicle, scapula, ribs, quadriceps, knee, interosseous, (C) body mass index (BMI), and (D) biochemical parameters such as serum albumin and total iron binding capacity (TIBC) or transferrin, which are estimated from the results of the various evaluations. Each of them is classified into four degrees of severity. Starting from 0 to 3 on the scale, the sum of all 10 MIS components can range from 0 (normal) to 30 (severely malnourished)—a higher score reflects a more severe degree of malnutrition and inflammation (27).

Body Composition

The participants were evaluated at the end of the HD session at the following time points: at baseline, and at the end of the follow-up—we included body weight and height to determine BMI. For the measurement of bioelectrical impedance analysis (BIE), two "emitting electrodes and two sensor electrodes" were carefully placed (standard, tetrapolar placement on the hand and foot). Measurements were conducted by the same

TABLE 3 | Changes (Δ) in sleep quality characteristics, nutritional status, and body composition in both study groups.

Sleep characteristics	At home (Δ) ($n = 11$)	ION (Δ) ($n = 12$)	*P*-Value
Sleep hours	0.5 (-1.5–3)	0.75 (-1–3)	0.42
PSQI components			
Subjective sleep quality	0 (-2–2)	0 (-2–1)	0.68
Sleep latency	0 (-1–2)	-0.5 (-2–1)	0.05
Sleep duration	0 (-2–1)	-0.5 (-2–1)	0.53
Habitual sleep efficiency	0 (-1–2)	0.5 (-3–1)	0.13
Sleep disturbances	0 (-1–1)	0 (-2–0)	0.08
Daytime dysfunction	0 (0–0)	0 (-1–0)	0.34
Use of medication for sleep	0 (-1–1)	0 (-1–0)	0.28
PSQI total score	1 (-5–6)	-2.5 (-10–2)	0.07
Nutritional status and body composition			
Body mass index kg/m^2	0.75 (-0.9–1.7)	0.89 (-0.04–2.0)	0.19
MIS score	-2 (-9–0)	-3 (– 8–0)	0.45
nPNA (g/kg/day)	0.09 (-0.09–0.32)	0.15 (-0.42–0.53)	0.93
Resistance/height*	-7.8 (-60.6–145.2)	-34.5 (-66.6–23.9)	0.04
Reactance/height*	-1.9 (-30.6–14.9)	-3.1 (-13.3–8.7)	0.79
Phase angle (degrees)*	0.2 (-1.91–2.4)	0.3 (-1.9–1.84)	0.92
Albumin mg/dL	0 (-0.4–0.36)	0 (0–1.6)	0.09

Wilcoxon rank-sum. Data are expressed as median (25th, 75th centile), or number (%), as appropriate.
**At 50 kHz.*

operator using an impedance device that emitted 800 μA and 50 kHz alternating sinusoidal current (Multifrequency Bodystat Quadscan 4000 BODYSTAT Ltd.), strictly following the methods reported elsewhere (28).

Study Covariates

Other study covariates were collected *via* standardized methods and included demographics such as self-reported civil status, employment, comorbidities, medications, and laboratory measurements. History of comorbidities and ongoing medications were obtained from consultation of the patient's clinical files. Kt/V as a measurement of dialysis adequacy (K = dialyzer urea clearance, t = time on dialysis, and V = total body water) was extracted from the medical records which was calculated by the machine (Fresenius Medical Care 4008 s) at the end of the sessions. All measurements were carried at baseline and 12 weeks after the completion of the nutritional intervention.

Sample Size

Since there are no previous studies that have evaluated the effect of ION supplementation on sleep quality, we analyzed the entire population of our HD unit, and subsequently performed a statistical power analysis. Power of the sample size was calculated using GPower[19]® (version 3.1.9.4; Heinrich-Heine-University, Düsseldorf, Germany). A statistical power calculation was made with the included sample size in which the effect size was 0.50, giving a total power of 0.608 with two tails considering the whole cohort.

Statistical Analysis

Descriptive statistics were performed, and continuous variables were presented as arithmetic means and standard deviation for those that had a normal distribution, and those that did not were presented as medians or the inter-quartile range. We carried out analysis following protocol, and to determine whether there were statistical differences between the groups, the chi-square test was performed for the qualitative variables. For the quantitative, the Mann Whitney U tests or the Student's *t*-test was employed, depending on their distribution. Wilcoxon or paired *T*-tests were used to determine the differences between the basal and final groups, according to the distribution of the data.

A subanalysis was performed by group and in all the patients as one group.

The analyses were performed using STATA (version 15.1; Stata Corp, College Station, TX) and $p \leq 0.05$ was established for statistical significance.

RESULTS

A total of 26 participants were randomized, of which 23 finished the study; in general, 94% adherence to treatment was observed. The description of patient selection and monitoring are explained in detail in **Figure 2**. This was a relatively young population with almost half of them with diabetes mellitus. Regarding biochemical measurements, a median of 3.6 (range 3.2–3.7) g/dl of serum albumin was observed, while iron profiles were in normal ranges. There were no differences at baseline in dietary energy and protein intake between groups (**Table 1**), nor in the

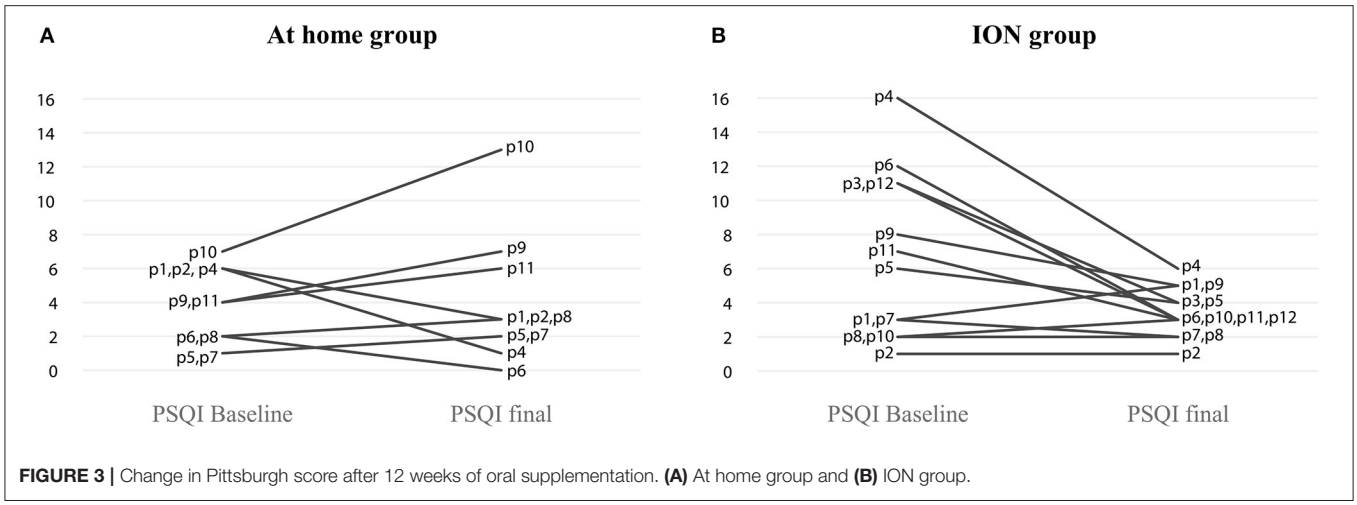

FIGURE 3 | Change in Pittsburgh score after 12 weeks of oral supplementation. **(A)** At home group and **(B)** ION group.

whole cohort comparing baseline and final measurements (data not shown).

Sleep Quality

The basal median of the total sample on PSQI was 4 (range 2–7) points. Sleepiness was present in 8% of the sample at baseline and 14 patients (46%) were poor sleepers (PSQI > 5).

Subsequently, after 12 weeks of follow-up, the Pittsburgh components were analyzed between groups, and a significant decrease difference was found in the sleep latency, as well as in the presence of sleep disturbances ($p < 0.05$ for both groups). The ION group showed improvement in the sleep duration and disturbances as well as a decreased prescription of sleep medications. The total score improved in the ION group (pre- vs. post-intervention, $p < 0.05$), whereas no differences were found in the home group (pre- vs. post-intervention) (**Table 2**). When we explored the difference in sleep changes between groups we found a statistical difference only in sleep latency in the ION group, with a trend toward improvement in the sleep disturbances and overall PSQI scores (**Table 3**).

When we evaluated the Pittsburgh score individually for each patient, we observed a trend to improve mainly in those of the ION group (**Figure 3B**), while the group that received supplementation at home, the score seemed to worsen by the end of the intervention (**Figure 3A**). Considering a decrease of two points in the PSQI an improvement, seven participants (58%) improved in the ION group; the number needed to treat indicated that we must treat eight [CI 95% (6.9–8.6)] patients with ION to decrease two points in the PSQI.

Nutritional Status and Body Composition

After randomization, no differences were found between groups. At the end of the study, we found an improvement in MIS score in both groups, pre- vs. post-intervention ($p < 0.05$), but no statistically significant differences were observed between the groups. In the ION group, BMI showed improvement; however, in the home group, the BMI decreased and nPNA improved after intervention, while no other differences were observed (**Table 2**). No differences were observed between groups in other nutrition status markers. Finally, considering it as an improvement of nutritional status, a decrease of two points was observed in the MIS score; almost all participants improved-−10 participants (83%) in the ION group and 8 participants (73%) in the home group. The number needed to treat indicated that we must treat 10 patients with ION to decrease two points in MIS 4.

When we analyzed the whole cohort as one group, we found a decrease in unsaturated iron-binding capacity, no other differences in biochemical measurements. According to PSQI components, we found an improvement in sleep duration and in the total score, when we classified poor vs good sleepers at the end of the study only 3 participants were poor sleepers, and the time to sleep increased, and according to MIS, only BMI not shown differences at the end of the study (**Table 4**).

DISCUSSION

Information regarding the use of oral supplements and the effect on sleep in HD patients is scarce, however, there is a large body of evidence that associates dietary intake with sleep quality in the general population, where the main outcomes are focused on nutritional status. This study suggests that intradialytic oral nutrition may improve sleep quality and both interventions (at home and nutrition supplementation during HD sessions) were effective in improving nutritional status.

When we analyzed sleep results, an effect was found only in the group that received ION. There is evidence linking the nutritional status assessed by the MIS score and sleep quality, where those patients who had the worst sleep quality were also those who had the worst nutritional status (29). Therefore, it was expected that those who would improve their nutritional status after reported using a nutritional supplement would also improve their sleep quality; however, it was observed that through this intervention, only the ION group showed improvement.

There are other studies that associate nutritional status with sleep quality in this population. Burrowes et al. (30) found a decreased appetite as sleep quality worsened. In this study, after ION improved patients' sleep quality,

TABLE 4 | Effects of ION on sleep quality index components, nutritional status, and body composition.

Characteristics	Baseline ($n = 23$)	Final ($n = 23$)	*P*-value
Albumin (g/L)	3.5 (3.3–4.3)	3.7 (3.5–4.5)	0.44
Serum Iron (μg/dL)	56 (44–74)	59.5 (46–74)	0.17
Unsaturated iron binding capacity (μg/dL)	214 (186–256)	194 (137–239)	0.02
Total iron binding capacity (μg/dL)	256 (237–315)	254 (224–292)	0.41
Saturation index %	23 (15–34)	21 (16–35)	0.85
Ferritin mg/dL	146 (50.5–334.4)	243.5 (98–395)	0.10
Serum creatinine (mg/dL)	11.6 (10.4–13.52)	10.8 (9.7–12.1)	0.09
Sodium (mmol/L)	139 ± 2.5	138 ± 3.13	0.25
Serum potassium (mmol/L)	5.2 (5.1–5.6)	5.1 (4.7–6)	0.76
Serum phosphorus(μg/L)	4.4 (3.7–5.9)	5.1 (3.9–5.8)	0.91
Total KT/V	1.9 ± 0.5	1.8 (1.5–1.93)	0.24
Pittsburg sleep quality index components			
Subjective sleep quality	1 (0–01)	1 (0–1)	0.16
Sleep latency	1 (0–2)	1 (0–1)	0.56
Sleep duration	1 (0–2)	0 (0–1)	0.02
Habitual sleep efficiency	0 (0–2)	0 (0–1)	0.25
Sleep disturbances	1 (1)	1 (0–1)	0.08
Daytime dysfunction	0 (0)	0 (0)	0.32
Use of medication for sleep	0 (0–1)	0 (0)	0.06
PSQI total score	4 (2–7)	3 (2−5)	0.05
Poor sleeper, *n* (%)	11 (48)	3 (13)	0.02
Sleep hours	7 (5–8)	8 (6.5–8.5)	<0.01
Nutritional status and body composition			
Body mass index (kg/m^2)	22 (20–30)	23 (20-31)	<0.01
nPNA (g/kg/day)	0.93 (0.8–1.04)	1.03 (0.88–1.12)	<0.05
Resistance/height*	358 (320–445)	335 (284–434)	0.03
Reactance/height*	41(32–49)	38 (34–44)	0.17
Phase angle (degres)**	6 ± 1.19	6.2 ± 0.89	0.63
Malnutrition inflammation score components			
Medical history	3 (2–4)	2 (1–2)	<0.01
Physical exam	2 (0–3)	0 (0–1)	<0.01
Body mass index	0 (0)	0 (0)	0.32
Laboratory parameters	1 (1–2)	1 (0-2)	<0.01
Total MIS score	6 (5–8)	3 (2–4)	<0.01

Wilcoxon rank-sum (Mann-Whitney test). Data are expressed as mean ± SD, median (25th, 75th centile), or number (%), as appropriate. MIS, Malnutrition Inflammation Score.
**Pre- vs post-intervention p value <0.05.*
***At 50 kHz.*
Medical history represents the sum of score: Change in dry weight, dietary intake, gastrointestinal symptoms, functional capacity and co-morbidity.
Physical exam represents the sum of score: decreased fat stores or loss of subcutaneous fat and signs of muscle wasting.
Laboratory parameters represents the sum of score: albumin and total iron binding capacity.

however, no differences were found in diet characteristics. At the same time, it has been reported that higher serum creatinine values are associated with better sleep quality, and that this has also been considered as a factor that indicates better muscle mass as nutritional status is variable.

Recent research has shown an association between dietary intake and sleep health that can influence risk factors for chronic diseases. In a recent review, it was described that meal time and sleep hygiene are two of the most important aspects to investigate in the link between diet and sleep (19). And in hemodialysis patients has been studied also how the patients use to skip meal on dialysis day (20).

The presence of sleep disturbances is highly prevalent in this population and dialysis modality or age can be important factors (31, 32). Both, the presence of sleepiness and poor sleep quality have been associated with worse quality of life of these type of patients (33) as this is a persistent problem; however, in this study, the presence of sleepiness was observed only in two individuals, so it is not possible to know if the improvement

in the nutritional status had any effect in this parameter (data not shown).

When we evaluated sleep quality characteristics, the group receiving ION presented improvement, mainly in terms of sleep duration and sleep disturbances as well as in a reduction of use of sleep medications ($p < 0.05$ intra-group effect). Similarly, there was an effect on the total PSQI score in the ION group. No effects were found between supplementation at home and sleep quality nor when exploring the difference between the groups. But, when we analyzed the whole cohort the results shown an improvement in sleep duration, sleep hours, fewer poor sleepers, and lower PSQI total score.

It is important to mention that this finding not only supports the use of an oral nutritional supplement in HD patients for nutritional improvement, but could also imply an improvement in sleep quality, if administered during the HD session. When we analyzed the results individually, we observed that a large proportion of participants in the ION group presented a decrease in the PSQI score, while the patients in the home group seemed to have a worse score. Although ION seems to have an effect on sleep quality in this study, it is clearly necessary to consider conducting clinical trials with a larger sample size. Nevertheless, this is one of the few studies that explore the relationship between ION and quality of sleep.

There are multiple proven benefits regarding the use of ION in HD patients; it has been shown to have persistent anabolic benefits for muscle protein metabolism in the post-HD phase, while the anabolic benefits of parenteral nutrition during HD sessions dissipated during the same period. These data support both the anabolic and anti-catabolic functions of ION (18). However, in this study, we observed that the use of a nutritional supplement is effective in both groups—patients who consume it during the HD session and those who had the supplementation at home. This was evidenced through the results obtained by the MIS score that improved significantly in both groups ($p < 0.05$, without statistical differences between the groups), and this is consistent when we analyzed the whole cohort, were we found improvements in all the components an exception of BMI, which was already normal at the beginning of the study. One explanation for these positive results could be adherence to treatment, which was higher than 90% in all the population.

There is a large body of evidence that states that oral supplementation decreases hospital admission rates, serum IL-6 levels (34), improves hypoalbuminemia (35), physical functionality (36), PEW (37), better body composition markers (38), quality of life (39), and reduces mortality (40). However, the evidence of ION compared with nutrition at home is scarce, although this strategy has already been shown to be safe and effective, as recently demonstrated by Ramos-Acevedo et al. (41).

On the other hand, BMI has been reported to be a nutritional status factor that can be maintained regardless of the supplement used during dialysis, as recently reported in a 6-month follow-up clinical trial, comparing consumption of a normal meal vs. a hyper-protein meal intra-dialysis; both groups maintained BMI but not for albumin, where the results were better for the group that received the hyper-protein meal (38).

Martin-Alemañy et al. demonstrated the combined effect of oral supplementation plus aerobic or resistance exercise on nutritional status, and physical functionality (42). Ocepek et al. found that in malnourished patients who had previously received oral supplementation but were not presently receiving them, serum albumin and hand-grip strength tended to worsen, and even for those who were well-nourished, the nutritional markers decreased (43), indicating, as expected, that the effect is not permanent. So, it should be done constantly, including the entire population as a part of a daily practice in HD patients.

A major limitation of our study is our small sample size and its low mean age, which may not reflect the usual on dialysis in most countries; however, this is the first trial that associates sleep quality with the use of oral supplementation. The use of oral supplementation improves nutritional status but it is necessary to perform more RCTs with larger sample sizes to explore the mechanisms that influence the relationship between ION and sleep quality, and is necessary to implement strategies to help compliance for a long time.

A clinical application of this paper is that oral supplementation has been studied in different outcomes, however, studies about sleep outcomes are missing.

In Conclusion, the results of this pilot study support the implementation of oral supplementation, as a strategy to improve nutritional outcomes and that could have an effect on sleep quality.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de ética, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán #2229. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AG-O and SR-A participated in study conception, design the research generation, analysis of the data, and writing the paper. ÁE-C participated in study conception and design, revision, analysis of the data, writing the paper, and approval of the final version of the manuscript. VS-A, GG, RC-R, MV-F, HX, and JJC participated in interpretation of the data and/or critical revision of the manuscript to its final form. All authors read and approved the final manuscript.

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Association between phase angle and coronary artery calcium score in patients on peritoneal dialysis

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Introduction: The phase angle (PhA) has been used as a nutritional marker and predictor of mortality in patients on peritoneal dialysis (PD). The coronary artery calcium (CAC) score has shown to predict the incidence of acute myocardial infarction and death from cardiovascular disease in these patients. However, the association between PhA and CAC score in patients with PD is not well-established, which is the objective of this study.

Materials and methods: Cross-sectional study with patients on PD, followed up at a University Hospital, between March 2018 and August 2019. PhA was evaluated by unifrequency bioimpedance (BIA). The CAC score was calculated based on cardiovascular computed tomography, considering positive when greater than or equal to 100 Agatston and negative when less than 100 Agatston.

Results: We evaluated 44 patients on dialysis, with a mean age of 56 years and median time on dialysis therapy was 11.7 months. In the statistical analysis, a significant association was only observed between the CAC score and the PhA.

Conclusion: The PhA is associated with a positive CAC score in patients with PD, and despite other factors, may be useful as a risk marker for coronary artery disease in this population.

KEYWORDS

bioelectrical impedance, phase angle, coronary artery calcium score, peritoneal dialysis, nutrition

Introduction

It is known that 23–76% of patients on dialysis are malnourished, and 6–8% have severe malnutrition (1). Malnutrition is a risk factor for mortality (2, 3) and its causes are multifactorial, including hemodynamic, hormonal, inflammatory changes, and water overload, leading to protein energy wasting (PEW) (4).

Bioelectrical impedance (BIA) has been used to assess body composition (5–8). Its application is based on the electrical properties of the biological tissue (9, 10), evaluating their conductivity to an alternating electrical current, and has two components: resistance and reactance. The first determines the hydration status of the tissue (11–14) and the second represents the energy reserve of the cell membrane (which indirectly reflects the number of cells) (15). The angle formed by the vector sum of reactance and resistance is called the phase angle (PhA) (11–14), a composite marker influenced by hydration and integrity of the body cell membrane (16) that can be used as an index of nutritional assessment (15).

In patients with end-stage chronic kidney disease (CKD), coronary artery disease (CAD) is a major cause of morbidity and mortality. These patients are usually asymptomatic until the event of acute myocardial infarction (AMI) or sudden cardiac death (17). The evaluation of coronary calcification through coronary artery calcium (CAC) score, measured by computed tomography with multiple detectors, is a marker for atherosclerotic plaque burden and has shown to predict the incidence of AMI and death from cardiovascular disease (18).

In 2017, a study showed that PhA was a predictor of vascular calcification and arterial stiffness in patients on peritoneal dialysis (PD) (16). In 2021, a Chinese study that evaluated patients on hemodialysis found a relationship between PhA and coronary calcification (15). This relationship is not yet well-established, but it seems to involve the malnutrition-inflammation-atherosclerosis syndrome (13, 14, 19).

In this study, our objective was to evaluate the relationship between PhA and coronary calcification in patients with PD.

Materials and methods

This cross-sectional study was approved by our Institutional Ethics and Research Committee (CAAE 80051517.1.0000.5411) and involved patients with CKD on PD of the Clinical Hospital of the Botucatu Medical School-UNESP, between March 2018 and August 2019.

Prevalent PD patients aged between 18 and 75 years, without previous coronary artery disease (CAD) or other overt atherosclerotic disease were included. Individuals with active or recent infections (< 7 days), autoimmune diseases, malignancy, or unstable heart disease (acute coronary syndrome, decompensated heart failure, and unstable arrhythmias) were not included in this study.

The registration of demographic and clinical data and the following complementary tests were performed with a maximum interval of 2 weeks: biochemical tests, nutritional assessment by bioimpedance (BIA), and anthropometry, calculation of dialysis adequacy (Kt/V), blood pressure measurement in upper extremities, ultrasound of carotid arteries, pulse wave velocity (PWV), ankle-brachial index (ABI), and CAC score.

Nutritional assessment

Nutritional status was assessed by uni-and multifrequency bioimpedance (BIA). Unifrequency BIA was performed with a Biodynamic device (model 450) and multifrequency BIA with Fresenius Medical Care device–Body Composition Monitor (BCM) model.

In the unifrequency BIA, the values of PhA, total body water (TBW), intra and extracellular water, fat, and lean mass were considered. These values were determined by the device, and the formulas used to calculate total body water and intracellular water are based on those proposed by Kushner and Schoeller (20) and Cohn et al. (21). In the evaluation of the body composition monitor (BCM), the values of the hyperhydration index (overhydration-OH) were considered. This device measures the electrical response of 50 different types of frequencies from 5 to 1,000 kHz. An OH index > 1.1L were considered volume overload, according to Wizemann et al. (22).

The patient was instructed not to perform physical exercises within 24 h of the examination; to urinate, when with residual renal function, at least 30 min before the exam; not to drink alcohol in the 48 h before the test; and, during the examination, remain in the supine position. BIA measurements were performed with no dialysate in the peritoneal cavity, and with the patient in the supine position on a non-conductive surface.

Coronary artery calcium score

The CAC score calculation was performed after a cardiovascular tomography scan (Multi slice, 64 channels, Optima, GE Medical Systems, Waukesha, WI, United States). Calcification consisted of a hyperattenuating lesion above the threshold of 130 Hounsfield units (HU) in an area of two or more adjacent pixels, observed in the coronary pathway. The product of the total area of calcium by a factor derived from the maximum attenuation (Maximal Computer Tomographic Number) is the calcium score published by Agatston et al. (23) and whose unit bears his name. The reported sensitivity and specificity in detecting this score are 98.7 and 100%, respectively

(24). The images, including their quality and accuracy, were analyzed by a single examiner specializing in cardiovascular tomography, being "blinded" to the patient's clinical, laboratory, and other complementary exam information. CAC score was considered positive when greater than or equal to 100 Agatston and negative when less than 100 Agatston.

Statistical analysis

The sample size was calculated in 40 patients to detect a difference in the proportion of 30% between the groups, divided according to the median of the PhA, considering an error α of 5% and β of 20%.

Statistical analysis was performed using the SPSS version 23.0 (SPSS Inc., Chicago, IL, United States). Data were expressed as frequencies, mean ± *SD* or median and interquartile range, when appropriate. Statistical comparisons between the study groups (PhA $\leq$ 5.5° and $>$ 5.5°) were performed using Student's *t*-test for continuous variables and the chi-square test for categorical variables. Through a multivariate logistic regression model, associations were made between the study variables. The positive and negative predictive values, sensitivity, specificity, and accuracy, between the PhA and the CAC score were analyzed through the ROC curve (Receiver Operating Characteristic) and the calculated area under the ROC curve (AUC). The significance level adopted was $p < 0.05$.

Results

A total of 76 patients were on PD at the inclusion period, and 59 were eligible. Of these, 11 refused to participate. Therefore, 48 patients were included; however, four withdrew their consent in the middle of the study. Thus, 44 patients were analyzed (**Figure 1**).

The median age of patients was 56 years, most of them male, white, and with less than 9 years of schooling. Most patients were hypertensive and dyslipidemic. Patients with lower PhA (≤5.5°) were older (59, 56–68 years vs. 53, 38–62 years), had more diabetes (60 vs. 31%), and lower BMI (26.3 ± 3.3 kg/m^2 vs. 28.7 ± 5.4 kg/m^2), smoked more (13.3 vs. 6.9%) and were more sedentary (80 vs. 65.5%) when compared to the group with the highest PhA. However, none of these

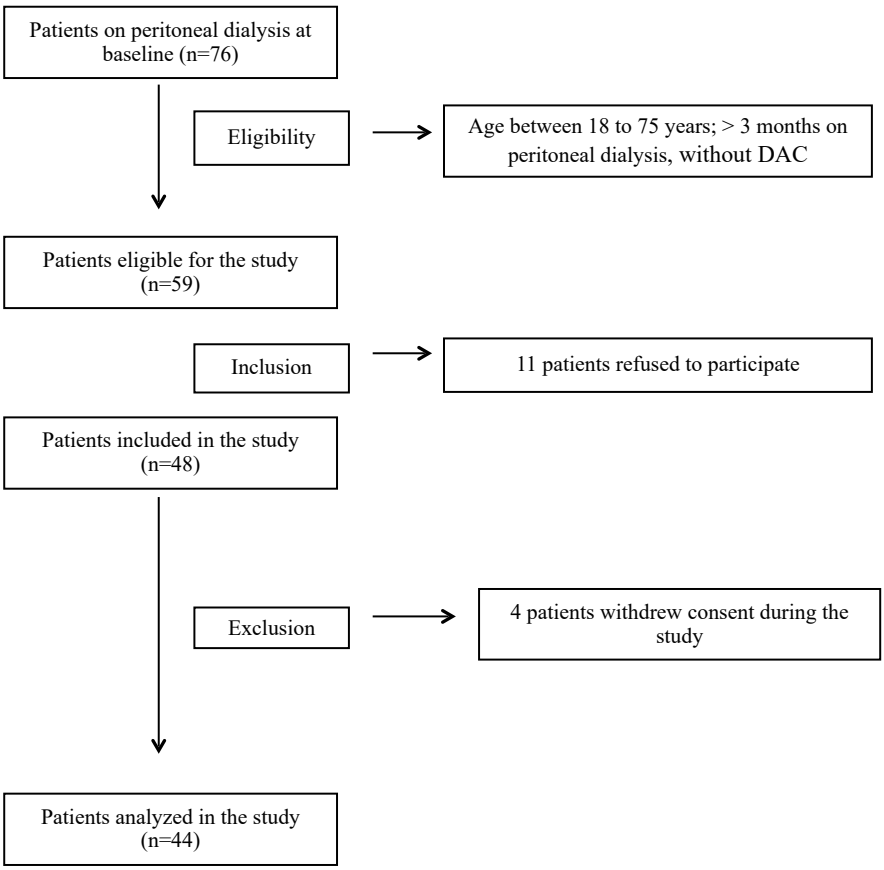

FIGURE 1
Flowchart of inclusion of patients in the study.

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TABLE 1 General characteristics of peritoneal dialysis (PD) patients.

	Total ($n = 44$)	Phase angle ≤5.5° ($n = 15$)	Phase angle >5.5° ($n = 29$)	p
Age (years)	56 (43–65)	59 (56–68)	53 (38–62)	0.393
Gender (% male)	54.5	60.0	51.7	0.752
Ethnic group (% white)	65.9	80.0	58.6	0.160
Diabetes (%)	40.9	60.0	31.0	0.105
Hypertension (%)	81.8	80.0	82.8	0.822
Dyslipidemia (%)	72.7	73.3	72.4	0.948
Body mass index (kg/m^2)	27.5 ± 4.8	26.3 ± 3.3	28.7 ± 5.4	0.234
Family story DAC (%)	22.9	20.0	24.0	0.799
Smoking (%)	9.1	13.3	6.9	0.481
Alcohol consumption (%)	9.1	13.3	6.9	0.481
Physical level (% sedentary)	70.5	80.0	65.5	0.550
Dialysis vintage (months)	11.7 (6.7–23.9)	11.3 (6.9–25.8)	12.6 (6.6–26.2)	0.525
Underlying condition				
Diabetes (%)	27.3	26.7	27.6	0.597
Hypertension (%)	31.8	33.3	31.0	
Glomerulopathies (%)	20.5	13.3	24.1	
Others (%)	20.4	26.7	17.3	
Previous hemodialysis (%)	13.6	13.3	13.8	0.966
Total Kt/V	2.2 (1.7–2.5)	2.8 (1.8–2.5)	2.2 (1.8–2.5)	0.988
Urine output (ml)	1071 ± 686	827 ± 535	1207 ± 732	0.086
Glomerular filtration rate (ml/min/1.73 m^2)	5.5 (2.1–8.0)	3.2 (1.9–7.4)	5.6 (2.8–8.7)	0.525

Data expressed as mean ± standard deviation or median (interquartile range). Student's t-test or chi-square.
DAC, coronary artery disease; Kt/V, dialysis adequacy.

TABLE 2 Bioimpedance parameters of peritoneal dialysis (PD) patients.

	Total ($n = 44$)	Phase angle ≤5.5° ($n = 15$)	Phase angle >5.5° ($n = 29$)	p
Lean mass (%)	70.0 ± 7.2	69.3 ± 7.2	70.4 ± 7.3	0.624
Fat mass (%)	30.0 ± 7.2	30.7 ± 7.2	29.6 ± 7.3	0.624
Intracellular water (%)	53.2 ± 3.6	50.1 ± 2.7	54.8 ± 3.0	<0.001
Extracellular water (%)	46.8 ± 3.6	49.9 ± 2.7	45.2 ± 3.0	<0.001
Total body water (litres)	37.3 ± 8.1	34.6 ± 6.8	38.7 ± 8.5	0.112
Overhydration (litres)	0.5 ± 1.5	1.5 ± 1.5	0.0 ± 1.2	0.001

Data expressed as mean ± standard deviation. Student's t-test or chi-square.

factors showed a statistically significant difference between the groups (Table 1).

Almost a third (31.8%) of patients had hypertension as the underlying disease for CKD, followed by diabetes (27.3%). The median dialysis vintage was 11.7 (6.7–23.9) months. The groups PhA ≤ 5.5° and > 5.5° were similar in terms of these variables, except for the urinary output that was higher in the group with PhA > 5.5 (1,207 vs. 827 ml), almost reaching statistical significance (Table 1).

In the evaluation of unifrequency BIA and OH parameters, patients with PhA ≤ 5.5° presented higher percentages of extracellular water and OH index and a lower percentage of intracellular water. The percentages of lean mass, fat mass, and total body water were similar between the groups (Table 2).

When analyzing the laboratory tests, the groups PhA ≤ 5.5° and > 5.5° were similar (Table 3). As for the atherosclerosis markers, only the percentage of positive CAC score (73.3 vs. 20.7%) showed a significant difference between the PhA ≤ 5.5° and > 5.5° groups (Table 4).

As some variables are known to be associated with PhA, such as age, sex, diabetes, glomerular filtration rate, BMI, hemoglobin, physical activity, calcium x phosphorus, PTH, and ultrasensitive CRP a hierarchical multiple logistic regression was performed to predict positive CAC score. In this evaluation, only PhA remained an independent predictor for positive CAC scores (Table 5).

In the analysis of the ROC curve for the diagnosis of a positive CAC score from the PhA, the area under the curve was.81 (CI: 0.68–0.94; $p < 0.01$). The best cut-off point was with

TABLE 3 Laboratory tests of peritoneal dialysis (PD) patients.

	Total ($n = 44$)	Phase angle ≤5.5° ($n = 15$)	Phase angle > 5.5° ($n = 29$)	p
Hemoglobin (g/dL)	11.7 ± 1.1	11.9 ± 1.1	11.5 ± 1.1	0.249
Urea (mg/dL)	103.6 ± 24.9	106.6 ± 26.2	102.2 ± 21.4	0.550
Creatinine (mg/dL)	9.3 ± 3.1	8.3 ± 2.2	9.3 ± 3.3	0.285
Corrected calcium (mg/dL)	9.2 ± 0.7	8.9 ± 0.8	9.3 ± 0.7	0.078
Magnesium (mmol/L)	2.0 ± 0.3	2.0 ± 0.3	2.0 ± 0.3	0.749
Phosphorus (mg/dL)	5.3 ± 1.1	5.1 ± 1.2	5.5 ± 1.2	0.243
Calcium × Phosphorus (mg^2/dL^2)	48.9 ± 10.5	44.7 ± 9.9	51.1 ± 10.3	0.057
HDL (mg/dL)	36.5 (30.7–43.2)	33 (30–38)	36 (32–53)	1.000
LDL (mg/dL)	73.7 ± 29.3	85.2 ± 30.0	77.0 ± 41.7	0.504
Triglycerides (mg/dL)	153.5 (117.0–210.7)	208 (118–240)	159 (124–287)	0.525
Total cholesterol (mg/dL)	147.7 ± 34.6	160.4 ± 34.1	154.7 ± 49.9	0.693
Glycosylated hemoglobin (%)	5.4 (5.1–6.3)	6.2 (5.5–6.6)	5.2 (5.0–5.9)	0.828
Albumin (g/dL)	3.7 ± 0.4	3.6 ± 0.5	3.7 ± 0.3	0.438
Uric acid (mg/dL)	6.0 ± 1.3	5.7 ± 1.2	6.4 ± 1.0	0.115
Alkaline phosphatase (U/L)	76.0 (68.7–127.2)	76 (67–143)	72 (47–96)	0.525
PTH (mg/dL)	241.0 (164.5–356.0)	311 (145–377)	201 (171–371)	0.326
Vitamin D (ng/mL)	25.8 (20.0–31.1)	25.7 (21.8–31.9)	25.3 (16.4–29.3)	0.897
High-sensitivity troponin (ng/L)	7.5 (2.3–17.1)	11.5 (7.2–16.2)	2.9 (1.8–16.6)	0.056
NT-Pro-BNP (pg/mL)	291 (205–411)	299 (146–539)	283 (182–678)	1.000
Interleukin 6 (pg/mL)	14.6 (8.5–27.9)	13.9 (5.3–16.2)	25.8 (9.6–37.7)	0.659
TNF-α (pg/mL)	4.6 (0.6–13.6)	3.5 (0.0–14.6)	8.5 (4.9–18.9)	0.970
Ultrasensitive CRP (mg/L)	1.9 (0.5–5.1)	3.4 (0.9–9.1)	1.2 (0.0–4.9)	0.743

Data expressed as mean ± standard deviation or median (interquartile range). Student's *t*-test or Mann-Whitney.
HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-Pro-BNP, N-terminal prohormone B-type natriuretic peptide; PTH, parathyroid hormone; CRP, c-reactive protein; TNFα, tumor necrosis factor-alpha.

TABLE 4 Atherosclerosis markers of peritoneal dialysis (PD) patients.

	Total ($n = 44$)	Phase angle ≤5.5° ($n = 15$)	Phase angle >5.5° ($n = 29$)	p
CAC score positive	38.6	73.3	20.7	0.001
VOP femoral (m/s)[a]	10.5 ± 4.0	11.1 ± 4.4	9.2 ± 3.5	0.166
Brachial ankle index	1.0 ± 0.2	1.1 ± 0.2	1.0 ± 0.1	0.573
Difference PAS-MMSS	15.9	20.0	13.8	0.594
EMIC left (cm)[b]	0.7 (0.6–0.8)	0.7 (0.6–1.0)	0.7 (0.6–0.7)	0.344
EMIC right (cm)[b]	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	0.106
Carotid plaque left[b]	36.1	54.5	28.0	0.127
Carotid plaque right[b]	44.4	63.6	36.0	0.124

Data expressed as mean ± standard deviation or median (interquartile range). Student's *t*-test or chi-square.
[a]n total = 36; n Pha > 5.5° = 23; n PhA ≤ 5.5° = 13.
[b]n total = 36; n PhA > 5.5° = 25; n PhA ≤ 5.5° = 11.
CAC, coronary artery calcium; EMIC, average carotid intimal thickness; PAS, systolic blood pressure; VOP, pulse wave speed.

PhA ≤ 5.5°, which showed a sensitivity of 64.7% and a specificity of 85.2%. Also, when the PhA was ≤ 7.2°, the sensitivity was 100% (Figure 2).

Discussion

This study showed that there is an inverse correlation between the PhA and CAC score in patients on PD, even after adjusting for several variables such as age, sex, diabetes, glomerular filtration rate, BMI, hemoglobin, physical activity, calcium x phosphorus, PTH, and ultrasensitive CRP.

The best cut-off point was with a PhA ≤ 5.5°, which had a sensitivity of 64.7% and a specificity of 85.2% to predict a positive CAC score. Also, when the PhA was ≤ 7.2°, the sensitivity was 100%.

A recent study (15) found the same result in hemodialysis patients. Sarmento-Dias et al. (16) studying patients with

TABLE 5 Hierarchical multiple logistic regression with some variables related to the phase angle to predict positive ECAC.

	Variables	OR	*p*	Confidence interval	
1° step	Age (years)	1.168	0.315	0.862	1.583
	Gender (% male)	2.087	0.689	0.057	76.780
	Diabetes (%)	0.011	0.219	0.000	14.921
	Glomerular filtration rate (ml/min/1,73 m^2)	1.204	0.397	0.784	1.848
	Body mass index (kg/m^2)	0.708	0.470	0.278	1.806
	Hemoglobin (g/dL)	0.672	0.695	0.093	4.877
	Physical level (% sedentary)	2.888	0.835	0.000	62705.095
	Calcium × Phosphorus (mg^2/dL^2)	1.151	0.331	0.866	1.529
	PTH (mg/dL)	1.008	0.286	0.993	1.023
	Ultrasensitive CRP (mg/L)	2.079	0.940	0.882	4.900
	Phase angle (°)	0.007	0.013	0.000	1.510
7° step	PTH (mg/dL)	1.010	0.060	1.000	1.020
	Ultrasensitive CRP (mg/L)	1.502	0.076	0.959	2.353
	Age (years)	1.096	0.102	0.982	1.224
	Diabetes (%)	0.078	0.081	0.004	1.367
	Phase angle (°)	0.055	0.008	0.007	0.462

Hierarchical multiple logistic regression. Model: Age, Gender, DM, Glomerular filtration rate, BMI, Hemoglobin, Physical level, Calcium × Phosphorus, PTH, Ultrasensitive CRP, and Phase angle.
DM, diabetes; BMI, body mass index; OR, odds ratio.

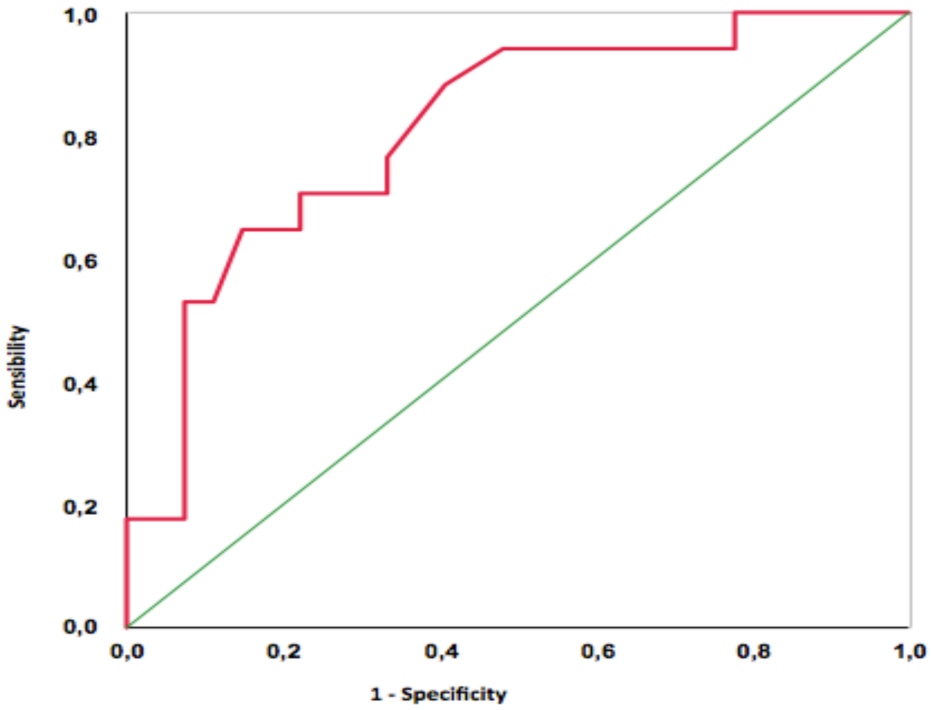

FIGURE 2
ROC curve for diagnosis of positive CAC score from the phase angle information.

PD showed that the PhA predicts arterial stiffness and vascular calcification. However, we did not find any study in the literature relating the phase angle with coronary calcification in PD patients.

One of the hypotheses for the relationship between PhA and CAC score is that the first reflects the nutritional status (25) and malnutrition plays an important role in the development of cardiovascular diseases, due to the

malnutrition-inflammation-atherosclerosis syndrome (26). Saitoh et al. (27) showed a correlation positive between the PhA with the percentage of lean mass and BMI, and negative with protein-energy malnutrition. Leal-Alegre et al. (28) found 29% of PEW in patients with vascular calcification undergoing PD. In 2021 (15), a study showed that the nutritional status of hemodialysis patients with vascular calcification was worse than those without calcification. In our study, there was no relationship between inflammatory markers (CRP, interleukins, and TNF-alpha) and PhA. However, some authors report that malnutrition may be a risk factor for cardiovascular mortality, independent of inflammation (29), and may have other still unknown mechanisms involved.

Another hypothesis is that the PhA is influenced by the hydration state (16), and the smaller the PhA, the higher the level of extracellular fluid. Excess extracellular fluid (ECF) results in pathological mechanical stimuli in vascular endothelium and smooth muscle cells. Such stimuli release angiotensin II, increase superoxide production, and reduce nitric oxide bioavailability, leading to atherosclerosis, and vascular calcification (30). In this study, higher levels of ECF and OH index showed a direct relationship with lower PhA and positive CAC scores.

The strengths of this study are the multivariate and hierarchical regression analysis for variables that could influence the CAC score in the population studied, and a large number of variables analyzed, enabling a better understanding of the relationship studied. Limitations include the cross-sectional study design, which does not allow establishing a causal relationship between the PhA and CAC score variables. Also, the small sample size, even though just peritoneal dialysis patients without overt atherosclerotic disease were included, and the fact that it was performed in a single center.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the research Ethics Committee of Botucatu Medical School. The patients/participants provided their written informed consent to participate in this study.

Author contributions

FR was responsible for the research idea and study design. FR, FC, and MS performed data acquisition. FR, MS, NR, LM, FC, and CS performed data analysis and interpretation, involved in statistical analysis, and drafted the manuscript. PB, LM, and SB were responsible for supervision and mentorship. All authors provided intellectual content to the work and gave final approval of the version to be published.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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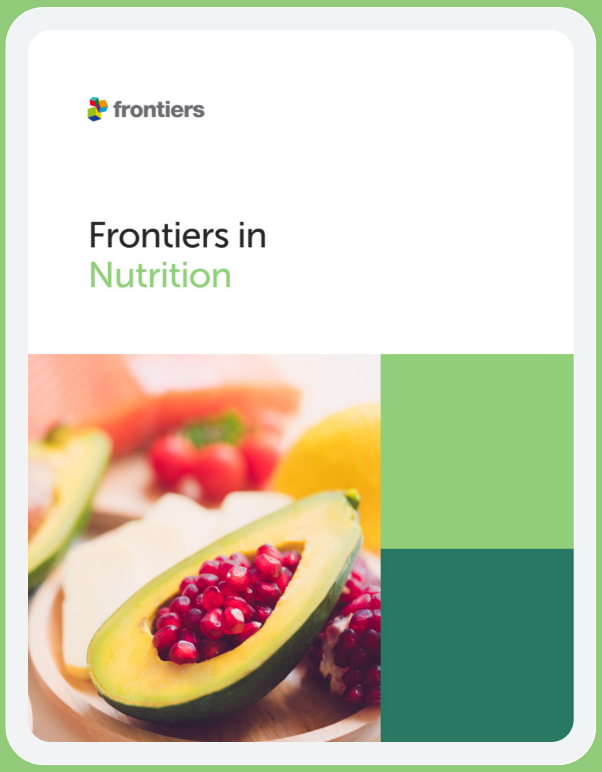

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