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Original Article

ORIGINAL ARTICLE

Comparing bioimpedance spectrometry and traditional creatinine kinetics methods for the assessment of muscle mass in peritoneal dialysis patients

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ABSTRACT

Background. Sarcopenia is a common and serious problem in patients receiving peritoneal dialysis (PD). Lean tissue mass (LTM) by bioimpedance spectrometry is a reasonably accurate method for measuring muscle mass. Fat-free edema-free body mass (FEBM) as determined by the creatinine kinetics method is a traditional method but evidence to support its use is limited.

Methods. We studied 198 new PD patients. Their serial LTM and FEBM were reviewed and compared by the Bland and Altman method. Multi-variable regression model was used to determine factors associated with the disparity between the two methods.

Results. There was a significant but moderate correlation between LTM and FEBM ($r = 0.309$, $P < .0001$). LTM was consistently higher than FEBM, with an average difference 13.98 kg (95% confidence interval −5.90 to 33.86 kg), and the difference strongly correlated with LTM ($r = 0.781$, $P < .0001$). By multivariable linear regression analysis, LTM and residual renal function were independent predictors of the LTM–FEBM difference. Where the measurements were repeated in 12 months, there was no significant correlation between ΔLTM and ΔFEBM ($r = -0.031$, $P = .799$).

Conclusion. There is a significant difference between LTM and FFBM. This discrepancy correlated with LTM and residual renal function, highlighting the limitations of FFBM in assessing skeletal muscle mass.

Keywords: frailty, malnutrition, renal failure, sarcopenia

INTRODUCTION

Sarcopenia—the loss of skeletal muscle mass and function—is a common and serious problem in the elderly population [1]. It is associated with adverse outcomes, including falls, functional decline, frailty and mortality, in the general population [1]. In patients with chronic kidney disease (CKD) or those on dialysis, skeletal muscle mass and function serve as indicators of the nutritional, and low values or

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KEY LEARNING POINTS

What was known:

- Lean tissue mass (LTM) by bioimpedance spectrometry is a reasonably accurate method for measuring muscle mass.
- Fat-free edema-free body mass (FEBM) as determined by the creatine kinetics method is a traditional method but evidence to support its use is limited.

This study adds:

- LTM was consistently higher than FEBM, and the difference strongly correlated with LTM.
- The change in FEBM after 12 months of dialysis did not correlate with the corresponding change in LTM.

Potential impact:

- There is a significant difference between LTM and FFBM.
- Our results suggest that FFBM may not be reliable in assessing skeletal muscle mass.

derangements over time are strong predictors of poor patient outcomes [2–4].

There are several methods able to determine the skeletal muscle mass of patients [4]. However, none of them is ideal. An anthropometric approach is simple, but it has low reliability [5]. Radiological imaging methods are accurate but cannot be applied for routine clinical use [6, 7]. In recent years, lean tissue mass (LTM) as determined by the use of multi-frequency bioimpedance spectrometry has emerged as a reliable and convenient method for the measurement of body compositions, including skeletal muscle mass [8]. In CKD patients, it has been reported that a low LTM is an independent predictor of mortality [9].

Unlike bioimpedance spectroscopy, the fat-free edema-free body mass (FEBM) as determined by the creatinine kinetics method is a traditional mean to assess skeletal muscle mass in dialysis patients [10]. FEBM has been referred to as lean body mass by creatinine kinetics (LBM-CK) in previous studies [11], but the term FEBM was recommended by the Dialysis Outcomes Quality Initiative (DOQI) guidelines [12]. The use of FEBM as a marker of skeletal muscle mass has the distinct advantage that its measurement can be integrated to the routine assessment of dialysis adequacy and does not require any additional test. However, the evidence to support FEBM as a prognostic marker of peritoneal dialysis (PD) patients is scarce, and previous studies showed that FEBM is affected by dietary intake, residual renal function and serum albumin level in hemodialysis patients [13]. There are few studies to investigate the relationship between FEBM and LTM. In the present study, we compared the skeletal muscle mass as measured by LTM and FEBM in a large cohort of PD patients, determined the clinical factors that are associated with the discrepancy between the two methods and explored their role in the serial monitoring of skeletal muscle mass in PD patients.

MATERIALS AND METHODS

The study was approved by the Joint Chinese University Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (approval number CRE-2023.363). All procedures in this study followed the guidelines outlined in the Declaration of Helsinki.

Patient selection

This is a retrospective analysis of a prospective, observational, cohort of 198 consecutive incident adult PD patients in a single center. Patients who were unlikely to survive for 6 months, planned to have living donor kidney transplant or transferal to other renal centers within 6 months were excluded. Multi-frequency bioimpedance spectroscopy, 24-h peritoneal dialysate and urinary collection, and other clinical assessment were performed on the same day around 4 weeks after the patients were stable on PD, and then repeated 12 months later. The procedures of peritoneal dialysate and urine collection followed standardized protocols as described previously [11, 14].

Lean tissue mass and bioimpedance study

LTM was determined by the multi-frequency bioimpedance study by a standardized protocol as previously described [15]. In brief, the right hand and the right foot of the patient were attached with electrodes in a supine position. The Body Composition Monitor (BCM, Fresenius Medical Care, Germany) was then used to measure the LTM. In addition, we also recorded the volume of overhydration, total body water, extracellular water, intracellular water and adipose tissue mass (ATM). The multi-frequency bioimpedance study was performed when the abdomen was full with PD solution. As shown in a previous study, peritoneal dialysate had minimal impacts on the bioimpedance measurements [16].

Fat-free edema-free body mass

FEBM was measured by the traditional creatinine kinetic method by the collection of 24-h urine and dialysate as previously described [11]. Briefly, FEBM was calculated by the following formula:

$$\text{FEBM} = 7.38 + [0.029 \times (\text{CE} + \text{CD})]$$

where CE refers to the creatinine excretion rate, and CD stands for creatinine degradation rate (both in mg/day). CE is calculated by:

$$\text{CE} = (\text{UCO} + \text{DCO}) \times 0.113$$

where UCO refers to the daily urinary creatine output, and DCO refers to the daily dialysate creatinine output (both in mmol/day). CD is calculated by:

$$\text{CD} = 0.38 \times (\text{PC} \times 0.0113) \times \text{body weight}$$

where PC is plasma creatinine concentration (μmol/L).

Table 1: Baseline demographic and clinical characteristics.

No. of patients	198
Sex (male:female)	111:87
Age (years)	59.45 ± 11.36
Height (cm)	162.00 ± 8.76
Body weight (kg)	63.21 ± 13.58
Body mass index (kg/m^2)	23.61 ± 4.26
Blood pressure (mmHg)	
Systolic	135.95 ± 20.90
Diastolic	73.52 ± 12.08
Renal diagnosis, no. of cases (%)	
Diabetic nephropathy	87 (43.94)
Glomerulonephritis	49 (24.75)
Hypertension	22 (11.11)
Urological problem	6 (3.03)
Polycystic kidney disease	11 (5.56)
Others or unknown	16 (8.08)
Major comorbidities, no. of cases (%)	
Diabetes mellitus	105 (53.03)
Coronary artery disease	52 (26.26)
Cerebrovascular accident	43 (21.72)
Peripheral vascular disease	11 (5.56)
Charlson's comorbidity score	6.05 ± 2.69
Type of PD, no. of cases (%)	
Machine-assisted automated PD	39 (19.7)
Low GDP solution	24 (12.12)

Data are presented as mean ± standard deviation or no. (%).
PD, peritoneal dialysis; GDP, glucose degradation products.

Table 2: Baseline bioimpedance and biochemical information.

No. of patients	198
LTM (kg)	39.16 ± 10.09
FEBM (kg)	25.39 ± 6.96
Overhydration (L)	3.71 ± 3.01
ATM (kg)	18.97 ± 10.64
E/I ratio	0.98 ± 0.16
Serum creatinine (μmol/L)	692.9 ± 255.5
Hemoglobin (g/dL)	9.54 ± 1.53
Serum albumin (g/L)	33.77 ± 5.00
Fasting plasma glucose (mmol/L)	5.85 ± 1.68
Lipid profile (mmol/L)	
Total cholesterol	4.94 ± 1.41
LDL cholesterol	2.81 ± 1.19
HDL cholesterol	1.34 ± 0.48
Triglyceride	1.75 ± 1.10
Total weekly Kt/V	2.16 ± 0.62
Residual GFR (mL/min/1.73 m^2)	4.30 ± 2.60
Iron profile	
Plasma iron (μmol/L)	13.76 ± 5.45
Plasma TIBC (μmol/L)	39.57 ± 7.59
Iron saturation (%)	0.36 ± 0.17
Serum ferritin (ng/mL)	1213.73 ± 1108.87

Data are presented as mean ± standard deviation.
E/I ratio, extracellular to intracellular fluid volume ration; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TIBC, total iron binding capacity.

Dialysis adequacy and other nutritional indices

From the same 24-h dialysate and urine collection, we also assess the dialysis adequacy of each patient as previously described [14]. Briefly, we calculated the total weekly Kt/V. Residual glomerular filtration rate (GFR) was calculated as the mean of 24-h urinary urea and creatinine clearances [17]. Serum albumin level was measured by the bromocresol purple method [18]. Other routine laboratory tests including hemoglobin, serum iron and ferritin levels were measured as part of the patients' standard clinical care.

Statistics

Statistical analysis was performed using the software SPSS (version 28.0. IBM Corporation, Armonk, NY, USA) and GraphPad Prism (version 10.1.1, GraphPad Software, CA, USA). All data are expressed as the mean ± standard deviation unless specified. Baseline LTM and FEBM were compared by the modified Bland and Altman method, with the differences between the two measures plotted against LTM, which was taken as the reference value. The Pearson's correlation coefficient was used to explore the association between the difference between LTM and FEBM and other nutritional and biochemical parameters. Similarly, he difference between changes in LTM (ΔLTM) and changes in FEBM (ΔFEBM) after 12 months of PD was analyzed by the modified Bland and Altman method. A P-value $<.05$ was taken as significant. All probabilities were two-tailed.

RESULTS

We reviewed 198 patients. Table 1 summarized their baseline clinical and demographic information; their bioimpedance and biochemical characteristics are summarized in Table 2. LTM was significantly higher for male than female patients (44.32 ± 8.75 vs 32.58 ± 7.54 kg; $P < .0001$). Similarly, FEBM was also slightly but significantly higher for male than female patients (25.86 ± 7.39 vs 24.32 ± 5.53 kg; $P < .0001$).

Agreement between LTM and FEBM

A significant but moderate positive correlation was observed between FEBM and LTM ($r = 0.309$, $P < .0001$) (Fig. 1A). However, LTM was consistently higher than FEBM, with an average difference of 13.98 kg [95% confidence interval (CI) −5.90 to 33.86 kg]. The relation LTM and FEBM is depicted in a modified Bland and Altman plot (Fig. 1B), which showed a strong positive correlation between LTM (taken as the reference measurement in this analysis) and the LTM–FEBM difference ($r = 0.781$, $P < .0001$). The LTM–FEBM difference was significantly higher in male (average 18.5 kg; 95% CI 16.68 to 20.25 kg) than female (average 8.3 kg; 95% CI 6.67 to 9.84 kg) patients ($P < .0001$). However, the correlation between the LTM–FEBM difference and LTM remained significant in both sex when analyzing separately ($r = 0.679$ and $r = 0.731$, respectively, $P < .0001$ for both).

To determine the factors that are associated with the LTM–FEBM difference, uni- and multivariable linear regression models were constructed (Supplementary data, Table S1). Since patient sex had a substantial impact on the body built and LTM–FEBM difference, uni- and multivariable linear regression models were constructed separately for each sex (Table 3). In essence, LTM and residual GFR were independently associated with the LTM–FEBM difference in both male and female patients. LTM–FEBM difference was also associated with age, body weight and total weekly Kt/V in male patients, and adipose tissue mass in female patients.

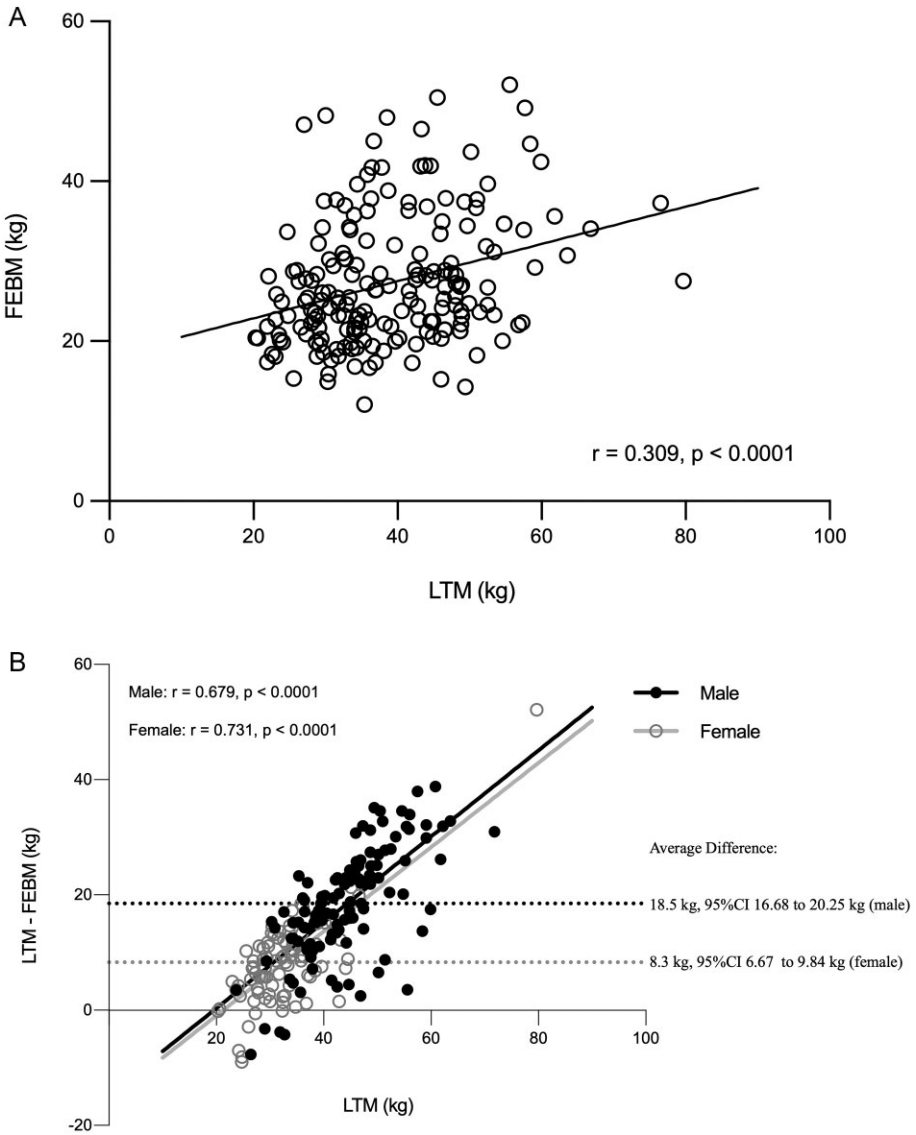

Figure 1: Scatter plot of (**A**) the muscle mass measured by the traditional creatinine kinetics method (FEBM) versus the muscle mass measured by the bioimpedance spectrometry method (LTM); (**B**) the difference between LTM and FEBM versus LTM. Patients were divided into two groups based on their biological sex. Black closed circles refer to males; grey open circles refer to females.

Agreement between serial changes in LTM and FEBM

After 12 ± 2 months, LTM and FEBM tests were repeated in 71 patients. The change in LTM (ΔLTM) was 1.13 ± 7.67 kg for the entire cohort; there was no statistically significant difference in ΔLTM between male and female patients (2.00 ± 8.72 vs −0.20 ± 5.59 kg; $P = .200$). The average change in FEBM (ΔFEBM) was 5.67 ± 5.60 kg for the entire cohort; there was also no significant difference in ΔFEBM between male and female patients (6.23 ± 5.78 vs 4.81 ± 5.30 kg; $P = .300$). The average difference between ΔLTM and ΔFEBM was −4.53 ± 9.64 kg, and there was no significant difference between male and female patients (−4.23 ± 10.77 vs −5.01 ± 7.76 kg; $P = .725$).

ΔLTM was generally lower than ΔFEBM, with an average difference of −4.53 kg (95% CI −6.79 to −2.27 kg). There was no significant correlation between ΔLTM and ΔFEBM ($r = -0.031$, $P = .799$) (Fig. 2A). The relation ΔLTM and ΔFEBM is depicted in a modified Bland and Altman plot (Fig. 2B), which showed a strong positive correlation between ΔLTM (taken as the reference measurement in this analysis) and the ΔLTM–ΔFEBM difference ($r = 0.814$, $P < .0001$). The correlation was similar when male and female patients were analyzed separately ($r = 0.844$ and $r = 0.730$, respectively, $P < .0001$ for both).

To determine the factors that are associated with the ΔLTM–ΔFEBM difference, uni- and multivariable linear regression models were constructed (Table 4). In essence, only changes in LTM were independently associated with the ΔLTM–ΔFEBM difference. No baseline parameters were identified as independent predictors (Supplementary data, Table S2).

DISCUSSION

In this study, we found that there was a substantial discrepancy between LTM determined by bioimpedance spectroscopy and FEBM determined by creatinine kinetics. LTM was consistently

Table 3: Linear regression model on the factors associated with the discrepancy between LTM and FEBM.

	Male					Female				
	Uni-variable		Multi-variable			Uni-variable		Multi-variable		
	B value	*P*-values	B value	95% CI	*P*-values	B value	*P*-values	B value	95% CI	*P*-values
Age	−0.038	.133	0.229	0.116 to 0.298	<.0001	−0.045	.678	0.082	−0.039 to 0.152	.513
Height	0.155	.104				0.152	.160			
Weight	0.202	.034	−2.073	−3.146 to −0.020	.047	0.134	.216			
Systolic BP	0.061	.523				0.000	.997			
Diastolic BP	0.089	.350				−0.016	.883			
Charlson's score	−0.055	.567				−0.033	.760			
Lean tissue mass	0.679	<.0001	2.212	0.656 to 4.301	.008	0.731	<.0001	0.677	0.491 to 0.779	<.0001
Overhydration	−0.051	.595				0.252	.019	0.074	−0.283 to 0.782	.351
Adipose tissue mass	−0.304	.001	1.284	−0.204 to 2.743	.091	−0.385	.000	−0.355	−0.353 to −0.108	.0004
E/I ratio	−0.345	<.0001	0.500	−4.691 to 65.397	.089	−0.159	.141			
Hemoglobin	0.147	.134				−0.084	.443			
Serum albumin	0.249	.012	0.024	−0.143 to 0.233	.637	−0.044	.692			
FPG	−0.092	.435				0.075	.550			
Total cholesterol	0.007	.950				−0.119	.346			
LDL cholesterol	−0.005	.968				−0.156	.216			
HDL cholesterol	−0.077	.515				0.219	.079	−0.052	−1.397 to 3.025	.462
Triglyceride	0.053	.651				−0.143	.256			
Total weekly Kt/V	0.345	<.0001	−0.247	−6.348 to −1.228	.004	0.197	.067	−0.129	−5.126 to 1.872	.354
Residual GFR	0.603	<.0001	0.677	1.666 to 2.827	<.0001	0.354	.001	0.469	0.714 to 2.852	.002
Plasma iron	−0.033	.784				−0.082	.521			
Plasma TIBC	−0.003	.978				0.212	.095	0.044	−0.067 to 0.391	.513
Iron Saturation	−0.082	.491				−0.158	.218			
Serum Ferritin	−0.084	.497				−0.206	.115			

CI, confidence interval; BP, blood pressure; E/I ratio, extracellular to intracellular fluid volume ration; FPG, fasting plasma glucose; LDL, low density lipoprotein; HDL, high density lipoprotein; GFR, glomerular filtration rate; TIBC, total iron binding capacity.

higher than FEBM. The discrepancy between LTM and FEBM was significantly higher in male patients and positively correlated with LTM and residual renal function, suggesting that FEBM is not reliable in patients with a higher skeletal muscle mass. Similarly, there was also a substantial discrepancy between the changes in LTM and FEBM after 1 year on PD, indicating that FEBM may not be a satisfactory parameter for the monitoring of skeletal muscle mass in longitudinal studies.

Assessment of skeletal muscle mass and detection of sarcopenia is important for the management of PD patients. In recent years, LTM as determined by multi-frequency bioimpedance spectrometry has been increasingly used for such a purpose [8, 19]. LTM has been found to be an independent predictor of mortality in CKD [9], while lean tissue index predicted survival in PD patients [20]. However, the measurement of LTM requires specific equipment that needs careful calibration. The use of FEBM, as determined by the creatinine kinetic method, is convenient and can be integrated into the routine assessment of dialysis adequacy without any additional cost. Unfortunately, although FEBM was recommended by the DOQI guidelines [12], its accuracy and clinical application have not been well studied.

In the present study, we compared muscle mass measurements using two methods, LTM and FEBM, in PD patients. Consistent with the general clinical impression, FEBM was higher in male than female patients, reflecting more skeletal muscle mass in men. The absolute FEBM values of our present study (25.9 and 24.3 kg for male and female, respectively) appear to be lower than in our previous report [11], probably because we recruited more elderly patients in our present cohort. Similar to the previous report of Arkouche *et al.* [21], we found a significant discrepancy between the two measurements, indicating a potential overestimation by the bioimpedance spectroscopy method or underestimation by the traditional method. The study by Yoowannakul and Davenport [22] also showed that muscle mass was lower when measured by creatinine kinetics than bioimpedance, and the prevalence of muscle wasting was much greater when the former method was used. Despite concerns about overhydration impacting LTM measurements, our findings did not support this claim. Taken together, there is good evidence that muscle mass is underestimated by the creatinine kinetic method, and a separate set of cut-off values will be required for the diagnosis of sarcopenia as determined by FEBM.

In the present study, the LTM determined by bioimpedance spectroscopy was considered as the reference measurement of muscle mass because the use of bioimpedance spectroscopy for the measurement of muscle mass is a long-established method [23], and both our present data and previous research showed that LTM measured by this method was not affected by the hydration status [24]. Recent research also showed that bioimpedance spectroscopy aligns well with dual-energy X-ray absorptiometry [25], which is another widely accepted standard for assessing muscle mass [6, 7]. More importantly, both baseline LTM and its longitudinal change are independent predictors of patients' all-cause mortality rate [19, 26]. Although FEBM by the creatine-based method is also an independent predictor of patient mortality and hospitalization rate [21], this parameter is more susceptible to errors due to factors like dietary habits, ethnicity, sex and coexisting inflammatory diseases, all of which may affect creatine production [27].

In this study, we used a multi-variable regression analysis to identify factors contributing to the difference between LTM and FEBM, as well as the changes (Δ) in these measurements. Our

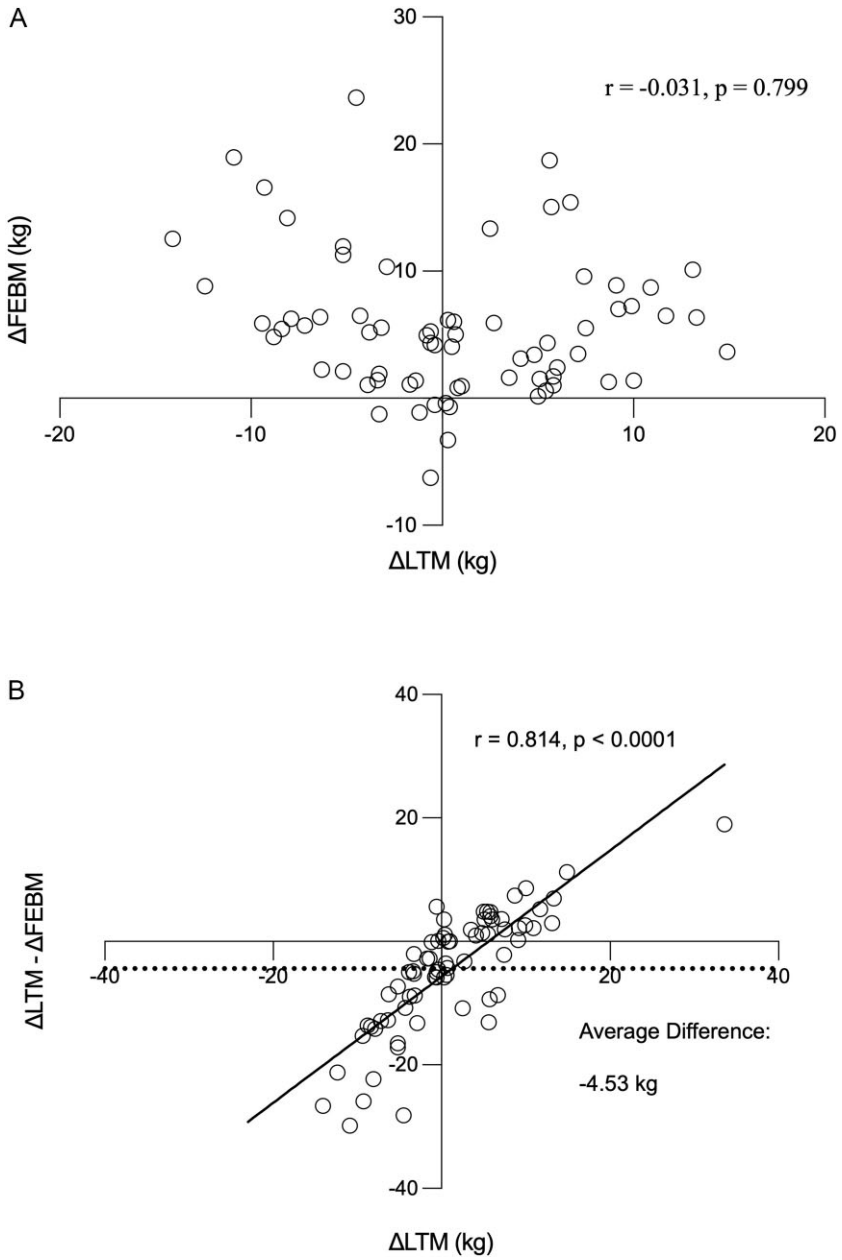

Figure 2: Scatter plot of (**A**) the change in muscle mass measured by the traditional creatinine kinetics method in 1 year since the first test (ΔFEBM) versus the change in muscle mass measured by the bioimpedance spectrometry method (ΔLTM) in 1 year; (**B**) the difference between ΔLTM and ΔFEBM versus ΔLTM.

analysis pinpointed the importance of residual GFR as the primary factor amplifying the disparity between LTM and FEBM, irrespective of sex. This aligns with prior research indicating that patients with higher residual GFR tended to exhibit greater differences in lean body mass determined by the anthropometric and creatine kinetic methods (the latter is equivalent to FEBM) [11]. In essence, the presence of residual renal function may lead to underestimation of muscle mass by FEBM. A previous study also showed that measured-to-predicted creatinine generation ratio increases with time and decline in residual renal function in PD, suggesting that creatinine metabolism or its non-renal excretion changes with time on PD [28]. In our present study, sex-specific analysis revealed that while neither LTM nor ATM independently predicted the LTM–FEBM difference in our regression model, both were significant predictors for female patients, while only LTM remained significant for males, probably because of the potential collinearity issues between LTM and sex. Regarding the discrepancy between ΔLTM and ΔFEBM, only ΔLTM emerged as the independent predictor positively associated with the difference. Furthermore, we found no significant correlation between ΔLTM and ΔFEBM, highlighting the inconsistent assessment of muscle mass by the two methods. Another important observation on the ΔLTM and ΔFEBM over 12 months was that the variation of ΔLTM was marginally higher than ΔFEBM (their standard deviations were 7.67 and 5.60 kg, respectively). This finding suggests that FEBM may be more reliable than LTM for the assessment of skeletal muscle mass.

Table 4: Linear regression model on the factors associated with the difference between changes in LTM and FEBM after 1 year of peritoneal dialysis.

	Uni-variable		Multi-variable		
	B value	P-values	B value	95% CI	P-values
Sex	0.040	.742			
Age	0.007	.953			
Height	−0.024	.844			
ΔWeight	0.348	.003	−1.856	−4.681 to 0.673	.135
ΔSystolic BP	0.053	.659			
ΔDiastolic BP	0.099	.412			
Charlson's score	−0.009	.942			
Baseline lean tissue mass	−0.404	<.0001	−0.126	−0.333 to 0.060	.164
ΔLean tissue mass	0.814	<.0001	2.623	0.280 to 6.210	.033
ΔOverhydration	−0.074	.540			
ΔAdipose tissue mass	−0.322	.007	2.257	−0.452 to 4.541	.104
ΔE/I ratio	−0.439	<.0001	0.523	−18.037 to 79.035	.207
ΔHaemoglobin	0.144	.245			
ΔSerum albumin	−0.088	.477			
ΔFPG	0.032	.845			
ΔTotal cholesterol	0.082	.616			
ΔLDL cholesterol	0.033	.841			
ΔHDL cholesterol	0.133	.421			
ΔTriglyceride	0.096	.554			
ΔTotal weekly Kt/V	0.107	.376			
ΔResidual GFR	0.281	.021	0.077	−0.491 to 1.153	.414
ΔPlasma iron	0.186	.238			
ΔPlasma TIBC	0.117	.462			
ΔIron saturation	0.085	.593			
ΔSerum ferritin	−0.333	.047	−0.183	−0.005 to 0.0005	.106

CI, confidence interval; BP, blood pressure; E/I ratio, extracellular to intracellular fluid volume ration; FPG, fasting plasma glucose; LDL, low density lipoprotein; HDL, high density lipoprotein; GFR, glomerular filtration rate; TIBC, total iron binding capacity.

Our study has several limitations. First, despite a reasonable sample size for the baseline study, a considerable proportion had missing data in residual GFR, hemoglobin level or blood iron profile, resulting in a smaller effective sample size for the multivariable regression model. The sample size for the analysis of ΔLTM and ΔFEBM was even smaller, reducing the reliability of our findings. Although we have demonstrated the inconsistency between LTM and FEBM, cautioning against their interchangeable use for the assessment of skeletal muscle mass, the underlying mechanism for this discrepancy remains uncertain. Further research is necessary to confirm our results and explore the potential molecular mechanisms responsible for the difference between LTM and FEBM.

In summary, our study revealed a significant difference between LTM and FFBM. This discrepancy was more pronounced in male patients and correlated with LTM and residual renal function, highlighting the limitations of FEBM in assessing skeletal muscle mass.

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online.

AUTHORS' CONTRIBUTIONS

Research idea and study design: L.X.X., J.K.-C.N., C.-C.S.; data acquisition: J.K.-C.N., W.W.-S.F., G.C.-K.C.; data analysis/interpretation: L.X.X., J.K.-C.N., C.-C.S.; statistical analysis: L.X.X., J.K.-C.N., C.-C.S.; supervision or mentorship: K.M.C., C.-C.S.; manuscript preparation: L.X.X., C.-C.S. Each author contributed important intellectual content during manuscript drafting or revision and 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.

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DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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