*Title Page (with author details)

Differences between measured total nitrogen losses in spent peritoneal dialysate effluent and estimated nitrogen losses

[1]Surachet Vongsanim MD, [2]Clara Salame MSc, [3]Simon Eaton PhD, [4]George Grimble PhD [2]Andrew Davenport FRCP

[1]Renal Division,Department of Internal Medicine, Chiang Mai University Hospital, Suthep Road, Chiang Mai,Thailand
[2]UCL Centre for Nephrology, Royal Free Hospital, University College London, Rowland Hill Street, London NW3 2PF
[3]UCL Great Ormond Street, Institute of Child Health, Development Biology & Cancer Programme, University College London, Great Ormond Street Institute of Child Health London WC1N 1EH
[4]UCL Institute for Liver and Digestive Health, Division of Medicine, University College London, London WC1E 6BT

Address for correspondence

Surachet Vongsanim	surachet.vongsanim@nhs.net
Clara Salame	csalame10@gmail.com
Simon Eaton	s.eaton@ucl.ac.uk
George Grimble	g.grimble@ucl.ac.uk
Andrew Davenport	andrewdavenport@nhs.net

contact andrewdavenport@nhs.net
UCL Centre for Nephrology,Royal Free Hospital, University College London, Rowland Hill Street, London NW3 2PF
tel 44-2074726457
fax 44-2073178591

Funding: Univeristy College London

short title nitrogen losses in peritoneal dialysate effluent

key words peritoneal dialysis muscle protein nitrogen nutrition bioimpedance nitrogen

word count	abstract	248
	body	21802
	figures	5
	Tables	1
	references	23

The authors have no conflicts of interest

Formatted: Font color: Red
Formatted: Font color: Red
Formatted: Font color: Red

*Manuscript (without author identifiers)
Click here to view linked References

Differences between measured total nitrogen losses in spent peritoneal dialysate effluent and estimated nitrogen losses

Abstract

Objective

Kidney dialysis patients treated by peritoneal dialysis (PD) are at increased risk of muscle wasting and clinical guidelines recommend assessing dietary intake, by calculating protein equivalent of nitrogen appearance (PNA) to assure protein sufficiency. The PNA equations were developed many years ago, and we wished to re-evaluate them by comparing estimated and measured peritoneal nitrogen losses.

Design

Cross sectional observational cohort study

Setting

Outpatient peritoneal dialysis centre of a University Hospital

Subjects

67 peritoneal dialysis patients, 61.2% male, median age 67.3 (53.2-79.4) years

Intervention

Measurements of the nitrogen content of 24 hour spent peritoneal dialysate, by automated chemiluminescence analyser compared to estimates of nitrogen losses based on dialysate urea loss using the Bergström, Randerson and Blumenkrantz equations.

Results

Measured total dialysate nitrogen was more than urea nitrogen equivalent, 5.79±4.07 vs 2.66±1.67 g/day (p<0.001). Each equation has an inflation factor to compensate for non-urea protein losses, however measured nitrogen loss was 27.7 (15.5-59.6) vs Bergström 16.5 (9.8-27.1), Randerson 16.4 (9.8-27.3) and Blumenkrantz 12.9 (7.9-25.4) g/day, p<0.001. Bland Altman analysis demonstrated systematic bias with increasing under-estimation by these equations with increasing measured nitrogen losses (r=0.74, p<0.001).

Conclusion

Our findings demonstrate that at higher protein losses, the currently used predictive equations underestimate the amount lost. It is important to attempt to compensate iatrogenic protein loss by recommending the appropriate intake of dietary protein to patients, in an attempt to minimise muscle wasting. This discrepancy may have arisen because of the characteristics of newer PD prescriptions and change in patient demographics. We propose a new equation PNA g/day = 0.31 x (urea loss mmol) + 7.17, which will require prospective validation in additional studies.

Introduction

Patients with chronic kidney disease (CKD) are at increased risk of losing muscle mass. The reasons for this are many [1] and include episodes of metabolic acidosis during progression of CKD which switch on the proteasome

pathway of muscle protein breakdown, low protein diets which aim to slow CKD progression [2], bone and mineral disorders and background inflammation which, itself, promotes muscle loss [3].In view of this, clinical guidelines recommend the regular assessment of dietary protein intake, with nutritional targets designed to reduce muscle breakdown and loss [4,5]. Assuming that a patient with CKD is not losing muscle mass if they are in neutral protein balance, then a useful laboratory measure of this is nitrogen balance, which is the difference between protein (i.e. nitrogen) intake and nitrogen losses. The protein equivalent of nitrogen appearance (PNA) has been traditionally estimated from combined urinary and peritoneal urea losses and it is assumed that urea represents a constant proportion of total nitrogen excreted [6]. In reality this is not always the case because nitrogen excretion switches between urea and ammonia, depending on fluctuating acid-base balance [7]. Thus, a relative increase in the proportion of nitrogen excreted as ammonia may be undetected, and urea excretion will yield an underestimate of nitrogen excretion. This would represent a false positive nitrogen balance which may mask concomitant muscle protein loss.

Despite this, three equations are currently used in clinical practice, all derived from small studies in peritoneal dialysis (PD) patients treated by continuous ambulatory peritoneal dialysis (CAPD) [8-10], with the largest study based on 23 measurements from 12 patients [8]. In those studies, the equations were derived from correlation of urea excretion and of total nitrogen excretion measured by the Kjeldahl method. This technique may not account for all

forms of nitrogen resistant to acid conversion to ammonia. In contrast, automated chemiluminescence analysis is superior because all forms of nitrogen are converted to nitric oxide, during pyrolysis at 1100°C [11]. An added advantage is that liquid samples require only simple dilution and can be analysed in duplicate within 4 minutes.

As patient demographics and PD prescriptions have changed over time we wished to determine whether there were differences in determining PNA using these equations, by measuring the excretion of nitrogen in urine and peritoneal dialysate effluent in the form of either urea or total nitrogen. In effect we have repeated the original studies [6,8,9] but with patients undergoing modern renal therapies.

Methods

We measured urea in twenty-four hour collections of spent dialysate effluent [1,2], using a standard laboratory analyser (P module analyzer, Roche Integra, Roche diagnostics, Lewes, UK [12]), and total nitrogen by chemiluminescence. In brief samples are pyrolysed at 1100°C in a mixture of oxygen and argon to quantitatively convert all nitrogen compounds to nitric oxide, and then reacted with ozone. Reaction with ozone yields nitrogen dioxide in which an electron relaxes to ground state emitting a photon. This chemiluminescence is measured by a photomultiplier tube [11,13]. Peritoneal dialysate protein was measured using pyrogallol red-molybdate (PRM) (Hitachi 726 auto analyser, Maidenhead UK). This method is linear up to 2.14 g/L, and

higher concentration samples were diluted to bring them into range [12]. C-reactive protein (CRP) was measured using the same assay as the UK National Amlyoid centre, with values reported down to < 1 mg/L, and haemoglobin was measured by an automated counter (Sysmex XN900, Sysmex Corporation, Kobe, Japan) [14]. Patients used standard lactate containing glucose dialysates (Baxter Health Care, Deerfield, Illinois, USA). In addition to 13.6 g/dL dextrose, 31 (59.6%) used 22.7 g/dL dextrose, median 4.0 (0-7.75) L/day, and 42 (80.8%) used 1.5 (0.6-2.0) L/day 7.5% icodextrin. Body composition was measured by multifrequency bioelectrical impedance (MFBIA) (InBody 720, Seoul, South Korea) [15]. Patients had not had peritonitis or other infections, or hospital admissions within the previous 8 weeks. MFBIA [16] was measured in a previously reported standardised manner; first patients were asked to empty the bladder and then peritoneal dialysate was drained out, as ascites and peritoneal dialysate can potentially alter bioimpedance derived body composition measurements [17]. Patients with amputations, pregnancy, and those with limb paralysis were excluded. Patient demographics and ethnicity were obtained from the Hospital patient records.

PNA was calculated using the following equations [4,5];

Bergström [6]

20.1 + 7.50 Urea Nitrogen Appearance g/24 hours

Randerson [8],

15.7 + 7.47 Urea Nitrogen Appearance g/24 hours

and Blumenkrantz [9]

34.6 + 5.86 Urea Nitrogen Appearance g/24 hours

Dialysate urea nitrogen was determined by adjusting for the nitrogen content of urea; 1 mole urea being equivalent to 28g nitrogen.

This project was registered with the UK Integrated Research Application System (IRAS) reference number 191812/893749/14/564 was approved by the National Research Ethics (Manchester) and the Hospital Research and Development Service and complied with NHS guidelines (UK NHS guidelines for clinical audit and service development). Individual consent was waived as only waste samples were analysed. In keeping with the Hospital Trust policy no patient identifiable data was used.

Statistical analysis

Data is presented as mean ± standard deviation, median (interquartile range), or percentage. Standard statistical tests; Wilcoxon rank sum pair test Anova, or Kruskal Wallis) with appropriate post hoc corrections made for multiple testing (Tukey or Dunn), where appropriate. Bland Altman analysis was used to determine agreement between methods. Statistical analysis used Prism 7.0 (Graph Pad, San Diego, USA) and SPSS 24 (IBM, Armonk, New York, USA). Statistical significance was taken as $p<0.05$.

Results

We measured urea and total nitrogen in 67 PD outpatients attending for the assessment of peritoneal membrane function, and dialysis adequacy (Table 1). The median number of peritoneal dialysate exchanges prescribed was 5.5 (4.0-6.0)/day with a median peritoneal dwell volume of 2.0 (2.0-2.0) L. Total nitrogen (measured by chemiluminescence) was greater than that for the urea nitrogen equivalent, 5.79±4.07 vs 2.66±1.67 g/day although there was a strong linear relationship between the two (Figure 1). The difference represents the non-urea nitrogen losses (e.g. ammonia, protein), which are approximately 55% of total nitrogen loss.

Each of the three equations converts urea nitrogen in spent dialysate effluent to total nitrogen on the basis of the regression equation derived from previous studies [6,8,9]. In each equation, the intercept and slope of the relationship between urea nitrogen and total nitrogen (which includes other nitrogen losses in the dialysate – an "inflation factor") differed. We then compared total nitrogen lost in the spent dialysates and the urea nitrogen equivalent adjusted by the individual inflation factor. For all three equations there was a positive correlation with total nitrogen losses, r=074, p<0.001. Bland-Altman analysis revealed a systematic bias for estimated total nitrogen when compared to measured total peritoneal nitrogen losses. At lower urea losses, total nitrogen loss was overestimated, whilst at higher peritoneal urea losses, total nitrogen loss was underestimated (Figures 2-4). The difference between measured total nitrogen and that estimated by each of the three equations differed significantly (Bergström -12.5 (-30.8 -4.1), Randerson –12.5 (-

30.8 -), Blumenkrantz – 16.2 (-36.6 -6.5), p<0.001). There was no correlation between this difference in peritoneal nitrogen loss and either serum urea (r=0.11, p=0.41), or total protein (r=0.06, p=0.66), but the difference between measured nitrogen losses and estimated losses increased with increasing total peritoneal dialysate protein loss (r=0.37, p=0.004), total drained dialysate volume (r=0.34, p=0.025), and greater muscle mass (r=0.28, p=0.026), but not body weight (r=0.13,p=0.29) or body fat mass (r=0.14, p=0.28).

Using our own data then the regression equation between PNA and daily peritoneal urea losses was PNA g/day = 0.31 (urea loss mmol) + 7.17. On Bland Altman analysis, this equation did not have the systematic bias of under estimating protein losses when patients had higher losses (Figure 5).

Discussion

Current clinical guideline recommend that PD patients should increase their dietary protein intake compared to those with chronic kidney disease to compensate for peritoneal protein losses [4,5]. These patients are potentially at increased risk of muscle wasting [1,2], and muscle wasting is an established risk factor for increased mortality [18]. Dietary protein intake is an important factor in maintaining muscle mass in the face of inflammation and other factors which stimulate net muscle protein breakdown. This is because amino acids released from skeletal muscle are either incorporated into acute phase proteins synthesised in the liver or oxidised for energy production with excretion of the amino group as urea or ammonia. In this situation, even though net muscle

protein breakdown is increased, the hope is that an adequate protein intake will provide substrate for muscle protein synthesis. Whereas the body has fat stores which can act as an energy store, the body holds no such equivalent store of protein which can provide amino acids and as such dietary protein intake is essential to prevent muscle loss.

Clinical guidelines recommend that PD patients should be assessed for dietary protein intake [4,5], and although dietary records and patient recall can be used, most dialysis centres use protein nitrogen accumulation (PNA) in clinical practice. PNA can be estimated by a number of equations [4-6], based on urea losses in spent peritoneal dialysate and urine, with an inflation factor to account for losses of proteins, small peptides and amino acids, and then a correction factor for other protein losses, including those from the gastrointestinal tract, and loss of skin, estimated to average around 1.3 g/day, although faecal losses may vary between 0.52-2.8g/day [6,8,10].

As discussed, the studies on which the equations for calculating PNA are based come from a limited number of small studies of patients treated by continuous ambulatory peritoneal dialysis (CAPD) [8,9,10]. Previous reports have shown that there are differences between the three most commonly used equations, and there is no universal consensus as to whether any one of these equations has superiority [6]. We found that all three equations under-estimated measured nitrogen losses with increasing losses, with the difference increasing from 4.4±8.5 to those with a PNA < 40 g/day to 33.9±13.3 g/day for those with a PNA > 40 g/day. This difference was greater with increasing volume

213 of drained peritoneal dialysate. We propose some explanations for this
214 discrepancy. Whereas the equations used to estimate PNA were derived from
215 studies using CAPD patients, who used between 6-8 litres of dialysate, the trend
216 in Europe and North America now is to use automated peritoneal dialysis cyclers,
217 using much higher volumes of dialysate, and in our study almost two thirds of
218 patients were treated by APD cyclers. In addition, the difference between
219 measured and estimated nitrogen losses was greater with increasing dialysate
220 protein losses, and although these equations have an inflation factor to
221 compensate for protein losses, this was based on lower dialysate volumes. Total
222 nitrogen includes not only proteins and urea, but also small peptides, nucleotides,
223 amino acids, organic acids, uric acid, nitrates and nitrites. However, even making
224 allowance for some of these compounds (which will be increased in the present
225 peritoneal dialysates) the Bergström, Randerson and Blumenkrantz equations
226 under estimate nitrogen losses with increasing losses, and as such would under
227 estimate nPNA. However, of more concern in patients with the lowest dialysate
228 nitrogen losses, these equations over-estimated losses in a proportion of these
229 patients. This group of patients would have the lowest PNA, and as such be at
230 greater risk of muscle loss due to lower dietary protein intake. From the
231 viewpoint of clinical risk, an overestimate of dietary intake in patients with the
232 lowest PNA may prevent recognition of dietary protein inadequacy or delay a
233 clinical decision to provide additional nutritional support.

234 We re-evaluated the standard equations used to estimate dietary protein
235 intake due to the increasing use of automated peritoneal dialysis in our own

centre. Our study is based on a relatedly small heterogenous patient cohort. We need to prospectively evaluate our equation, as this may well have to be modified for age, gender, ethnicity, physical activity and dietary variations, for example vegans [19,20,21]. As previous reports have demonstrated that age, gender, and ethnicity all potentially have an effect on body composition, especially muscle mass. In addition, energy expenditure also has been shown to have an effect on body composition muscle mass [22,23].

The development of alternative methods to measure nitrogen losses in body fluids has allowed us to re-examine the estimation of PNA, using the three most commonly used equations, in peritoneal dialysis patients. These equations were based on a series of small studies in a generally younger cohort of patients treated by CAPD. Over the course of time there have been changes in patient demographics and PD prescriptions. By measuring total nitrogen, we have found that these equations developed more than 20 years ago may underestimate higher protein losses. We developed an equation from our data that does not have systematic bias, but this will need to be prospectively validated in separate patient cohorts, and it may be that different equations will be required for patients treated by continuous ambulatory peritoneal dialysis and those using cycler PD prescriptions.

Practical application

When estimating dietary protein intake by calculating the nitrogen appearance rate in peritoneal dialysis patients using the Bergstrőm, Randerson or

Blumenkrantz equations, then these patients under estimate protein nitrogen losses in patients with greater protein nitrogen losses, and so under estimate dietary protein intake.

The authors have no conflict of interest

The data presented in this paper has not been previously published in part or full form

References

1. Mitch WE, Remuzzi G. Diets for patients with chronic kidney disease, should we reconsider? BMC.Nephrol. 2016; 17:(1) 80
2. Bellizzi V, Chiodini P, Cupisti A, Viola BF, Pezzotta M, De Nicola L, Minutolo R, Barsotti G, Piccoli GB, Di Iorio B. Very low-protein diet plus ketoacids in chronic kidney disease and risk of death during end-stage renal disease: a historical cohort controlled study. Nephrol Dial Transplant. 2015;30(1):71-7
3. Deger SM, Hung AM, Gamboa JL, Siew ED, Ellis CD, Booker C, Sha F, Li H, Bian A, Stewart TG, Zent R, Mitch WE, Abumrad NN, Ikizler TA. Systemic inflammation is associated with exaggerated skeletal muscle protein catabolism in maintenance haemodialysis patients. JCI Insight. 2017;2(22)
4. Dombros N, Dratwa M, Feriani M, Gokal R, Heimbürger O, Krediet R, Plum J, Rodrigues A, Selgas R, Struijk D, Verger C; EBPG Expert Group on Peritoneal Dialysis. European best practice guidelines for peritoneal dialysis. 8 Nutrition in peritoneal dialysis. Nephrol Dial Transplant. 2005;20 Suppl 9:ix28-ix33
5. NKF-DOQI CLINICAL PRACTICE GUIDELINES FOR PERITONEAL DIALYSIS ADEQUACY. Assessment of Nutritional status. AmJKidDis 2007; 30(3 Suppl 2), S125-9
6. Bergström J, Heimbürger O, Lindholm B. Calculation of the protein equivalent of total nitrogen appearance from urea appearance. Which formulas should be used? Perit Dial Int 1998; 18: 467–473
7. Weiner ID, Mitch WE, Sands JM. Urea and Ammonia Metabolism and the Control of Renal Nitrogen Excretion. Clin J Am Soc Nephrol. 2015;10(8):1444-58
8. Randerson DH, Chapman GV, Farrell PC: Amino acid and dietary status in CAPD patients, in Peritoneal Dialysis (Eds) Atkins R, Thomson N, Farrell PC, Edinburgh, Churchill Livingstone, 1981, pp. 179—191
9. Blumenkrantz MJ, Kopple JD, Moran JK, Grodstein GP, Coburn JW. Nitrogen and urea metabolism during continuous ambulatory peritoneal dialysis. Kidney Int. 1981;20(1):78-82.
10. Bergström J, Fürst P, Alvestrand A, Lindholm B. Protein and energy intake, nitrogen balance and nitrogen losses in patients treated with continuous ambulatory peritoneal dialysis. Kidney Int. 1993 ;44 (5): 1048-57
11. Grimble GK, West MF, Acuti AB, Rees RG, Hunjan MK, Webster JD, Frost PG, Silk DB. Assessment of an automated chemiluminescence nitrogen analyzer for routine use in clinical nutrition. JPEN J Parenter Enteral Nutr. 1988;12(1):100-6
12. Rajakaruna G, Caplin B, Davenport A. Peritoneal protein clearance rather than faster transport status determines outcomes in peritoneal dialysis patients. Perit Dial Int. 2015;35(2):216-21

13. Konstantinides FN, Boehm KA, Radmer WJ, Storm MC, Adderly JT, Weisdorf SA, Cerra FB. Pyrochemiluminescence: real-time, cost-effective method for determining total urinary nitrogen in clinical nitrogen-balance studies. Clin Chem 1988; 34, (12) 2518-2520

14. Booth J, Pinney J, Davenport A. Changes in red blood cell size and red cell fragmentation during hemodialysis. Int J Artif Organs. 2010 ;33(12):900-5

15. Davenport A, Willicombe M. Comparison of fluid status in patients treated by different modalities of peritoneal dialysis using multi-frequency bioimpedance. Int J Artif Organs. 2009;32(11):779-86

16. Fürstenberg A, Davenport A. Assessment of body composition in peritoneal dialysis patients using bioelectrical impedance and dual-energy x-ray absorptiometry. Am J Nephrol. 2011;33(2):150-6

17. Davenport A. Effect of intra-abdominal dialysate on bioimpedance-derived fluid volume status and body composition measurements in peritoneal dialysis patients. Perit Dial Int. 2013;33(5):578-9

18. Fahal IH. Uraemic sarcopenia: aetiology and implications. Nephrol Dial Transplant. 2014;29(9):1655-65

19. Hung R, Wong B, Goldet G, Davenport A. Differences in Prevalence of Muscle Wasting in Patients Receiving Peritoneal Dialysis per Dual-Energy X-Ray Absorptiometry Due to Variation in Guideline Definitions of Sarcopenia. Nutr Clin Pract. 2017;32(4):539-544

20. Abro A, Delicata LA, Vongsanim S, Davenport A. Differences in the prevalence of sarcopenia in peritoneal dialysis patients using hand grip strength and appendicular lean mass: depends upon guideline definitions. Eur J Clin Nutr. 2018 Jun 19. doi: 10.1038/s41430-018-0238-3

21. Yoowannakul S, Tangvoraphonkchai K, Davenport A. The prevalence of muscle wasting (sarcopenia) in peritoneal dialysis patients varies with ethnicity due to differences in muscle mass measured by bioimpedance. Eur J Clin Nutr. 2018;72(3):381-387

22. El-Kateb S, Sridharan S, Farrington K, Fan S, Davenport A. Comparison of equations of resting and total energy expenditure in peritoneal dialysis patients using body composition measurements determined by multi-frequency bioimpedance. Clin Nutr. 2018 ;37(2) :646-650

23. El-Kateb S, Sridharan S, Farrington K, Davenport A. Comparison of resting and total energy expenditure in peritoneal dialysis patients and body composition measured by dual-energy X-ray absorptiometry. Eur J Clin Nutr. 2016;70(11):1337-1339

Figure 1. Total nitrogen measured in 24 hour spent dialysate compared to urea nitrogen equivalent.

Figure 2. Bland Altman plot daily mean peritoneal nitrogen loss, and difference between measured total nitrogen and estimated peritoneal urea nitrogen equivalent losses using the Bergström equation [10]. Dotted line – mean bias, dashed and dotted line 95% limits of association.

Figure 3. Bland Altman plot daily mean peritoneal nitrogen loss, and difference between measured total nitrogen and estimated peritoneal urea nitrogen equivalent losses using the Randerson equation [8]. Dotted line – mean bias, dashed and dotted line 95% limits of association.

Figure 4. Bland Altman plot daily mean peritoneal nitrogen loss, and difference between measured total nitrogen and estimated peritoneal urea nitrogen equivalent losses using the Blumenkrantz equation [9]. Dotted line – mean bias, dashed and dotted line 95% limits of association.

Figure 5. Bland Altman plot daily mean peritoneal nitrogen loss, and difference between measured total nitrogen and estimated peritoneal urea nitrogen equivalent losses using data obtained from this study. Dotted line – mean bias, dashed and dotted line 95% limits of association.

Figure 1

Click here to download high resolution image

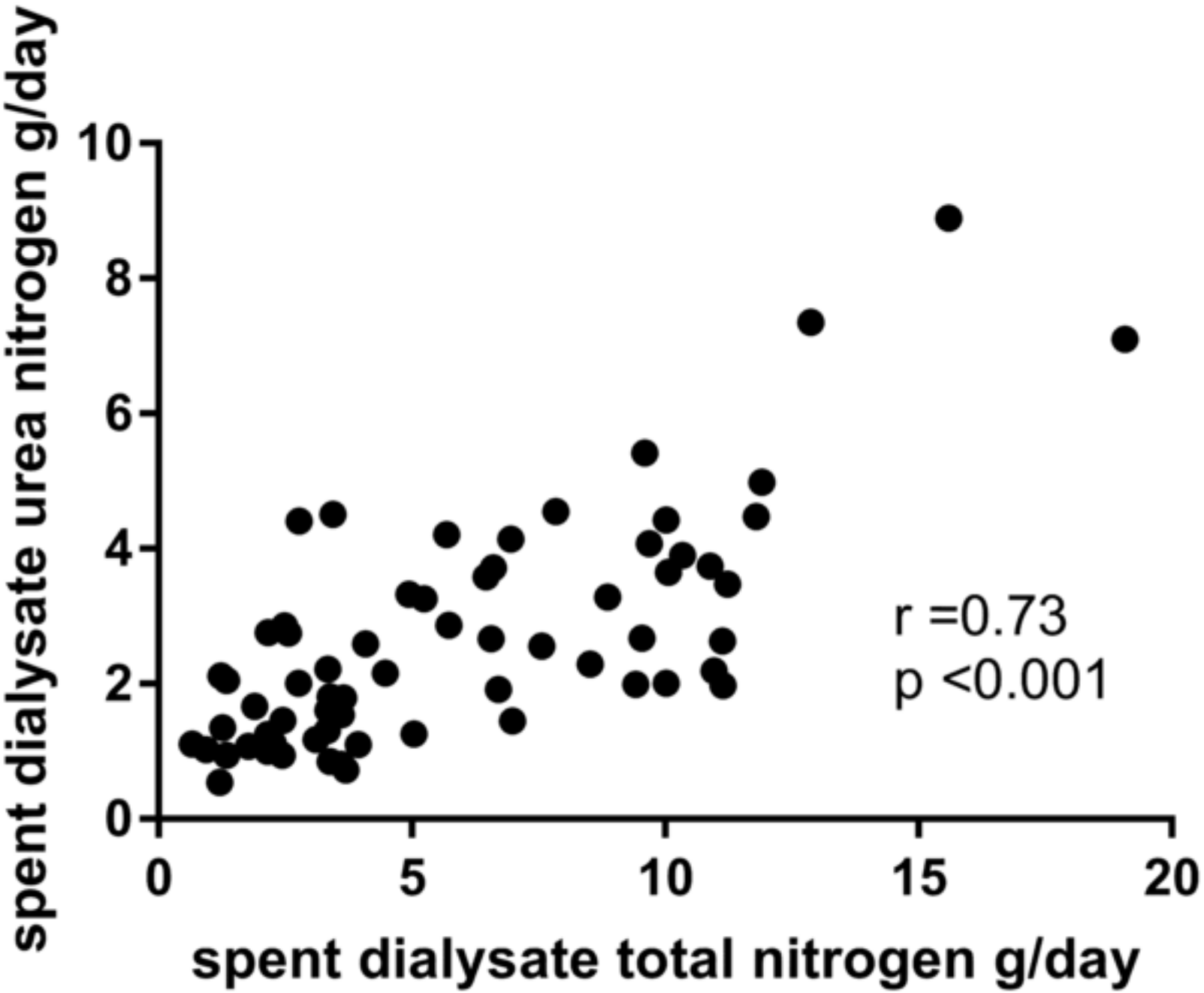

Figure 2

Click here to download high resolution image

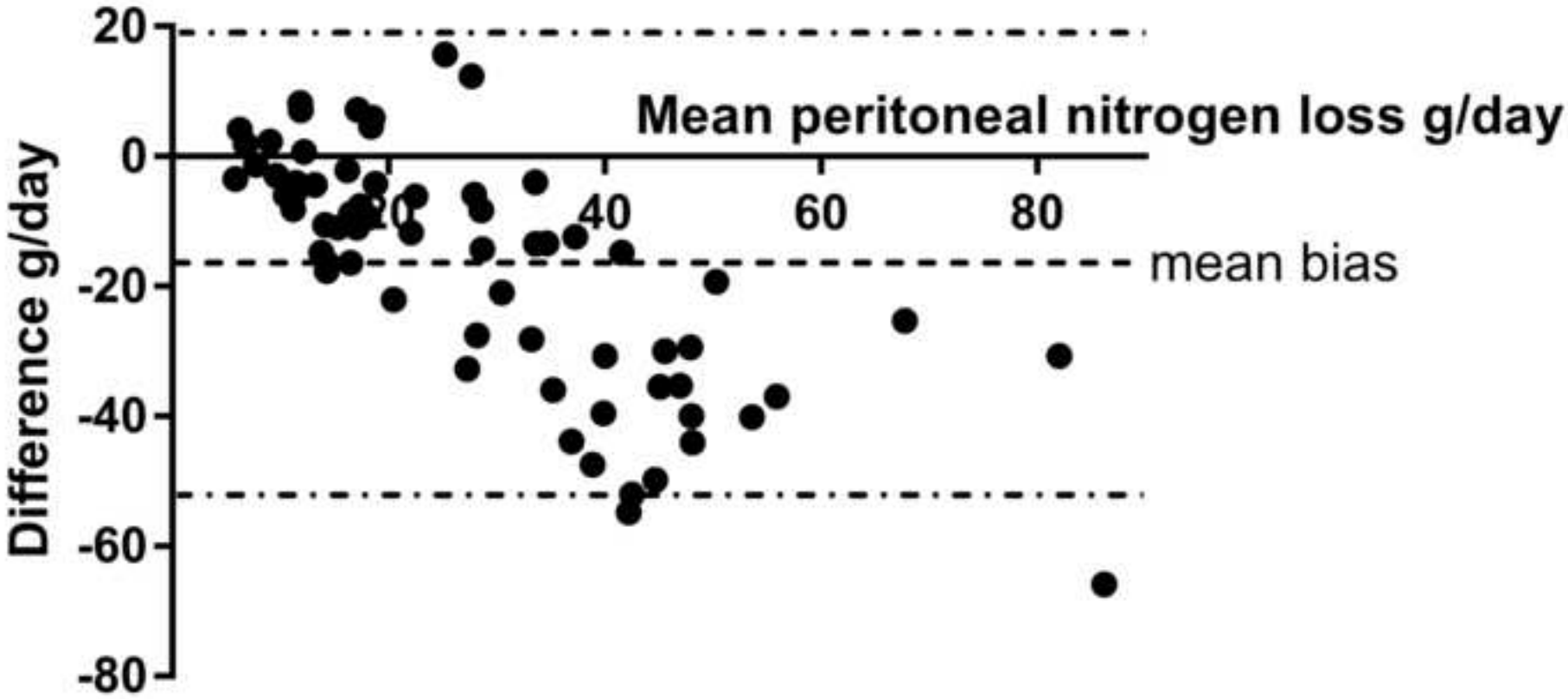

Figure 3

Click here to download high resolution image

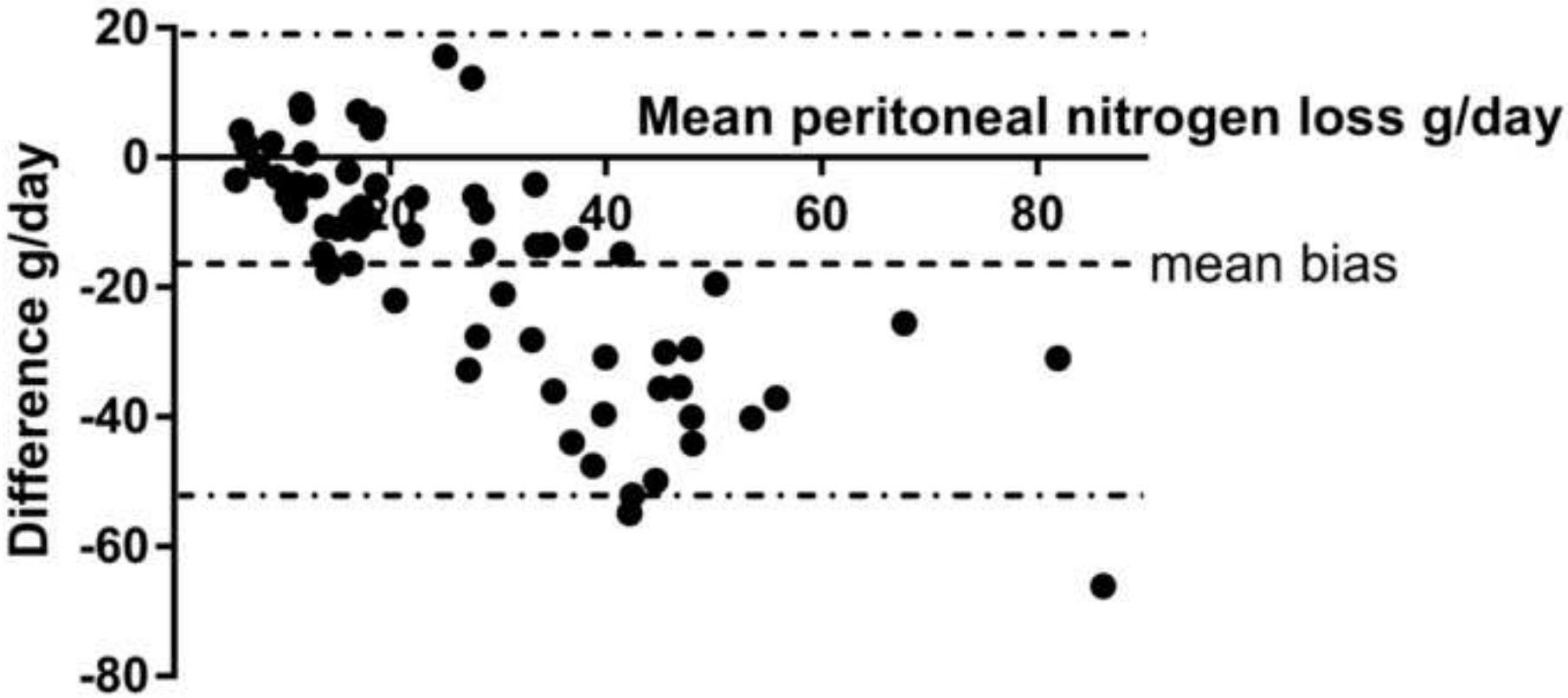

Figure 4

Click here to download high resolution image

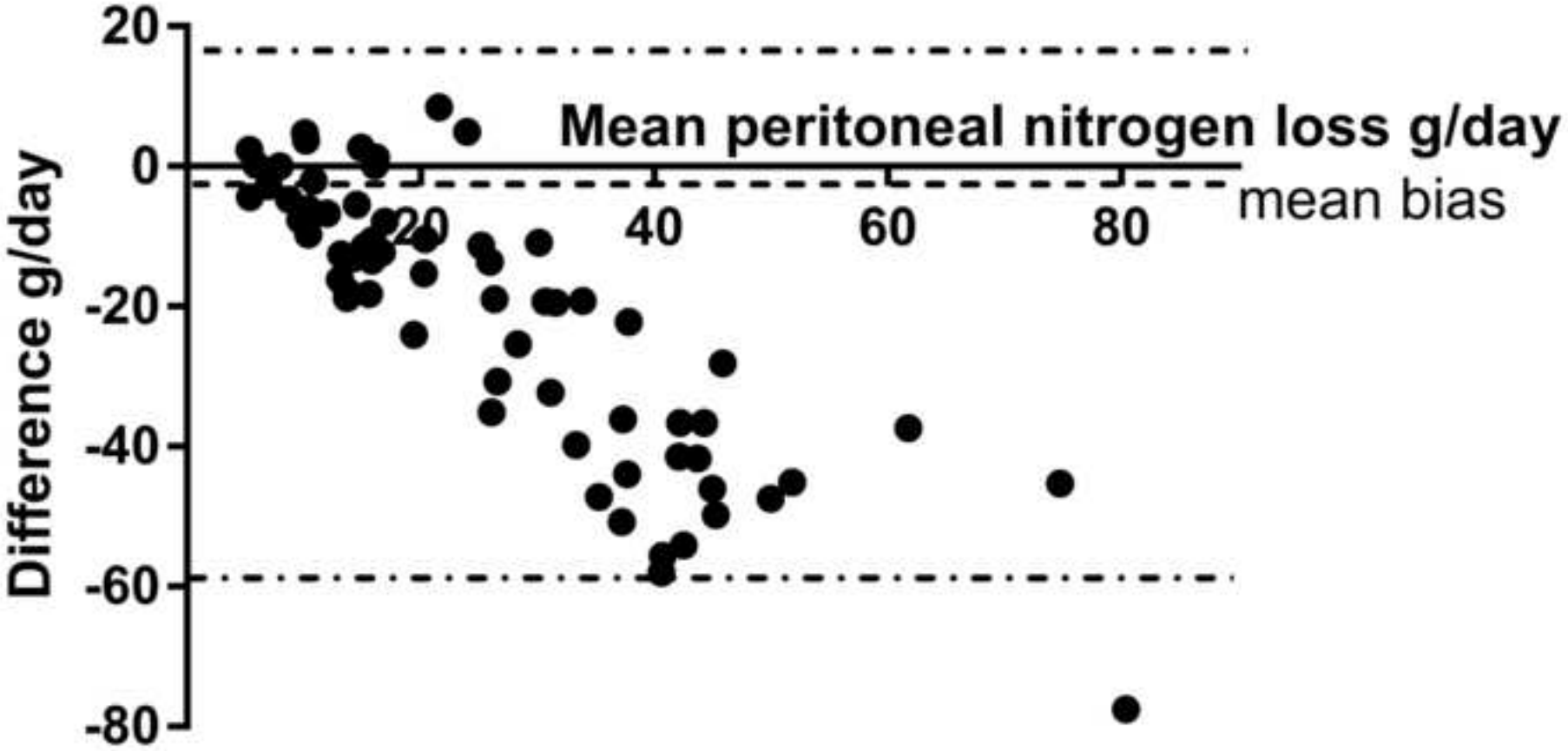

Figure 5

Click here to download high resolution image

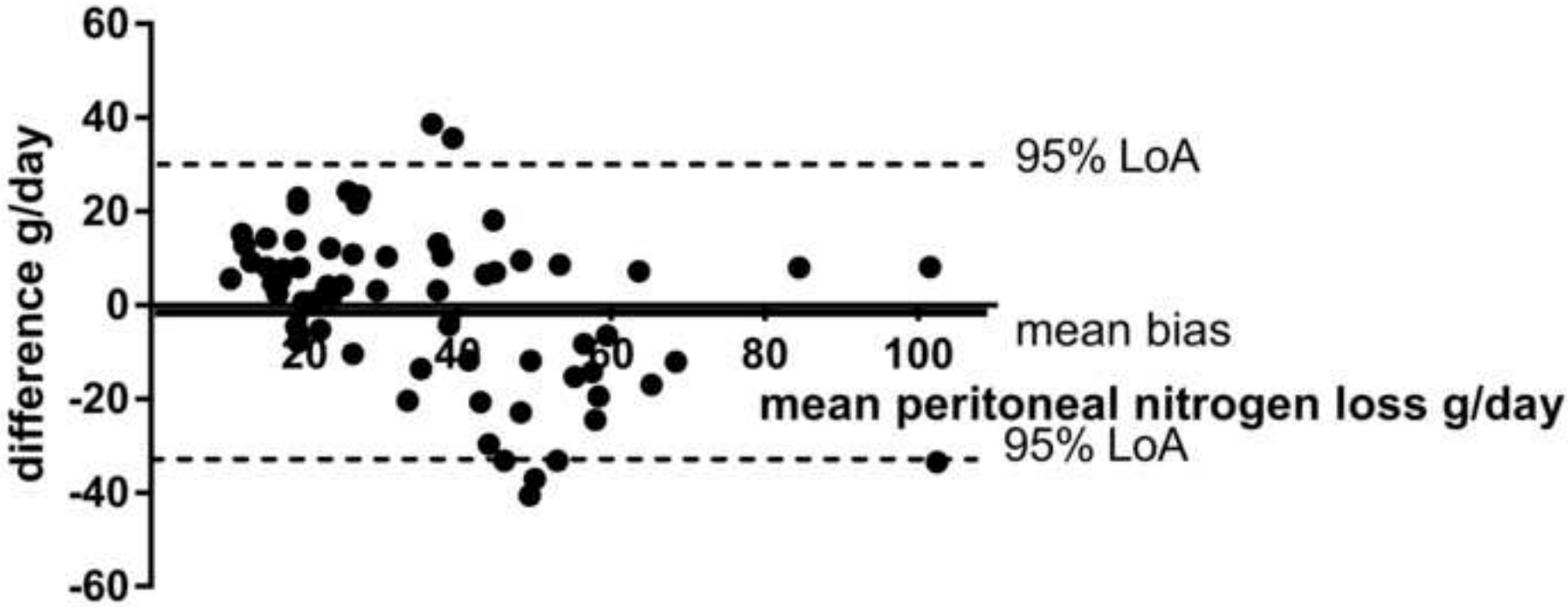

Table 1. Patient demographics, dialysis prescriptions, body composition and laboratory investigations. Number (no), Continuous ambulatory peritoneal dialysis (CAPD), Duration of PD treatment (vintage) months, Results expressed as integers, mean ± standard deviation, median (interquartile range) or percentage.
.

variable	
Male - number. (%)	41 (61.2)
Age, years	67.3 (53.2-79.4)
Ethnicity no (%) White Black Asian	 31 (46.3) 16 (23.9) 20 (29.9)
Diabetes mellitus - no.(%)	26 (38.8)
Body mass index kg/m2	27.3±5.2
Skeletal muscle mass kg	26.7±6.7
% Body fat	32.6±10.2
Haemoglobin, g/dL	10.9±13.9
Albumin, g/L	37.7 ± 3.2
C reactive protein mg/L	4.0 (2.0-12.3)
Serum calcium mmol/L	2.28 ±0.17
Serum phosphate mmol/L	1.63 ±0.42
Dialysis vintage, months	15.1 (5.5-31.9)
Peritoneal equilibrium test no (%) Fast Fast Average Slow Average Slow	 20 (29.9) 31 (46.3) 7 (10.4) 9 (13.4)
CAPD no (%)	23 (34.3)

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

Data Statement

Click here to download Data Statement: Data statement.docx