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# Polyunsaturated fatty acid food frequency questionnaire validation in people with end stage renal disease on dialysis

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**Methods**: Participants (n = 32) completed the PUFA FFQ and three 24-hour recalls. Erythrocyte samples (n = 29) were used for erythrocyte fatty acid analysis. The triangular relationship between the PUFA FFQ, 24-hour recalls and the biomarker was assessed using the method of triads. Agreement between the two dietary methods was also assessed using Bland-Altman plots and classification by quintiles. Reproducibility was tested on a subset of the group (n = 8).

**Results**: The PUFA FFQ was a valid measure of all PUFA except for docosapentaenoic acid (DPA) and arachidonic acid (AA). Strong validity coefficients were found for n-3 long-chain PUFA (LCPUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of 0.914 (95% CI: 0.665, 0.997) and 0.889 (95% CI: 0.706, 0.994), respectively. In the Bland-Altman plots 91-100% of observations fell between the limits of agreement for all PUFA. There were significant correlations between the initial FFQ and the repeat FFQ for all PUFA except DPA and AA.

**Conclusions**: The PUFA FFQ is a valid tool for assessing PUFA intake in people with end stage renal disease.

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# Polyunsaturated Fatty Acid Food Frequency Questionnaire Validation in People with End Stage Renal Disease on Dialysis

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*Abstract*

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*Conclusion:* The PUFA FFQ is a valid tool for assessing PUFA intake in people with end stage renal disease.

Keywords: Dietary Assessment, Renal, Polyunsaturated Fatty Acids, Food Frequency Questionnaire, Validation

## Introduction

Polyunsaturated fatty acids (PUFA) include the essential linoleic acid (LA) (omega-6) and alpha-linolenic acid (ALA) (omega-3). These are called essential as they cannot be synthesised by the body and therefore they must be obtained through the diet.[1] ALA is a substrate for the omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA).[1] However, conversion of ALA to EPA and in particular to DHA is poor,[2] highlighting the importance of consuming preformed n-3 LCPUFA primarily found in fish and seafood.[3]

DHA and EPA have been identified as beneficial for conditions such as rheumatoid arthritis,[4] exercise induced asthma[1] and are important for optimal brain function.[5] Of particular interest is the reduced risk of cardiovascular disease from n-3 LCPUFA. This was originally discovered from studying the Greenland Eskimo population who consumed large amounts of seal fat and whale blubber rich in EPA and DHA. It was discovered that atherosclerosis was rare in this population and there was a low incidence of ischaemic heart disease.[6] This was attributed to the EPA and DHA intake.[6] In addition a meta-analysis conducted by Kotwal et al. (2012) found that treatment with n-3 LCPUFA were protective against vascular death.[7] The GISSI prevenzione trial[8] was conducted on a large Italian cohort who had recently suffered a myocardial infarction. Results from this study indicated that, supplementation with n-3 LCPUFA from fish oil in this group led to reductions in total deaths (20%), cardiovascular deaths (30%) and sudden deaths (45%).[8] More recently, there have been two meta-analyses and a review paper that have reported that n-3 LCPUFA are not effective in reducing deaths from CVD.[9-11] However, a recent review by Meyer & de Groot (2017) report that the trials included in these meta analyses do not all have a high enough dose of n-3 LCPUFA, some are not powered to show a change in CVD outcomes and some do not

measure n-3 levels in the blood to gauge the baseline level of their cohort and also to track compliance.[12] A dose of at least 500mg per day of DHA was still shown to be effective in reducing cardiovascular mortality.[12]

Individuals with end stage renal disease who are undertaking dialysis also suffer from a high cardiovascular disease burden;[13] and the risk of cardiovascular disease has been reported as up to 30 times greater than that of the normal population.[14] Furthermore sudden cardiac death is the number one cause of mortality for dialysis patients.[15] These high rates of CVD are due to many factors common in ESDR patients, firstly chronic kidney disease in itself has been identified as a risk factor for CVD.[16] Patients with ESRD often have not only more traditional risk factors such as hypertension and diabetes,[17] but also present with lipid abnormalities[18] and abnormal mineral and bone metabolism.[17] In addition there are a number of non-traditional CVD risk factors related to uremia in ESRD patients such as: increased homocysteine levels, chronic inflammation and increased oxidative stress.[19]

As a result, levels of n-3 LCPUFA are of interest in this population due to their cardioprotective properties.[20] A study investigating serum phospholipid n-3 LCPUFA found an association between increasing n-3 LCPUFA levels and decreased risk of sudden cardiac death within the first year of dialysis.[15] Other benefits of n-3 LCPUFA in dialysis patients have been demonstrated in clinical trials and includes: an alleviation in pruritis symptoms,[21] improvements in dialysis stunt patency rates[22] and a reduction in inflammatory markers.[22] In addition, n-3 supplementation has been associated with reduced risk of end stage renal disease and delays the progression of kidney disease.[23] However, once on dialysis treatment n-3 serum levels decline with duration on dialysis.[24]

Due to the numerous health benefits attributed to n-3 LCPUFA an accurate measure of assessing dietary PUFA intake is warranted as an estimate of n-3 LCPUFA status. Initially

Sullivan and colleagues developed and validated the n-3 LCPUFA food frequency questionnaire (FFQ) as a simple method of assessing a person's n-3 intake and it was also found to be reproducible.[25] This n-3 LCPUFA FFQ was also validated using plasma and erythrocyte fatty acid levels as biomarkers.[26] The n-3 LCPUFA FFQ was then updated to include other PUFA including omega-6 (n-6) PUFA and was re-named the PUFA FFQ. The PUFA FFQ was transformed to an electronic version to make it easier to administer. This new electronic PUFA FFQ was then validated using the method of triads, which compared the PUFA FFQ to a biomarker and an alternate dietary intake method.[27] This PUFA FFQ has also been adapted and validated in a New Zealand population.[28] However this PUFA FFQ has yet to be validated in a population with a chronic illness such as end stage kidney disease. Hence the aim of this study was to validate the PUFA FFQ in a cohort of individuals with end stage kidney disease undertaking dialysis and test for reproducibility.

## Methods

Participants were recruited from the renal unit of two regional NSW Hospitals, with the use of flyers, letters of invitation and participant information sheets. Participants were then directly approached by the investigator regarding their willingness to participate. Willing participants signed a consent form before completing the study. To be involved in the study participants had to be stable on dialysis for at least 3 months and participants were excluded if pregnant or breastfeeding or were incapable of completing the FFQ, for example patients with dementia.

This study was approved by the University of Wollongong Human Research Ethics Committee (Approval: HE 14/051). The methodology and reporting of this study is compliant with STROBE (strengthening the reporting of observational studies in epidemiology) guidelines.

Participants completed the PUFA FFQ with assistance from the investigator. Assistance was required as the questionnaire was completed during the dialysis process; hence participants were unable to move their arm due to their fistula. The investigator also tallied and confirmed with participants the reported numbers of serves of meat, takeaway meals and desserts eaten per week to check for over reporting. Data from the PUFA FFQ was then rerecorded into the online version of the PUFA FFQ which automatically calculates the amount of PUFA including n-3 LCPUFA consumed by each participant. Participants also completed three separate 24 hour recalls in an interview with the investigator. The interviews took approximately 30 to 45 minutes to complete both the FFQ and the 24 hour recalls. These 24 hour recalls covered one dialysis day, one non-dialysis weekday and one non-dialysis weekend day. Data obtained from the 24 hour recalls were then analysed in Foodworks software (version 7.0.3016, Xyris software, Highgate Hill, Brisbane, Australia).

Non-fasted blood samples were taken at the same time as a participant's usual monthly blood test, with 6-8mls of blood being collected in ethylenediamine tetraacetic acid (EDTA) tubes. This was collected on the first dialysis session of the week after the long dialysis break. Blood was separated with centrifugation for 10 minutes at 4°C at 3000 rpm, and the plasma and white blood cells were removed and discarded. The remaining erythrocytes were stored in a -80°C freezer until ready for fatty acid analysis.

The erythrocyte fatty acids were analysed according to Lepage and Roy (1986).[29] Briefly 400µl of packed erythrocytes were resuspended in a Tris buffer then placed on the centrifuge at 4°C for 30 min at 49000 rpm. The erythrocyte membrane pellet was then resuspended in 200µl of distilled water, to be used in direct transesterification.

150µl of the resuspended erythrocyte membranes were added to 2.2ml of Methanol: toluene (4:1) (BHT 0.01% w/v), including internal standard (21:0, Nu-Check and Sigma) and then

vortexed with the addition of acetyl chloride (200µl). These samples were then heated on a heat block for 60 minutes and then placed in an ice bath for 5 minutes. A 6% Potassium carbonate solution was then added (5mL) to the samples which were then subjected to centrifugation at 4°C for 10 minutes at 3000rpm, the upper toluene phase was removed and placed in gas chromatography vials. These samples were then stored in the -20°C freezer ready for gas chromatography. One microlitre of the samples were injected onto the column (50m x 0.25mm internal diameter capillary column) and fatty acids were analysed using flame-ionisation gas chromatography (GC-17A, Shimadzu). Fatty acids were identified through the comparison with known standards for fatty acids and quantified using internal standard (Nu-chek and Sigma), using Shimadzu analysis software (Class-VP 7.2.1 SP1, USA).

A subset of the cohort (n=8) completed a second PUFA FFQ (FFQ2) with the investigator. This occurred within 3-4 months of the initial FFQ (FFQ1). This group was a convenient sample of participants who were available during the repeat clinic visits.

Data was tested for normality using the Shapiro-Wilk test. Data is expressed as median ($25^{th}$, $75^{th}$ percentile), percentage or validity coefficient (confidence interval) where applicable. The variables weight and body mass index (BMI) were log-transformed to normal distribution for analysis. Baseline characteristics were compared between groups using ANOVA for normally distributed variables, the Kruskal Wallis test for non-normally distributed variables or the Chi-Squared test for categorical variables.

Individual PUFA intakes from the PUFA FFQ and the 24 hour recalls were compared using independent t-tests for normally distributed data and the Wilcoxon Signed Rank test for non-normally distributed data. Statistical significance was set at $p<0.05$. SPSS statistical software for windows (Version 22.0) and JMP Pro (Version 11.0) were used for statistical analysis.

The statistical method used to validate the PUFA FFQ was the method of triads, which has been used to successfully validate this questionnaire in a healthy population.[14] The method of triads compares a questionnaire (PUFA FFQ), an alternative method of dietary intake (average of 3 x 24 hour recalls) and a biomarker (erythrocyte omega-3 levels) in a triangular model. Pearson's correlation is used in this triangulation method which then estimates a validity coefficient between each pair of the hypothetical true intake, the dietary methods and the biomarker. If the validity coefficient for the PUFA FFQ is close to one this means the dietary method (FFQ) is a good measure of actual intake.[27] The software package used for the method of triads analysis was R (R core team, 2013).

To calculate the 95% confidence interval (CI) for each of the fatty acids tested, the bootstrap method[30] was used from 10 000 bootstrap samples (n=29). Cases where the square of the estimated validity coefficient were negative or > 1 are known as Heywood cases. These Heywood cases were discarded because the specific bootstrap samples that generated the Heywood cases have violated the assumptions underlying the methods of triads.

Absolute agreement and systematic bias between the 2 dietary assessment methods (PUFA FFQ and 24 hour recalls) were assessed by Bland-Altman plots. Values were log transformed due to the proportional relationship between the differences and the mean of the two methods for most PUFA.[31] The limits of agreement within the Bland-Altman plot are representative of a range, with the property that if 95% of differences of intakes occur in this range it indicates there is good agreement between the 2 dietary assessment methods.[32] Pearsons's correlation was used to test for significant correlations within the Bland-Altman plots.

Quintile assignments assessed relative agreement between the 2 methods (PUFA FFQ and 24 hour recalls).[33] For individual PUFA, total n-3 PUFA, total n-3 LCPUFA, total n-6 PUFA and total PUFA the numbers of participants in the same or adjacent quintiles were measured

as were the numbers of misclassified participants.[28] The number of participants was n=32, which did not divide evenly into 5 groups, so the last two quintiles were assigned an extra value. The extra values were assigned to the last quintiles as there was a clear gap in the data where the splits occur.

Reproducibility was assessed by testing for differences between the 2 FFQ measures using the paired t-test, or Wilcoxon's signed rank test for non-normally distributed data. Spearman's correlation coefficients were used to examine whether there is a linear relationship between the 2 FFQ measures.

## Results

A total of 101 people were accessible to researchers in the renal unit for participation in the trial. Of these 9 were excluded due to health reasons, 5 were not present at the time of recruitment, 2 were deceased during the recruitment period and a further 53 were unwilling to participate. Thirty two subjects agreed to participate in the study. The characteristics of participants and the subset of participants who completed FFQ2 are outlined in **Supplementary Table 1** and are compared to the whole dialysis cohort at the 2 study sites. There were no significant differences between the groups. The total 32 subjects completed the PUFA FFQ and the multiple 24 hour recalls, however only 29 subjects provided blood samples. This was due to 2 samples being missed during the blood collection phase and one participant had died after the diet interviews but prior to the blood collection.

The Validity Coefficients for the method of triads are shown in **Table 1**, with high validity coefficients found for EPA, DHA, n-3 LCPUFA, and total PUFA. For the PUFA FFQ the lowest 95% CI for these fatty acids ranged from 0.481 to 0.706, whilst the highest CI ranged

from 0.961 to 0.997. The median percentage of erythrocyte EPA and DHA was 0.5% and 4.3% respectively.

**Table 2** shows the median intakes of fatty acids for both the PUFA FFQ and the multiple 24 hour recalls. The PUFA FFQ had estimated significantly higher intakes of ALA, EPA, DPA, DHA, total n-3 LCPUFA, total n-3, AA and total PUFA compared to the multiple 24 hour recalls. There was no difference in intake between LA and total n-6.

Bland-Altman plots (**Figure 1**) were created using log transformed data for all PUFA. For EPA and DPA 91% and 94% of observations fell between the limits of agreement respectively, for the remaining fatty acids (total n-3, total n-6, total PUFA, ALA and LA, DHA and n-3 LCPUFA) 97-100% of observations fell between the limits of agreement. Data points were distributed above and below the line of mean difference for all plots, however in some Bland Altman plots a significant negative correlation was found (EPA, DPA, DHA, n-3 LCPUFA).

As shown in **Supplementary Table 2** the quintile assignments for n-3 LCPUFA between the PUFA FFQ and the food record obtained from multiple 24 hour recalls classify 50% of subjects into the same quintile, 28% into adjacent quintiles and 22% of subjects were misclassified and therefore were not in the same or adjacent quintiles. For the remaining PUFA (ALA, EPA, DPA, DHA, LA, AA, total n-3, total n-6 and total PUFA), the percentages of subjects classified in the same or adjacent quintile ranged from 66% (DPA) to 81% (EPA).

Repeat FFQ data is shown in **Table 3** (n=8). There were no significant differences between the two PUFA measures for all PUFA except total n-3. Significant correlations between the original (FFQ1) and repeat FFQ (FFQ2) data were found for all PUFA except AA (r=0.36, p=0.39) and DPA (r=0.62, p=0.10). The subset of participants completing the FFQ2 were not

significantly different from the main cohort completing FFQ1, and the dialysis population as a whole for any baseline characteristics (Supplementary Table 1).

## Discussion

The results of this investigation confirm that the PUFA FFQ has good validity for assessing PUFA intake except for DPA and AA in a people with end stage renal disease on dialysis. Strong validity coefficients were found in particular for n-3 LCPUFA EPA, DHA and total LC omega-3 (Table 1). These values are higher than previous findings of Ingram et al.[28] and for DHA and n-3 LCPUFA values are higher than Swierk et al.[27] The fact that this study had the investigator assist with the completion of the PUFA FFQ and the 24 hour recalls may have lessened the variability of the results and contributed to these high validity coefficients.

The method of triads validity coefficients rely on the presence of a linear relationship between dietary intake and erythrocyte fatty acids. As it has been established that EPA and DHA are incorporated into cell membranes,[34] the high validity coefficients for EPA, DHA and total n-3 LCPUFA are not surprising. Validity coefficients for DPA, LA and AA are not presented as they have previously been found to be low.[26-28] DPA stays relatively stable in the cell membranes and is therefore not influenced by dietary intake, which would suggest selective uptake by tissues.[27] As for AA and LA, LA is readily converted to AA,[35] and it is AA that is taken up by erythrocyte membranes. Hence there is a lack of direct correlation between dietary LA and erythrocyte LA. Likewise there is a lack of direct correlation between dietary AA and erythrocyte AA.

Although for all Bland-Altman plots 91-100% of observations fell between the limits of agreement, these limits of agreement must be considered practically. The limits of agreement for all PUFA were anti-logged to give the magnitude of difference between the methods. The upper limits of agreement ranged from 3.8 to 57.7 times while the lower limits of agreement

were 0.19 to 0.61. This illustrates a clear discrepancy between the 24 hour recalls and the PUFA FFQ, at best the FFQ intakes were between 0.45 to 3.91 times the 24 hour recall intakes for total PUFA and at worst 0.19 to 57.7 times the 24 hour recalls for DHA. When comparing only the two dietary methods for nutrients such as n-3 LCPUFA where the main food source may not be consumed everyday there can be large discrepancies. For example if a participant recorded eating fish 3 times a week in the FFQ this could potentially be missed in the 24 hour recalls and would result in a greater bias.

Also, it is interesting to note that there was a significant negative correlation in the Bland Altman plots for EPA, DPA, DHA and n-3 LCPUFA (figure 1b). This would imply that the FFQ at smaller intakes overestimated intake and at larger intakes underestimated intakes. Ingram *et al.* previously found overestimation of the n-3 LCPUFA EPA, DPA and DHA by the FFQ compared to the weighed food record.[28] The discrepancy between the two methods is also highlighted in (Table 2) as there was significant differences between the intakes of ALA, EPA, DPA, DHA, total n-3 LCPUFA, AA and total PUFA. This highlights the importance of using the method of triads to validate a questionnaire measuring nutrients such as n-3 LCPUFA, where the main food sources are not consumed daily. When there is a direct relationship between the intake and the biomarker the method of triads is preferable as it utilises the three measures.[27]

The level of quintile assignment found in this study (Supplementary Table 2) for n-3 LCPUFA is comparable to previous studies.[25, 28] The number of participants classified in the same quintiles for n-3 LCPUFA was 50%, compared to 43-49%.[25, 28] The quintile assignments for the other PUFA were also comparable to previous literature, with the number of subjects classed in the same quintile ranging from 31-47%, compared to 31-43%.[28] This would suggest good agreement between the methods, but the PUFA FFQ may be over-reporting intakes. It is accepted that FFQ tend to overestimate the frequency of consumption

of foods, particularly when different types of foods within a food group are listed,[36] which occurs in the PUFA FFQ. Alternatively, diet recall methods such as the multiple 24 hour recalls used tend to underestimate consumption.[37] Overestimation is also suggested in Table 2, as there was a difference in reported intakes between the PUFA FFQ and the 24 hour recalls.

The PUFA FFQ was found to be reproducible using a subset of n=8. It is important to note that with 8 repeat measures type 2 errors are more likely, and so looking at the amount of difference between the repeated measures may be more useful. When examining the difference in median intake for FFQ1 and FFQ2 there was a difference of 50, 80 and 170mg for DHA, EPA and n-3 LCPUFA respectively. This is comparable to the difference in repeated measures found by Ingram *et al*. with 90, 100 and 230mg for DHA, EPA and n-3 LCPUFA respectively.[28]

The differences between the present study and the previous validation studies may possibly be explained largely by the severity of the poor health of the participants and that they were mostly older individuals. The dialysis participants are generally older with a median age of 73, which may impact their ability to recall what foods have been consumed.[38] However, older participants tend to have established eating habits and so their relayed diet should be an accurate measure of usual intake.[38] This would be particularly true for the dialysis patients as they are consuming similar foods served at the dialysis centre as well.

Also the method used to measure dietary intake differs between this study and previous validation studies.[25, 27, 28] Previous studies have used a weighed food record to record dietary intake of participants. This is considered the gold standard of dietary intake methods[37] and so may be more accurate than the multiple 24 hour recalls used in the present study. A weighed food record was not used in this population due to its high participant burden in a chronically

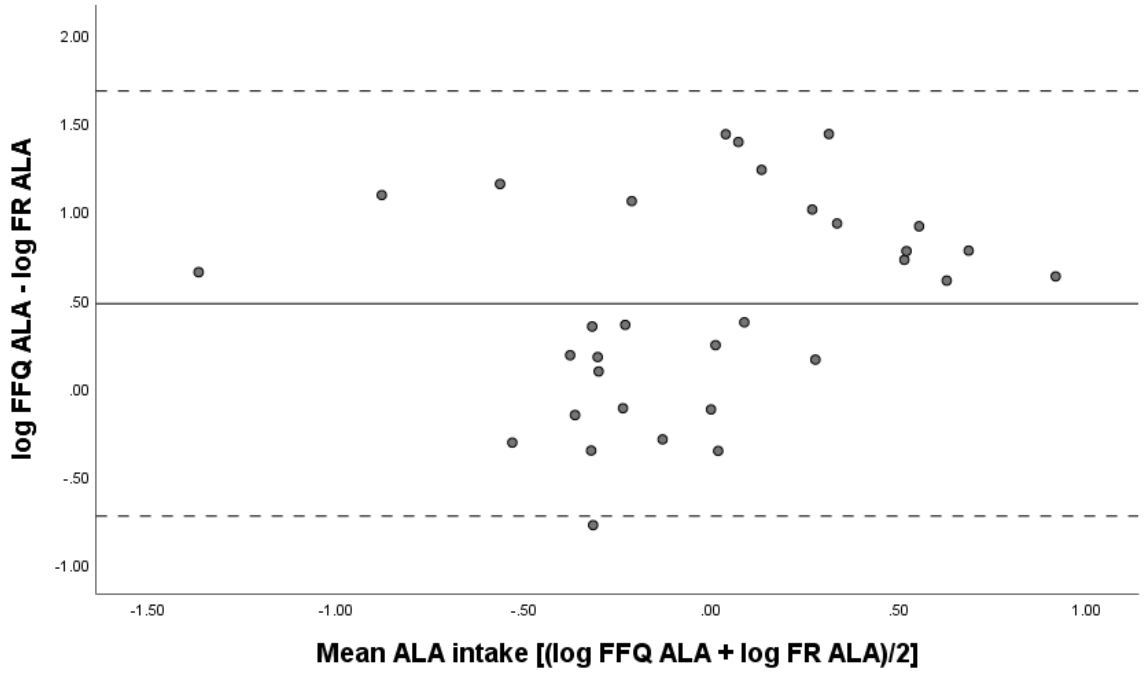
ill cohort with a terminal illness. Impairments with long term memory and attention[39] and low levels of health literacy[40] are also common in this population. Given that dialysis patients in the units study consumed main meals and snacks each week in the unit with known nutrient data on the items consumed, the 24 hour recall method was used instead. Three 24 hour recalls were used due to their quick administration time, low burden for participants and its good representation of usual intake when averaged.[41] Moreover, this study has displayed similar levels of agreement to the previous validation studies,[25, 27, 28] suggesting that multiple 24 hour recalls may be a sufficient diet assessment method to validate the PUFA FFQ, and 3 day weighed food records may not be necessary. This is important for future studies using diet assessment methods on a similar population.

A clear limitation of the study is the small number of study participants. We recruited approximately one third of the participants who were available to participate in the study, with the remainder unwilling to participate. However, the population included in the study were found to be representative of the larger Australian Haemodialysis cohort[42]. This suggests that the results are generalisable to the larger cohort. We compared demographics such as age, gender, weight, BMI and dialysis adequacy between the study group and the wider population of dialysis patients and found no difference. Furthermore the subgroup selected for the reproducibility assessment, although a convenient sample, are once again representative of the 32 study participants.

In conclusion, the PUFA FFQ described in this study is a valid and reproducible instrument to assess the intake of all PUFA in people with end stage renal disease on haemodialysis, although assistance in completing the PUFA FFQ is usually required.

Figure 1

A


log FFQ ALA - log FR ALA
2.00
1.50
1.00
.50
.00
-.50
-1.00
-1.50
-1.00
-.50
.00
.50
1.00
Mean ALA intake [(log FFQ ALA + log FR ALA)/2]

B

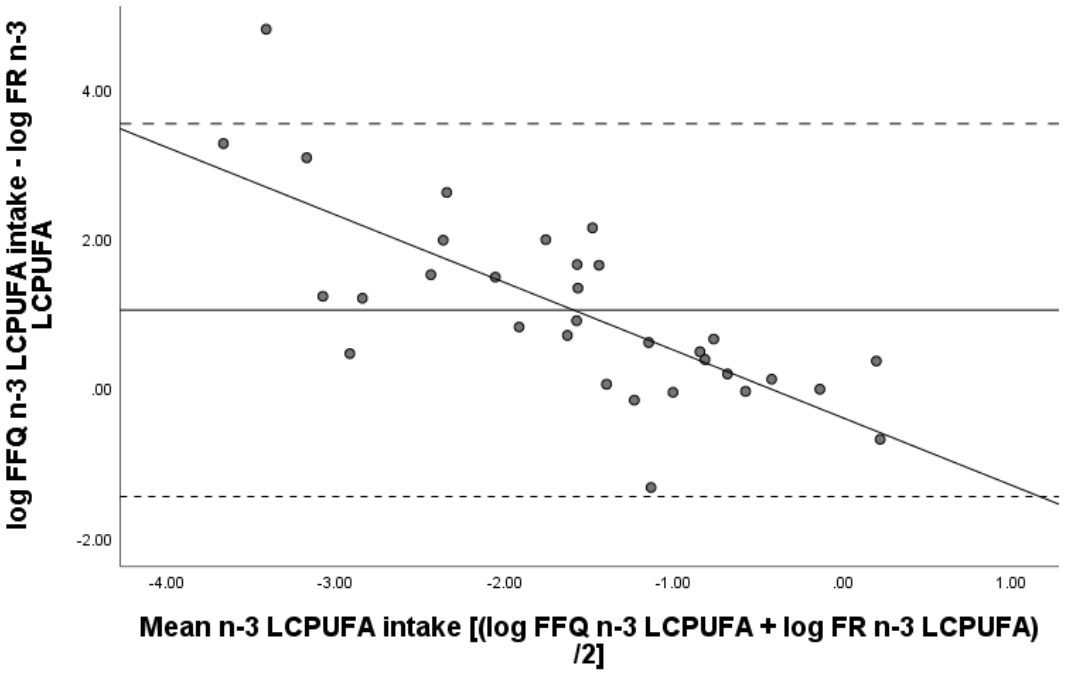

log FFQ n-3 LCPUFA intake - log FR n-3 LCPUFA
4.00
2.00
.00
-2.00
-4.00
-3.00
-2.00
-1.00
.00
1.00
Mean n-3 LCPUFA intake [(log FFQ n-3 LCPUFA + log FR n-3 LCPUFA) /2]

**Figure 1** Validation of the PUFA FFQ against a food record (average of 3 24 hour recalls) for ALA (A), and total n-3 LCPUFA (B). The solid line shows mean difference in intake, the dashed lines represent limits of agreement. For ALA and total n-3 LCPUFA approximately 95% of observations fall between the limits of agreement. A significant correlation was found for total n-3 LCPUFA (B), $p<0.01$, $r=-0.743$. ALA, alpha linolenic acid; n-3 LCPUFA, omega-3 long-chain polyunsaturated fatty acids; FR, food record.

**Table 1: Method of triads validity coefficients for the PUFA FFQ, FR and erythrocyte fatty acids[a]**

| | Validity Coefficient PUFA FFQ vs. T | Validity Coefficient FR vs. T | Validity Coefficient Biomarker vs. T |
|---|---|---|---|
| EPA (20:5n-3) | 0.914 (0.665, 0.997) | 0.863 (0.695, 0.990) | 0.652 (0.120, 0.921) |
| DHA (22:6n-3) | 0.889 (0.706, 0.994) | 0.860 (0.721, 0.989) | 0.386 (0.084, 0.695) |
| Total n-3 LCPUFA | 0.873 (0.650, 0.995) | 0.848 (0.690, 0.990) | 0.443 (0.085, 0.762) |
| Total PUFA | 0.720 (0.481, 0.961) | 0.839 (0.616, 0.992) | 0.502 (0.201, 0.806) |

[a]Data is presented as validity coefficient (95% CI), n= 29. LC, long-chain; FFQ, food frequency questionnaire, FR, food record, T, true intake

**Table 2: PUFA intakes from PUFA FFQ and 24 hour recalls[a]**

| Nutrient | FFQ intake* | 24 hour recalls intake* |
|---|---|---|
| n-3 PUFA | | |
| ALA | 1.09 (0.76, 2.21) | 0.81 (0.62, 1.10)[b] |
| EPA | 0.11 (0.07, 0.19) | 0.04 (0.01, 0.18)[b] |
| DPA | 0.07 (0.04, 0.11) | 0.03 (0.01, 0.05)[b] |
| DHA | 0.16 (0.09, 0.27) | 0.04 (0.01, 0.21)[c] |
| Total n-3 LCPUFA | 0.36 (0.23, 0.55) | 0.12 (0.04, 0.37)[b] |
| Total n-3 PUFA | 1.55 (1.15, 2.65) | 1.07 (0.72, 1.37)[b] |
| n-6 PUFA | | |
| LA | 9.49 (6.04, 14.6) | 8.18 (5.88, 10.4) |
| AA | 0.14 (0.10, 0.20) | 0.07 (0.04, 0.11)[b] |
| Total n-6 PUFA | 9.62 (6.13, 14.8) | 8.25 (6.41, 10.5) |
| Total PUFA | 11.2 (7.18, 18.1) | 9.31 (7.28, 11.6)[b] |

[a]Values are all g/day, *Values expressed as median value ($25^{th}$, $75^{th}$ percentile), [b] significantly different from FFQ intake using independent t test $p<0.05$, [c] significantly different from FFQ using Wilcoxon signed rank test $p<0.05$

**Table 3: PUFA Measures from FFQ1 (n= 8) and FFQ2 (n=8)[a]**

| Nutrient | FFQ1 intake | FFQ2 intake | P value | Correlation coefficient | Correlation P value |
|---|---|---|---|---|---|
| n-3 PUFA | | | | | |
| ALA | 0.86 (0.50, 1.00) | 1.27 (0.56, 2.27) | 0.34 | 0.83 | 0.01[c] |
| EPA | 0.14 (0.05, 0.35) | 0.22 (0.03, 0.50) | 0.83 | 0.83 | 0.01[c] |
| DPA | 0.07 (0.04, 0.09) | 0.08 (0.02, 0.13) | 0.65 | 0.62 | 0.10 |
| DHA | 0.22 (0.10, 0.40) | 0.27 (0.04, 0.56) | 0.40 | 0.71 | <0.05[c] |
| Total n-3 LCPUFA | 0.42 (0.19, 0.83) | 0.59 (0.10, 1.19) | 0.28 | 0.81 | 0.01[c] |
| Total n-3 | 1.16 (0.94, 2.21) | 1.89 (1.41, 2.81) | 0.04[b] | 0.71 | <0.05[c] |
| n-6 PUFA | | | | | |
| LA | 5.08 (3.22, 6.17) | 7.11 (4.06, 16.2) | 0.34 | 0.90 | <0.01[c] |
| AA | 0.10 (0.08, 0.23) | 0.15 (0.07, 0.28) | 0.42 | 0.36 | 0.39 |
| Total n-6 | 5.20 (3.28, 6.44) | 7.20 (4.20, 16.3) | 0.40 | 0.79 | 0.02[c] |
| Total PUFA | 6.46 (4.43, 8.51) | 9.14 (5.64, 18.8) | 0.25 | 0.76 | 0.03[c] |

[a]Values are all g/day

Values expressed as median ($25^{th}$, $75^{th}$ percentile)

Reproducibility data n=8 for FFQ 1 and FFQ 2

[b]Significantly different determined by paired t-test $p<0.05$

[c]Significant Spearman correlation $p<0.05$

**Supplementary Table 1: Characteristics of Dialysis Cohort[a]**

| | Full Dialysis Cohort n=101 | Study Participants n=32 | Subset for FFQ 2 n=8 | P-Value |
|---|---|---|---|---|
| Age, y | 72.0 (58.0, 79.0) | 73.0 (62.5, 78.8) | 79.5 (74.0, 83.8) | 0.20 |
| Gender, M (%) | 55 (54) | 17 (53) | 2 (25) | 0.41 |
| Weight (kg) | 71.5 (60.1, 85.9) | 76.5 (61.5, 93.9) | 63.5 (46.0, 70.4) | 0.16 |
| BMI | 25.8 (22.7, 32.2) | 28.6 (22.5, 34.1) | 24.6 (20.4, 29.5) | 0.50 |
| Kt/V | 1.55 (1.40 1.83) | 1.53 (1.33, 1.80) | 1.68 (1.44, 2.43) | 0.82 |

[a]Values are median ($25^{th}$, $75^{th}$ percentile) or percentage, n=101 for Full Dialysis Cohort, except for weight, n=100, BMI, n=97, Kt/V, n=98. n= 32 for participants

BMI, Body Mass Index, Kt/V, Dialysis adequacy

**Supplementary Table 2: Agreement of quintile assignment between the FFQ and food record based on PUFA intakes**

| Fatty Acid | Same Quintile n (%) | Adjacent Quintile n (%) | Misclassified [a] n (%) |
|---|---|---|---|
| EPA (20:5n-3) | 11 (34) | 15 (47) | 6 (19) |
| DPA (22:5n-3) | 15 (47) | 6 (19) | 11 (34) |
| DHA (22:6n-3) | 13 (41) | 9 (28) | 10 (31) |
| Total n-3 LCPUFA | 16 (50) | 9 (28) | 7 (22) |
| Total n-3 PUFA | 10 (31) | 12 (38) | 10 (31) |
| LA (18:2n-6) | 12 (38) | 12 (38) | 8 (25) |
| AA (20:4n-6) | 11 (34) | 10 (31) | 11 (34) |
| Total n-6 PUFA | 13 (41) | 12 (38) | 7 (22) |
| Total PUFA | 11 (34) | 14 (44) | 7 (22) |

Values expressed as number and percentage of participants

[a]Percentage of participants not classified into the same or adjacent quintile

n=32

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