Official title of the Study:

Omega 3 Fatty Acids in Patients with Chronic Kidney Disease

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Material and methods:

We invited to participate patients with Chronic Kidney Disease (CKD) who had a urine albumin excretion of 30 mg/g creatinine or over. All these participants were part of Non- communicable Chronic Disease programs at public primary health care clinics of East Metropolitan Santiago. Participants were invited to a first interview to assess their eligibility for the study. We excluded participants younger than 18 years and older than 85 years of age. We also excluded those who had chronic debilitating diseases such as active cancer, heart failure grade III or more according to the classification of the New York Heart Association [1], chronic debilitating infections or a history of drug or alcohol abuse. In the case of diabetic patients, we excluded those who were decompensated, defined as a fasting blood glucose over 150 mg/dl or a glycosylated hemoglobin over 8%. The study was approved by the ethics committee of East Metropolitan Santiago Health Service.

Participants deemed eligible were cited after an overnight fast to the outpatient clinic of the Institute of Nutrition and Food Technology (INTA). After signing an informed consent, they were subjected to the following procedures:

1. Weight, height and waist circumference were measured.
2. A fasting blood sample was obtained to measure blood glucose, serum lipids, glycosylated hemoglobin, creatinine, cystatin C, β2 microglobulin, C reactive protein, interleukin-6 and fatty acid profile in red cell membranes. Cystatin C and interleukin 6 were measured using ELISA kits from R&D Systems. Red cell membrane fatty acids were measured by liquid chromatography after extracting lipids from erytrhocytes using Bligh y Dryer [2] method and subsequently derivatized. All other determinations were carried out using routine clinical laboratory methods in a certified clinical laboratory (Vida Integra).

3. A spot urine sample was obtained to measure albumin, creatinine and urea nitrogen at Vida Integra laboratory.
4. Blood pressure and pulse wave velocity were measured using a Mobil-O-Graph® oscillometric device. Augmentation pressure and index and reflection magnitude of the pulse wave are also measured. The measurements were done in the sitting position after a five minutes rest in a quiet room.
5. Measurement of carotid intima media thickness using a General Electric LogiQ ultrasonographic device with border detection software. A 14.2 x 47 mm lineal GE 12L-RS transducer was used. International measurement norms were used [3].
6. Measurement of liver fat infiltration using the same ultrasonographic device but with a convex multifrequency (2 – 5.5 MHz) 4C RS GE transducer. Fatty infiltration was ascertained using Hamaguchi score [4]. We have previously validated this technique against liver fat measurement using magnetic resonance spectroscopy.

Participant features were reassessed with the new laboratory determinations and those who continued to comply with inclusion criteria were randomized into two groups, balancing by gender, age, body mass index and urine albumin excretion expressed per mg of creatinine. Randomization was carried out using an iteration software based on random number generation. The output was an individual number for each patient. This number was sent to the oil manufacturer, who had the codes for each number and prepared the supplement to be used.

1. Active supplement contained 282 mg of docosahexanoic and eicosapentanoic acids per ml.
2. Placebo supplement: Contained corn oil

Participants were instructed to drink 13 ml of the supplement per day in one or two doses. Both supplements had a lemon flavor and were indistinguishable due to deodorization technology used

to prepare them. Therefore the investigators and the participants were blinded to the treatment allocation.

The intervention period lasted 3 months. Participants were cited every two weeks to provide them with a new bottle of supplement. They returned the used bottle and the residual content was measured to determine compliance. They were also interrogated about adverse events or other eventualities.

At the sixth week and at the end of the intervention at 12 weeks, participants were subjected to the same assessments done at baseline.

All participants could call the investigators in any moment if they an intercurrent problem. If a subject failed to attend an appointment, he or she was called by phone or visited at their houses to find out the reason for not showing. I a subject declined to continue in the study, he or she was considered as a loss from follow up.

Data analysis:

Until the end of the intervention period for all participants, they were only identified with their allocation number. Once the intervention ended, each participant was classified as pertaining to group 1 or 2, without knowing which was the group receiving the active supplement. All red cell membrane fatty acid composition measurements, was analyzed when all statistical analysis of the rest of the variables were done, to maintain the blinding. An intention to treat analysis was performed.

Normality of variable distribution was analyzed using Shapiro Wilk test. Since most variables had a non-normal distribution, results are expressed as median (interquartile range). By the same token, non-parametric statistical methods were used to compare variables. Chi square or fisher tests were used to compare proportions. Differences between groups were compared using Kruskal

Wallis test. To assess changes in repeated measures of a single parameter a mixed linear regression model for repeated measures was used [5]. Significance level was set at 0.05

The main outcome of the study was set as a reduction in urine albumin excretion by 20% or more. We anticipated that 35% more participants receiving the active product than those receiving placebo would attain this reduction. Thus, the sample size to obtain results with an α of 0.05 and a power of 0.8 was 38 participants per group.

Results:

Participant flow is shown in figure 1. We randomized 50 participants per group and lost five in corn oil group and four in Ω-3 groupl The demographic features, body mass index and baseline albumin excretion of participants randomized to each group are shown in table 1. No differences between groups were observed, therefore the premises of randomization were correctly attained. Red blood cell membrane phospholipids fatty acid composition during the study are shown in table 2. Patients receiving the active supplement had significant increases in eicosapentanoic and docosahexanoic fatty acid levels.

A 20% reduction in urine albumin excretion was attained in 13 participants of group 1 and 19 participants of group 2 (fisher p= 0.274). The clinical and laboratory parameters in the three assessment periods for groups 1 and 2 are observed in table 3.

Summarizing, participants receiving corn oil maintained their pulse wave velocity during the follow up while controls experienced an increase in this parameter. Triacylglycerol levels decreased significantly in the Ω-3 group and glycosylated hemoglobin increased significantly in both groups

The compliance with the study product for groups 1 and 2, calculated measuring the leftovers was 88.7 (74-97) and 89.6 (77-98) %, respectively (p= 0.99). Eighty nine adverse events were recorded (48 in group 1 and 27 in group 2, p = NS). Of these, two were severe (a severe hyperkalemia and

the discovery of a cervical mass that resulted in a medullary thyroid carcinoma, both in participants of group 1). Mild gastrointestinal symptoms were reported by 12 and 14 participants in groups 1 and 2 respectively.

Preliminary conclusions:

1. The primary outcome measure of the study was not attained, since urine albumin excretion decreased by 20% or more in a similar proportion of participants in each intervention group.
2. The striking reduction in serum triacylglycerol observed in participants receiving Ω-3 fatty acids is a known pharmacological effect of Ω-3 fatty acids.
3. The deterioration of glycosylated hemoglobin in both groups, may be associated with the fact that participants received 110 Kcal/day of extra calories with the supplement.
4. The lack of increase in pulse wave velocity in group 2 may indicate that the supplement that this group received had a favorable effect on arterial stiffness.
5. The supplement was well tolerated. The lack of differences between groups in compliance and gastrointestinal complains indicates that the fish oil effectively almost completely deodorized.

Figure 1

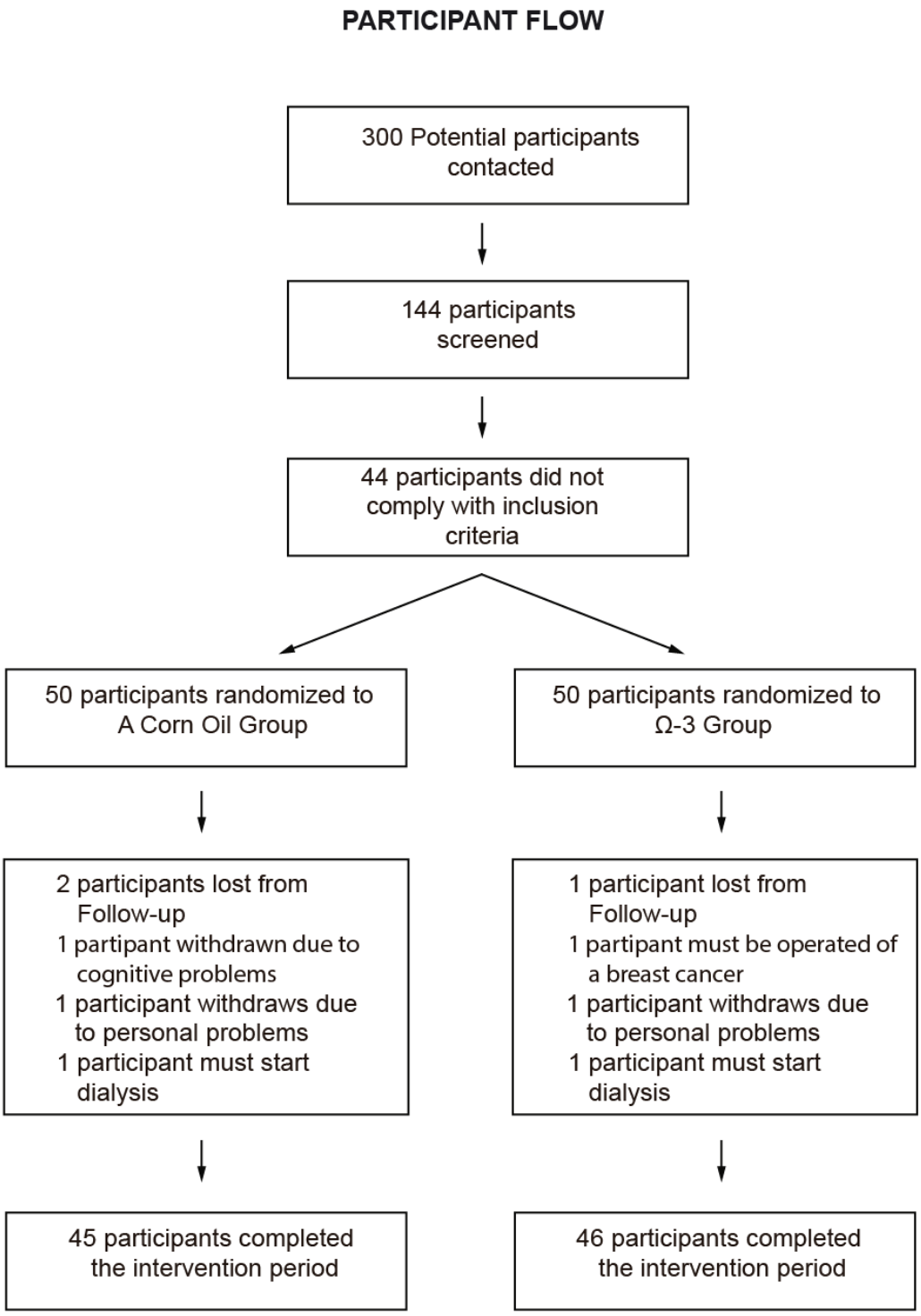
PARTICIPANT FLOW
300 Potential participants contacted
144 participants screened
44 participants did not comply with inclusion criteria
50 participants randomized to A Corn Oil Group
50 participants randomized to Ω-3 Group
2 participants lost from Follow-up
1 partipant withdrawn due to cognitive problems
1 participant withdraws due to personal problems
1 participant must start dialysis
1 participant lost from Follow-up
1 partipant must be operated of a breast cancer
1 participant withdraws due to personal problems
1 participant must start dialysis
45 participants completed the intervention period
46 participants completed the intervention period

Table 1: Initial features of randomized participants expressed as median (interquartile range)

	Gender (F/M)	Age (years)	Body mass index (kg/m2)	Urine albumin (mg/g creatinine)
Corn oil Group	26/24	66 (58-74)	31 (27.2-35.3)	604.5 (212-1234)
Ω-3 group	23/27	67 (56-74)	33.5 (30-37.3)	365.5 (157-1079)
Difference		NS	NS	NS

Table 2: Changes in red blood cell membrane phospholipids fatty acid composition. Values expressed as median (interquartile range) in mg/100 mg of total fatty acids

	Corn oil group (n=45)			Ω-3 Group (n=46)			p¶
	Basal	**Six weeks**	**Final**	**Basal**	**Six weeks**	**Final**	
Pentadecanoic Acid (C15:0)	0.2 (0-3)	2.3 (0-3.2)	2 (0-3)	0.2 (0-2.9)	1.2 (0-3.2)	2.3 (0-3.3)	b
Palmitic Acid (C16:0)	27.4 (25.2-29.9)	28.9 (27-31.1)	25.7 (23.7-28.3)	27.8 (25.5-29.2)	28.8 (26.7-30.8)	27.7 (24.8-28.9)	
Heptadecanoic Acid (C17:0)	2.4 (0.4-4.2)	3.1 (0.8-4.7)	2.3 (0.3-4.7)	1.7 (0.4-3.6)	3.1 (0.5-4.5)	2.7 (0.5-4.3)	b
Estearic Acid (C18:0)	15.6 (12.9-18.4)	15.3 (13.7-17.1)	17.5 (13.6-18.8)	16.3 (13.8-17.9)	15.6 (14-17.9)	16.9 (14.1-18.1)	
Arachidic Acid (C20:0)	0 (0-0.3)	0 (0-0.3)	0 (0-0.3)	0 (0-0.3)	0 (0-0.3)	0.2 (0-0.3)	
Eneicosanoic Acid (C21:0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	
Behenic Acid (C22:0)	0.9 (0.6-1.2)	1.1 (0.8-1.2)	0.8 (0.3-1)	1 (0.7-1.2)	1.1 (0.9-1.3)	0.9 (0.4-1.2)	
Lignocerico Acid (C24:0)	2.6 (1.4-3.4)	2.8 (2.4-3.4)	2.2 (1-3.4)	2.6 (1.3-3.3)	2.8 (2.2-3.3)	2.6 (0.8-3.3)	
Total Saturated	54.9 (50.7-58.3)	55 (51.9-57.7)	55.8 (51.7-59.8)	54.1 (49.7-58.2)	55.2 (50.1-57.8)	55.9 (52.9-62.2)	b
Miristoleic Acid (C14:1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	
Pentadecanoico Acid (C15:1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	
Palmitoleic Acid (C16:1)	0 (0-0.4)	0 (0-0.4)	0 (0-0.4)	0 (0-0.4)	0 (0-0.4)	0 (0-0.3)	
Cis 10-Heptadecanoic A Acid (C17:1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	
Oleico Acid (C18:1)	14.3 (13.3-15.7)	14.7 (13.7-15.7)	13.7 (12.1-14.5)	14.7 (13.3-15.9)	14.8 (14-16.1)	14.2 (13-15)	
Gadoleic Acid (C20:1)	0 (0-0.2)	0 (0-0.2)	0 (0-0.2)	0 (0-0.2)	0 (0-0)	0 (0-0.2)	
Erucic Acid (C22:1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	
Nervonic Acid (C24:1)	2.5 (1.5-3.1)	2.7 (2.3-3.2)	2.2 (1.7-2.7)	2.6 (1.7-3)	2.8 (2.4-3.2)	2.5 (0.9-2.8)	
Total monounsaturated	17.4 (16.2-19)	17.5 (16.9-19)	16.6 (15.3-18.5)	17.6 (16.3-19)	17.7 (17.2-19)	17.4 (15.3-18.1)	
Linoleic Acid (C18:2 n6 c)	11.8 (9.3-13.9)	12.2 (10-13.9)	11.8 (8.6-13.4)	10.9 (9.4-12.6)	11 (9.5-12.3)	9.9 (8.5-11.5)	
Gama linolenic Acid (C18:3 n6 c)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	
Alfa linolenic Acid (C18:3 n-3)	0 (0-0.2)	0 (0-0)	0 (0-0.1)	0 (0-0.1)	0 (0-0)	0 (0-0.1)	
Eicosadienoico Acid (C20:2)	0 0-0.3)	0 (0-0.3)	0.1 (0-0.3)	0 (0-0.3)	0 (0-0.3)	0 (0-0.2)	
Table 2 continuation							
	Corn oil group (n=45)			**Ω-3 Group (n=46)**			**p¶**
	Basal	**Six weeks**	**Basal**	**Six weeks**	**Basal**	**Six weeks**	

Dihomo gamalinolenic Acid (C20:3 n-6)	1.5 (1.1-1.9)	1.4 (1.1-1.7)	1.4 (1-1.9)	1.3 (0.8-1.9)	1.1 (0.8-1.5)	1.2 (0.7-1.6)	
Cis-11-14 icosatrienoico Acid (C20:3 n-3)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	
Arachidonic Acid (C20:4 n-6)	9.4 (6.1-12.5)	10.1 (6.2-12.1)	9.1 (6.4-12.6)	8.9 (5.5-11.7)	8.8 (6.6-11.1)	8.1 (5.1-10)	
Eicosapentanoic Acid (C 20:5 n-3)	0.4 (0-0.7)	0.4 (0-0.7)	0.5 (0.2-0.8)	1.3 (0.4-3)	2.8 (1.4-3.3)	2.6 (1.1-3.5)	c
Docosadienoico Acid (C22:2)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	
Docosahexanoic Acid (C22:6 n-3)	1.9 (1.3-2.8)	1.8 (1.4-2.6)	1.6 (1-2.4)	2.7 (2-3.7)	2.9 (2.2-3.8)	2.8 (2.2-4.2)	c
Total polyusaturated	27.5 (23.4-30.8)	26.6 (24.1-30.3)	26.6 (22.4-29.9)	27.6 (23.2-31.5)	27 (24.3-31.4)	26.1 (22.1-29.9)	b

¶ = significance for differences between groups determined by a mixed effects lineal regression model for repeated measures. [a] = difference between groups without intervention effect, [b]= intervention effects without differences between groups [c]= intervention effect with differences between groups (Ω-3 fatty acid effect)

Table 3: Measured parameters in both treatment groups, expressed as median (interquartile range)

	Corn oil group (n=45)			Ω-3 Group (n=46)			p¶
	Basal	Six weeks	Final	Basal	Six weeks	Final	
Clinical parameters							
Systolic blood pressure (mm Hg)	129 (121-142)	134 (123-140)	133 (123-150)	135.5 (126-153)	137 (122-150)	138.5 (128-149)	
Diastolic blood pressure (mm Hg)	81 (73-87)	80 (73-86)	83 (74-90)	81.5 (73-95)	80.5 (75-92)	84.5 (74-93)	
Weight (kg)	76.4 (69.7-91.5)	77.4 (71.7-90.5)	77.6 (70.1-93.3)	90.4 (79.1-101.2)	91.05 (77.5-100.5)	91.2 (77.6-101.9)	
Waist circumference (cm)	104 (97-113.2)	104 (97.6-114)	105 (96-113)	110.5 (103.5-122.4)	112 (104.2-120)	111.25 (104-120.6)	
Carotid intima media thickness:							
Left (mm)	0.66 (0.6-0.83)	0.67 (0.57-0.84)	0.71 (0.58-0.83)	0.71 (0.61-0.88)	0.735 (0.62-0.85)	0.695 (0.59-0.82)	
Right (mm)	0.62 (0.55-0.715)	0.62 (0.54-0.73)	0.645 (0.555-0.755)	0.68 (0.6-0.78)	0.7 (0.61-0.78)	0.71 (0.61-0.79)	
Arterial stiffness measures							
Augmentation pressure (mm Hg)	8 (5-12)	9 (5-15)	12 (5-19)	9 (5-16)	11 (6-20)	12 (4-18)	b
Augmentation index (%)	18 (9-29)	23 (11-35)	25 (14-39)	20 (8-31)	27 (12-38)	23 (9-34)	b
Reflection magnitude (%)	66 (61-73)	70 (59-75)	69 (57-75)	67 (61-73)	67 (63-73)	66 (61-74)	
Pulse wave velocity (m/s)	9.3 (8.5-10.7)	9.7 (8.3-11)	9.7 (8.3-11.2)	9.75 (8.1-10.6)	10 (7.6-10.8)	10.1 (8-10.9)	c
Liver ultrasound							
Liver steatosis score (units)	3 (2-4)	3 (2-4)	3 (3-4)	3 (2-4)	4 (2-4)	3 (3-4)	
Laboratory values							
Fasting glucose (mg/dl)	94 (86-115)	97 (89-120)	95 (86-132)	99 (83-118)	107.5 (90-143)	103.5 (90-137)	
Total cholesterol (mg/dl)	165 (138-203)	162 (135-195)	160.5 (134-195)	170 (140-204)	167.5 (141-203)	154.5 (127-176)	
Hdl cholesterol (mg/dl)	43.1 (37.1-52.3)	44.2 (36-51.6)	41.7 (35.3-53.2)	41.3 (35.2-54.4)	42.7 (35.6-53.8)	41.55 (37.8-51.8)	
Triacylglycerol (mg/dl)	147 (117-219)	133 (96-220)	150 (106-219)	163.5 (111-238)	125.5 (90-173)	117.5 (90-167)	c
Serum creatinine (mg/dl)	1 (0.82-1.76)	1.09 (0.79-1.78)	1.08 (0.81-2.06)	1.01 (0.79-1.33)	0.98 (0.75-1.57)	1.04 (0.8-1.37)	
Urine albumin excretion (mg/L)	370.6 (140.9-756.2)	344.3 (132.1-1096.5)	513.9 (185.4-978.7)	375.35 (146.5-584.4)	360.85 (162.9-1099.6)	467.9 (144.6-1005.2)	
Blood urea nitrogen (mg/dl)	22.3 (16.7-36.7)	20.5 (16.3-37.5)	20.7 (16.1-36.9)	18.95 (15-25.5)	19.6 (15.4-28.6)	19.15 (13.6-28)	
Urine creatinine excretion (mg/dl)	78 (56-100)	95 (69.5-113.5)	80 (57-118)	91.5 (62-135)	99.5 (62-126)	90.5 (68-121)	
Glycosylated hemoglobin (%)	5.9 (5.4-6.7)	6.2 (5.4-6.8)	6.2 (5.6-6.9)	6.35 (5.7-7.2)	6.45 (5.8-7.2)	6.5 (5.9-7.4)	b
Urine albumin/creatinine ratio (mg/g)	541 (173-1125)	561.5 (129-1063)	678 (149-1217)	363 (157-1030)	435 (164-950)	492 (129-1071)	

Table 3 continuation							
	Corn oil group (n=45)			**Ω-3 Group (n=46)**			**p¶**
	Basal	**Six weeks**	**Final**	**Basal**	**Six weeks**	**Final**	
Urine urea nitrogen (mg/dl)	569.6 (439.3-784.2)	570.5 (467.95-783.4)	615.6 (433.6-803.3)	629.45 (526.6-824.9)	659.4 (444.6-881.3)	594.1 (488.3-763.6)	
C Reactive protein (mg/L)	1.68 (1.11-4.11)	1.81 (0.99-4.49)	1.91 (0.99-5.22)	3.265 (1.54-5.27)	2.5 (1.31-5.35)	2.14 (1.15-5.85)	
β2 microglobulin (mg/L)	2.7 (1.9-5.1)	2.7 (2-5)	2.4 (2-5.1)	2.35 (1.8-3.4)	2.3 (1.8-3.8)	2.2 (1.7-3)	
Interleukin-6 (pg/ml)	0.3 (0.2-0.4)	0.3 (0.2-0.3)	0.2 (0.2-0.4)	0.3 (0.2-0.4)	0.3 (0.2-0.4)	0.3 (0.2-0.4)	
Cistatin-C (mg/L)	1 (1-1.9)	1.1 (1-2.1)	1.2 (1-1.8)	1.1 (0.9-1.6)	1.2 (1-1.8)	1.2 (0.9-1.6)	
Estimated glomerular filtration rate (ml/min/1.73 m^2)	68 (31.2-80)	67 (28.6-75.9)	60.1 (34.6-76.5)	60.7 (42.4-81.9)	58.5 (33.7-73.9)	60.6 (39.6-78.9)	

¶ = significance for differences between groups determined by a mixed effects lineal regression model for repeated measures. [a] = difference between groups without intervention effect, [b]= intervention effects without differences between groups [c]= intervention effect with differences between groups (Ω-3 fatty acid effect

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