



HAL
open science

Dietary omega-3 PUFA improved tubular function after ischemia induced acute kidney injury in mice but did not attenuate impairment of renal function

Katharina Rund, Shu Peng, Robert Greite, Cornelius Claassen, Fabian Nolte, Camille Oger, Jean-Marie Galano, Laurence Balas, Thierry Durand, Rongjun Chen, et al.

► To cite this version:

Katharina Rund, Shu Peng, Robert Greite, Cornelius Claassen, Fabian Nolte, et al.. Dietary omega-3 PUFA improved tubular function after ischemia induced acute kidney injury in mice but did not attenuate impairment of renal function. *Prostaglandins and Other Lipid Mediators*, 2019, pp.106386. 10.1016/j.prostaglandins.2019.106386 . hal-02396035v2

HAL Id: hal-02396035

<https://hal.science/hal-02396035v2>

Submitted on 19 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Dietary omega-3 PUFA improved tubular function after ischemia induced acute kidney**
2 **injury in mice but did not attenuate impairment of renal function**

3
4 Katharina Rund^{1#}, Shu Peng^{2#}, Robert Greite², Cornelius Claaßen¹, Fabian Nolte¹, Camille
5 Oger³, Jean-Marie Galano³, Laurence Balas³, Thierry Durand³, Rongjun Chen², Faikah
6 Gueler^{2*#}, Nils Helge Schebb^{1*#}

7 ¹ Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of
8 Wuppertal, Wuppertal, Germany

9 ² Nephrology, Hannover Medical School, Hannover, Germany

10 ³ Institut des Biomolécules Max Mousseron (IBMM), UMR 5247 CNRS, Université de
11 Montpellier, ENSCM, France

12
13
14 # Authors contributed equally

15
16 *Corresponding author (Tel: +49 202-439-3457; E-mail: nils@schebb-web.de, postal address:
17 Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of
18 Wuppertal, Gaußstr. 20, 42119 Wuppertal)

20 **Abstract**

21 Background: Acute kidney injury (AKI) is an important complication after major surgery and solid
22 organ transplantation. Here, we present a dietary omega-3 polyunsaturated fatty acid (n3-PUFA)
23 supplementation study to investigate whether pre-treatment can reduce ischemia induced AKI in
24 mice.

25 Methods: Male 12-14 week old C57BL/6J mice received a linoleic acid rich sunflower oil based
26 standard diet containing 10% fat (STD) or the same diet enriched with n3-PUFA (containing
27 1% EPA and 1% DHA) (STD+n3). After 14 days of feeding bilateral 30 min renal ischemia
28 reperfusion injury (IRI) was conducted to induce AKI and mice were sacrificed at 24 h. Serum-
29 creatinine and blood urea nitrogen (BUN) as well as liver enzyme elevation were measured.
30 Kidney damage was analyzed by histology and immunohistochemistry. Furthermore,
31 pro-inflammatory cytokines (IL-6, MCP-1) were determined by qPCR. FA and oxylipin pattern
32 were quantified in blood and kidneys by GC-FID and LC-MS/MS, respectively.

33 Results: n3-PUFA supplementation prior to renal IRI increased systemic and renal levels of
34 n3-PUFA. Consistently, eicosanoids and other oxylipins derived from n3-PUFA including
35 precursors of specialized pro-resolving mediators were elevated while n6-PUFA derived
36 mediators such as pro-inflammatory prostaglandins were decreased. Feeding of n3-PUFA did
37 not attenuate renal function impairment, morphological renal damage and inflammation
38 characterized by IL-6 and MCP-1 elevation or neutrophil infiltration. However, the tubular
39 transport marker alpha-1 microglobulin (A1M) was significantly higher expressed in proximal
40 tubular epithelial cells of STD+n3 compared to STD fed mice. This indicates a better integrity of
41 proximal tubular epithelial cells and thus significant protection of tubular function. In addition,
42 heme oxygenase-1 (HO-1) which protects tubular function was also up-regulated in the
43 treatment group receiving n3-PUFA supplemented chow.

44 Discussion: We showed that n3-PUFA pre-treatment did not affect overall renal function or renal
45 inflammation in a mouse model of moderate ischemia induced AKI, but tubular transport was

46 improved. In conclusion, n3-PUFA food supplementation altered the oxylipin levels significantly
47 but did not protect from renal function deterioration or attenuate ischemia induced renal
48 inflammation.

49

50 **Introduction**

51 Acute kidney injury (AKI) is a frequent complication after major cardiac surgery, solid organ
52 transplantation and also after trauma surgery. Onset of AKI increases morbidity and mortality of
53 the patients and is characterized by an increase in serum creatinine and/or reduction of the
54 glomerular filtration rate (GFR) [1]. In a review of 25,182 trauma patients post-traumatic AKI was
55 as high as 24% and these patients had a 3.4-fold higher risk of death compared to non-AKI
56 patients [2]. Also, after myocardial infarction mortality was 3-fold increased in patients with AKI
57 and incidence of major cardiac events (MACE) was with 26% higher than in non-AKI patients
58 during the three year follow-up [3]. It has been shown that AKI is not only an acute event but
59 also increases the risk for chronic kidney disease (CKD) [4]. Even if the serum creatinine
60 elevation returns to normal levels shortly after AKI a significant proportion of patients will develop
61 CKD or will even proceed to end stage renal disease with the need of renal replacement therapy
62 [5]

63 Renal ischemia reperfusion (IRI) injury due to hypotension, major bleeding and hypoxia causes
64 intrarenal vasoconstriction with activation of pro-oxidative mechanisms, release of pro-
65 inflammatory cytokines and subsequent leukocyte invasion [6-9]. The severity of AKI correlates
66 with the long-term outcome and progression to CKD [5].

67 Established strategies to prevent or attenuate AKI even in scheduled surgeries such as cardiac
68 valve replacement are lacking. Several studies discussed a beneficial role of omega-3
69 polyunsaturated fatty acids (n3-PUFA) in the context of kidney diseases [10-19]. For the
70 prevention of cardiovascular diseases the nutritional-status of n3-PUFA is a well-established risk
71 factor [20, 21]. The higher the incorporation of n3-PUFA, determined by the relative content of
72 EPA and DHA in the cells, typically red blood cells [20, 22] the lower the risk for cardiovascular
73 diseases and mortality [23]. It has to be noted that n3-PUFA are essential constituents of the
74 human diet and that the nutritional status and not the intake of a n3-PUFA supplement is related

75 to their physiological effects. Thus, if only the intake of n3-PUFA by supplementation is
76 correlated to the risk for cardiovascular diseases the outcome is less clear [24, 25].
77 In the context of renal diseases effects of n3-PUFA supplementation on the outcome in kidney
78 transplantation are inconsistent [26]. However, in some experimental AKI models the
79 administration of n3-PUFA correlated with an improved kidney function [10-15].
80 The molecular mechanisms underlying beneficial effects of n3-PUFA include direct actions, e.g.
81 binding to ion channels or transcription factors, e.g. NFκB or PPARγ, thereby reducing the
82 expression of pro-inflammatory and activating the expression of anti-inflammatory genes,
83 respectively. As constituents of membranes, e.g. predominantly in phospholipids, n3-PUFA also
84 impact membrane structure and fluidity [27]. However, it is beyond doubt that a relevant part of
85 the physiological effects of n3-PUFA are mediated by their oxygenated products, i.e.
86 eicosanoids and other oxylipins [27-29]. In the so-called arachidonic acid (ARA) cascade PUFA
87 serve as substrates for enzymatic and non-enzymatic conversion resulting in a multitude of lipid
88 mediators from both n6- and n3-PUFA (Fig. 1) [27, 28, 30]. Enzymatic conversion of PUFA in the
89 ARA cascade comprises three major pathways: (I) cyclooxygenases (COX) lead to the formation
90 of prostanoids and thromboxanes, (II) cytochrome P450 monooxygenases (CYP) give rise to
91 epoxy-PUFA and terminal hydroxy-PUFA and (III) lipoxygenases lead via hydroperoxy-PUFA to
92 hydroxy-PUFA and leukotrienes [31]. Structurally similar to these enzymatically formed products
93 oxidized PUFA also arise during non-enzymatic autoxidation, e.g. isoprostanes, which also have
94 been shown to possess biological activity [32, 33]. In the kidney, oxylipins have important
95 functions in (patho-)physiology, e.g. regulation of renal blood flow, glomerular filtration rate and
96 tubular transport function [34-37]. As n3-PUFA compete with ARA for conversion dietary n3-
97 PUFA supplementation impacts the overall oxylipin profile resulting in a shift from predominantly
98 pro-inflammatory, e.g. 2-series prostaglandins, towards n3-PUFA derived oxylipins with less
99 potency or even anti-inflammatory properties [27]. Furthermore, in recent years activity of LOX
100 with n3-PUFA have been related to the formation of multiple hydroxylated PUFA, e.g. resolvins,

101 maresins and protectins which have been attributed to be actively involved in the resolution of
102 inflammation [38], though several studies fail to detect them in biological samples [39, 40].
103 Changes in the oxylipin pattern in the context of kidney function in AKI have been sparsely
104 investigated. Therefore, we used a well characterized model of ischemia induced AKI in mice
105 [41, 42] to study the effect of dietary n3-PUFA (EPA and DHA) supplementation based on a
106 linoleic acid rich western-like diet [43] for 14 days prior to surgery on the fatty acid composition
107 and oxylipin pattern in blood and tissue in comparison to renal damage and inflammation at 24 h
108 after injury.
109

110 **Materials and methods**

111 Chemicals

112 Oxylin and deuterated oxylin standards were purchased from Cayman Chemicals (local
113 distributor: Biomol, Hamburg, Germany). Methyl pentacosanoate (FAME C25:0) was obtained
114 from Santa Cruz Biotechnology (Heidelberg, Germany). PD1 and isoprostane standards were
115 included in the method based on in-house synthesis as described [40, 44]. HPLC grade and LC-
116 MS-grade methanol (MeOH), LC-MS-grade acetonitrile (ACN), LC-MS-grade isopropanol, LC-
117 MS-grade acetic acid and HPLC grade methyl *tert*-butyl ether (Acros Organics) were purchased
118 from Fisher Scientific (Schwerte, Germany). Sodium hydrogen phosphate and *n*-hexane (HPLC
119 grade) were obtained from Carl Roth (Karlsruhe, Germany). Ammonium acetate (p.a.) was
120 obtained from Merck (Darmstadt, Germany) and potassium hydroxide (85%) from Gruessing
121 GmbH (Filsum, Germany). Ethyl acetate, acetyl chloride and potassium carbonate (anhydrous)
122 were purchased from Sigma Aldrich (Schnelldorf, Germany).

123

124 Feeding experiment and renal ischemia reperfusion injury in mice

125 C57BL/6J^{ham-ztm} male mice (12–14 weeks of age) were obtained from the institute of laboratory
126 animal science (Hannover Medical School, Germany). Mice were cared for in accordance with
127 the institution's guidelines for experimental animal welfare and with the guidelines of the
128 American Physiological Society. All experiments were approved by the animal protection
129 committee of the local authorities (Lower Saxony state department for food safety and animal
130 welfare, LAVES; approval 33.19-42502-04-14/1657). Mice were housed under conventional
131 conditions with a 14/10 h light/dark cycle. Mice were divided into two feeding groups receiving
132 either a sunflower oil based standard diet (STD) or the same diet enriched with n3-PUFA
133 containing 1% eicosapentaenoic acid (EPA) and 1% docosahexaenoic acid (DHA) as ethyl
134 esters (10% each in fat; STD+n3) based on a standard experimental diet (ssniff Spezialdiaeten
135 GmbH, Soest, Germany). Both diets contained in total 10% fat. During the whole feeding period

136 every 2-3 days fresh chow was provided and animals had free access to the food and domestic
137 quality drinking water.

138 After 14 days of feeding, renal IRI was initiated in general isoflurane anesthesia (5% induction,
139 2% maintenance) combined with iv butorphanol as analgetic treatment. IRI was induced by
140 transient bilateral renal pedicle clamping for 30 min using a non-traumatic vascular clamp [45].
141 Mice were sacrificed 24 h after reperfusion by deep general anesthesia and total body perfusion
142 with ice cold PBS [45].

143 IRI experiments were conducted in three sets each with n=7-8 animals in the STD and STD+n3
144 fed group. In one of these experiments additionally a sham group (n=5) receiving STD chow was
145 included which underwent midline laparotomy but without renal pedicle clamping.

146 Blood was collected at baseline (before starting the feeding) and at 24 h after reperfusion. An
147 aliquot of 30 μ L whole blood was diluted with 150 μ L deionized water for fatty acid analysis and
148 EDTA-plasma was generated by centrifugation (4000 \times g, 10 min, 4°C) and stored at -80°C until
149 analysis for clinical chemistry and oxylipins. Kidneys were collected at endpoint, dissected and
150 immediately processed as follows: one piece was fixed in paraformaldehyde for histology, one
151 piece was stored in RNAlater for qPCR and one piece was shock frozen in liquid nitrogen and
152 stored at -80°C for fatty acid and oxylipin analysis. Renal function (serum-creatinine and BUN)
153 and liver enzymes (aspartate transaminase: AST, alanine aminotransferase: ALT) were
154 measured in EDTA-plasma by an Olympus analyzer (AU400) in an automated fashion according
155 to the manufacturer's instruction.

156

157 Histology and immunohistochemistry

158 The middle part of the kidney was fixed in 4% paraformaldehyde (PFA) overnight and embedded
159 in paraffin. Two μ m paraffin sections were cut and PAS (Perjodic Schiff's Acid) stain according
160 to standard diagnostic protocols was done. For determination of AKI scores a semi-quantitative
161 grading system was used: 0 = focal AKI with <5% of tubuli of the cortex affected, 1 = mild AKI

162 with 5-25% of tubuli affected, 2 = moderate AKI with 26-50% of tubuli affected, 3 = severe AKI
163 with 51-75% of the tubuli affected, 4 = very severe AKI with > 75% of tubuli affected.
164 Immunohistochemistry for neutrophil infiltration (Gr-1 antibody, Biorad), the tubular function
165 marker alpha-1 microglobulin (A1M; gift from Magnus Gram, Lund University) and heme
166 oxygenase-1 (HO-1 antibody; Enzo Life Sciences, Switzerland) was done on paraffin sections.
167 Sections were incubated with trypsin for 15 min at 37°C for antigen retrieval. Nonspecific binding
168 sites were blocked with 10% normal donkey serum (Jackson ImmunoResearch Lab, West
169 Grove, USA) for 30 min and then primary antibodies (Invitrogen, California, USA) were
170 incubated for 60 min at room temperature in the dark. Afterwards secondary antibodies
171 (Invitrogen, California, USA) were incubated for additional 60 min in the dark. Analysis was
172 performed in a blinded manner using a Leica imaging microscope. Gr-1 positive neutrophil
173 infiltration was scored with a 0-4 grading system in 10 different view fields (VF) per section in
174 200-fold magnification: 0 = no infiltrates, 1 = mild infiltrates with <5 cells/VF, 2 = moderate
175 infiltrates with 6-10 cells/VF, 3 = severe infiltration with 11-25 cells/VF, 4 = very severe infiltration
176 with >25 cells/VF. Semiquantitative analysis of the percentage of A1M or HO-1 positive proximal
177 tubuli in the cortex was done in 10 different VF per section.

178

179 Detection of pro-inflammatory cytokines by qPCR

180 For mRNA work-up one part of the kidneys was fixed in RNAlater immediately. For total RNA
181 extraction the RNeasy mini kit system (Qiagen, Hilden, Germany) was used and RNA was
182 transcribed with Qiagen mini kits. For quantitative PCR (qPCR) 1 µg of DNase-treated total RNA
183 was reverse transcribed using Superscript II reverse transcriptase (Invitrogen) and qPCR was
184 performed on a Lightcycler 420 II (Roche Diagnostics, Penzberg, Germany) using FastStart
185 Sybr-Green chemistry. Gene-specific primers for MCP-1 (QT00167832) and IL-6 (QT00098875)
186 were used. For normalization HPRT (QT00166768) was used as housekeeping gene.
187 Quantification was carried out using qgene software.

188
189 Extraction and quantification of fatty acids
190 The fatty acid composition was analyzed in diluted whole blood (15 μ L blood + 75 μ L water) and
191 kidney tissue (15 \pm 2 mg) as fatty acid methyl esters using gas chromatography with flame
192 ionization detection (GC-FID) as described with slight modifications [46]. Briefly, 10 μ L internal
193 standard (FAME C25:0, 750 μ M) was added to diluted whole blood and kidney tissue samples.
194 Kidney tissue was homogenized in 300 μ L MeOH and 50 μ L water using two stainless steel
195 beads (3 mm, 5 min, 25 Hz) with a vibration ball mill (MM 400, Retsch, Haan, Germany). Lipids
196 were extracted with methanol/methyl *tert*-butyl ether and the lipid extract was trans-esterified to
197 fatty acid methyl esters using methanolic hydrogen chloride. The calculation of the absolute fatty
198 acid concentrations and relative pattern was based on response factors as described [46].

199
200 Extraction and quantification of oxylipins
201 Oxylipins in plasma and kidney tissue were extracted using anion exchange Bond Elut Certify II
202 SPE cartridges (Agilent, Waldbronn, Germany) as described with modifications [40, 44]. In the
203 first step of oxylipin extraction 10 μ L antioxidant solution and internal standards were added to
204 each sample.

205 For the determination of free oxylipins in plasma (150-200 μ L) the 2.8-fold volume of MeOH was
206 added and the samples were stored at -80°C for 30 min. After centrifugation the supernatant
207 was diluted with disodium hydrogen phosphate buffer yielding a MeOH content \leq 18% (pH 6.0)
208 and loaded on the preconditioned SPE cartridge.

209 For analysis of free oxylipins in kidney tissue (50 \pm 5 mg) samples were homogenized with
210 300 μ L MeOH as described [47]. After centrifugation the supernatant was diluted with 2.7 mL
211 sodium phosphate buffer (pH 6.0) and loaded on the preconditioned SPE cartridge.

212 For quantification of total, i.e. free and esterified oxylipins in kidney tissue (20 \pm 2 mg) samples
213 were homogenized in 400 μ L isopropanol and stored for 30 min at -80°C. Samples were

214 centrifuged and the supernatant was hydrolyzed (300 μ L 1.5M KOH (75/25, MeOH/water, v/v),
215 immediately neutralized with acetic acid, diluted with 2 mL sodium phosphate buffer (pH = 5.5)
216 and loaded on the preconditioned SPE cartridge.

217 The SPE procedure was carried out as described and samples were analyzed by LC-MS/MS
218 (QTRAP, Sciex, Darmstadt, Germany) in scheduled selected reaction monitoring mode following
219 negative electrospray ionization as described [40, 44].

220

221 Data analysis

222 Data evaluation and statistical analyses were performed using GraphPad Prism software for
223 Windows (version 5.0, La Jolla California USA). Data are presented as mean \pm standard error of
224 mean (SEM). For oxylipin and fatty acid analysis for the calculation of the mean $\frac{1}{2}$ lower limit of
225 quantification (LLOQ) was used if the concentration was below the LLOQ. The concentration
226 was set to LLOQ if the analyte could not be quantified in more than 50% of the samples in one
227 group. For comparison of two groups two-tailed unpaired student's t-test was used. For multiple
228 comparisons ANOVA with post hoc Tukey correction was applied. Differences were considered
229 significant at a p-value <0.05 (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

230

231 **Results**

232 Bodyweight and food intake of both feeding groups were not changed between the STD and the
233 STD+n3 diet.

234 **Blood and tissue pattern of fatty acids and their oxidative metabolites**

235 After 14 days of feeding an n3-PUFA enriched sunflower oil based diet (1% EPA and 1% DHA in
236 the chow, STD+n3) absolute and relative levels of n3-PUFA in the kidney and in the circulation
237 were significantly increased predominantly at the expense of n6-PUFA compared to mice on the
238 STD diet (Fig. 2 A+C). Considering the very low levels of EPA in tissue and in the circulation of
239 mice on the STD diet, especially relative levels of EPA were massively increased in response to
240 the STD+n3 diet. Interestingly, in whole blood the STD+n3 diet led to an overall decrease in the
241 absolute FA level. The relative concentration of the sum of EPA+DHA, reflecting the
242 endogenous n3-PUFA status, increased 2.5- and 3.7-fold in the kidney and whole blood,
243 respectively (Fig. 2 B+D) indicating successful modulation of the n3-PUFA content by the
244 supplementation.

245 Consistent to the changes in the PUFA pattern, levels of eicosanoids and other oxylipins derived
246 from ARA, EPA and DHA were massively altered in response to the STD+n3 diet, while
247 concentrations of oxylipins in the plasma and kidney were almost similar in the IRI treated group
248 compared to the sham group both fed with the STD diet (Fig. 3). Comparing levels of individual
249 oxylipins 24 h after IRI a similar trend was observed for free metabolites in the plasma as well as
250 for free and esterified oxylipins in kidney tissue, though levels of esterified oxylipins were
251 massively higher than the respective free mediators: Overall STD+n3 diet led to a significant
252 decrease of ARA derived metabolites from all major formation pathways while EPA and DHA
253 derived oxylipins were significantly elevated. Especially, regarding levels of free prostanoids in
254 the kidney tissue, levels of ARA derived prostaglandins such as pro-inflammatory PGE₂ or the
255 prostanoid TxB₂ were elevated following IRI compared to sham, while STD+n3 feeding caused
256 their significant decrease and concomitant formation of the less potent EPA derived
257 counterparts, i.e. PGE₃ and TxB₃ (Fig. 3 B). Amongst others in the circulation as well as in the

258 kidney also a significant increase of 17-HDHA and 18-HEPE, precursors for the formation of pro-
259 resolving mediators (SPM), was observed in response to STD+n3. However, except for DHA
260 derived protectin D1 (PD1) and its isomer PDX no signals above the lower limit of quantification
261 (LLOQ), i.e. 0.25-2 nmol/kg in kidney tissue and 0.07-0.56 nM in plasma, were detected for all
262 SPM. LC-MS/MS signals for PD1 indicated relevant apparent concentrations in plasma and
263 kidney of STD+n3 fed mice (Fig, 3, SI Fig. S1). However, the ratios between monitored
264 transitions differed significantly between the standard and the biological samples (Fig. S1).
265 Similar to enzymatically formed oxylipins, levels of prostaglandin-like autoxidation products
266 formed esterified in phospholipids were massively altered by n3-PUFA feeding. Total levels of
267 ARA derived 5(*R,S*)-F_{2t}-IsoP were decreased, while EPA and DHA derived 5(*R,S*)-5-F_{3t}-IsoP
268 and 4(*R,S*)-4-F_{4t}-NeuroP were elevated in response to STD+n3. Interestingly, levels of these
269 peroxidation products were lower following IRI compared to sham on the same STD diet (Fig.
270 S2).

271 **Renal function, renal morphology and inflammation**

272 Despite successful n3-PUFA supplementation indicated by the changes of PUFA and oxylipin
273 patterns renal function impairment was similar in both groups. IRI caused a 6-fold elevation of
274 serum creatinine (Fig. 4 I). In line with renal function deterioration, renal damage measured by
275 AKI score was similar in both groups (Fig. 4 A, D, G). Furthermore, Gr-1 positive neutrophil
276 infiltration showed moderate to severe leukocyte infiltration in both groups. On mRNA-level the
277 pro-inflammatory cytokines IL-6 and MCP-1 were elevated in both groups. The STD+n3 group
278 even showed a trend towards higher values compared to the STD group (Fig. 4 C, F).

279 **Tubular function and heme oxygenase-1 expression**

280 Alpha-1 microglobulin (A1M) is synthesized in the liver, filtered by the glomeruli and reabsorbed
281 by proximal tubuli. In healthy kidneys A1M is present in vesicles in the cytoplasm of 60-70% of

282 the tubuli. Upon AKI tubuli have a breakdown in energy metabolism and impaired transport
283 function leading to lower cytoplasm concentration of A1M [48]. Due to IRI A1M expression
284 decreased but was significantly higher in the STD+n3 group indicating better preservation of the
285 tubular homeostasis and energy metabolism (Fig. 5 A, C, F). Heme oxygenase-1 (HO-1) is a
286 renoprotective enzyme which is upregulated after AKI and mediates healing [49]. HO-1
287 expression was significantly higher in STD+n3 fed mice (Fig. 5 B, D, G). Liver enzymes were
288 elevated after IRI due to distant organ injury [50]. STD+n3 treatment caused less elevation of
289 liver enzymes compared to STD (Fig. 5 E). Overall, we observed some protective effects by the
290 STD+n3 diet in the context of renal IRI (Fig. 5).

291

292 **Discussion**

293 Renal IRI is a common cause of AKI which is associated with high morbidity and mortality rates
294 [7]. Particularly in the context of solid organ transplantations IRI is inevitable contributing to
295 impaired allograft function [51]. Cardiac surgery in elderly patients with slightly decreased renal
296 function has incidence rates of AKI of ~30% [52]. In the context of scheduled surgeries a dietary
297 intervention would be possible, while the patients are waiting for the surgery. Beneficial effects of
298 n3-PUFA supplementation have been reported, however in the context of kidney transplantation
299 the outcome is inconsistent [26].

300 In the present study we investigated if dietary n3-PUFA supplementation improves renal function
301 impairment in an ischemia induced AKI model following renal IRI in mice [42]. The control group
302 received a chow, reflecting the omega-6 rich western diet [43] containing 10% linoleic acid rich
303 sunflower oil (STD). For the investigation of potential beneficial effects of n3-PUFA
304 supplementation on the base of a western diet this chow was enriched with n3-PUFA (1% EPA
305 and 1% DHA; STD+n3) as previously described [22]. In order to ensure a maximal modulation of
306 the tissue fatty acid composition by the dietary PUFA supplementation a pre-feeding period of 14
307 days was included prior induction of renal IRI. After 14 days of feeding the STD+n3 chow, the
308 relative fatty acid pattern in the kidney, e.g. relative levels of the sum of saturated fatty acids,
309 monounsaturated fatty acids, n6-PUFA and n3-PUFA (38.3%, 10.3%, 24.6% and 26.9%
310 respectively), was comparable to the relative levels observed after 45 days of feeding the same
311 diet to mice (38.1%, 13.0%, 25.2% and 23.9% respectively) [22] indicating that a steady-state in
312 the modulation of the kidney fatty acid composition by the feeding was reached after 14 days.
313 This is also consistent with results from feeding fish oil to mice where a steady state of the PUFA
314 status in heart and brain tissue was reached between 1 and 2 weeks [53]. Overall, feeding of the
315 STD+n3 diet led to a massive elevation of absolute and relative levels of n3-PUFA (18:4n3,
316 20:4n3, 20:5n3, 22:5n3, 22:6n3) with simultaneous decrease in n6-PUFA (18:3n6, 20:2n6,
317 20:3n6, 20:4n6, 22:4n6, 22:5n6) in the circulation and in the kidney. These changes are in line

318 with previous reports showing extensive modulation of PUFA composition in blood
319 compartments and tissues by n3-PUFA feeding [22, 54-58] or by endogenous n3-PUFA
320 accumulation in transgenic fat-1 mice [22, 54, 59]. Compared to the STD chow, in response to
321 the STD+n3 feeding the relative content of the main long-chain PUFA ARA, EPA and DHA in the
322 kidney changed from 16.4% to 8.6% (0.5 fold), 0.04% to 4.1% (99 fold) and 10.1% to 21.0% (2
323 fold), respectively. Similar alterations in the renal fatty acid composition were also observed in
324 mice and rats fed chow containing 1-3% each EPA and DHA (in each case in almost equal
325 amount) [22, 55-57]. Relative levels in the kidney in response to STD+n3 are similar to earlier
326 findings for n3-PUFA feeding in rodents ranging for EPA from 1-4.6% in mice and from 5.2-9.7%
327 in rats and for DHA from 6.7-24.6% in mice and from 5.5-7.8% in rats depending primarily on the
328 diet composition (e.g. ratio of n3/n6-PUFA). The differences in relative renal levels of EPA and
329 DHA between mice and rats mainly originate from differing PUFA compositions under basal
330 conditions (steady-state without n3-PUFA supplementation) [60]. The relative renal PUFA
331 composition in response to STD+n3 was similar to the relative amount in murine renal
332 phospholipids, especially phosphatidylcholines (9-12.4, 2.3-7.5 and 10-18.1% of ARA, EPA and
333 DHA respectively) observed after feeding fish oil supplemented chow [54, 58], reflecting that
334 phospholipids as main membrane constituents are dominant lipids in the kidney [61, 62].
335 It should be noted, that the dietary supplementation by STD+n3 led to a more pronounced
336 modulation of the n3-PUFA pattern compared to endogenous synthesis in transgenic fat-1 mice
337 [22, 54]: The relative levels in transgenic fat-1 mice for ARA, EPA and DHA of 20.3%, 1% and
338 12.1% in the kidney [22] and of 11.3%, 1%, 12.9% in kidney phospholipids, respectively [54]
339 reveal that relative levels reached by STD+n3 feeding are around 4.1 fold and 1.7 fold higher for
340 EPA and DHA respectively.
341 The successful modulation of the endogenous n3-PUFA status by the feeding was also clearly
342 indicated by the pronounced changes in %EPA+DHA levels in blood. In whole blood, which is
343 dominated by the PUFA of erythrocytes, in response to the STD+n3 diet 3.7 fold higher levels of

344 %EPA+DHA were observed compared to the STD diet (4.5 ± 0.1 in STD vs. $16.9 \pm 0.3\%$ in
345 STD+n3). Extrapolating from mice to man, these concentrations reflect a very high endogenous
346 n3-PUFA status, e.g. determined by the omega-3 index, i.e. the relative content of EPA+DHA in
347 membranes of erythrocytes. With a value of 16.9% the endogenous n3-PUFA levels reached in
348 response to the STD+n3 feeding are significantly higher than those which efficiently reduce
349 overall mortality and the risk for cardiovascular diseases (i.e. an omega-3 index ≥ 8) in men [20,
350 21]. In contrast, the STD diet reflects the “western-diet” causing a low omega-3 index, even in
351 mice, which more efficiently convert short chain n3-PUFA to long chain n3-PUFA than men [63].
352 Thus, it can be concluded that the n3-PUFA feeding strategy used in this study reflects the
353 maximal shift in the PUFA pattern which can be reached. On a side note, a well described
354 clinical effect of n3-PUFA is a reduced serum triglyceride concentration [64, 65]. Indeed, the total
355 FA and thus TG concentration in whole blood was reduced in the animals receiving the STD+n3
356 compared to the STD diet (Fig. 2).

357 It is believed that a major portion of beneficial effects of n3-PUFA are mediated by changes in
358 the pattern of oxygenated PUFA, i.e. eicosanoids and other oxylipins [27, 29]. As eicosanoids
359 exert important functions in renal (patho-)physiology including regulation of renal blood flow,
360 glomerular filtration rate and tubular transport function [34-37] modulation of the oxylipin pattern
361 by n3-PUFA may also impact kidney function in renal IRI.

362 Reflecting changes in the PUFA composition, the feeding strategy used in this study resulted in
363 massive alterations of oxylipins in blood and renal tissue from all major formation pathways
364 (COX, LOX, CYP as well as non-enzymatic): ARA derived eicosanoids were significantly
365 decreased while EPA and DHA derived oxylipins were significantly elevated in response to
366 STD+n3 feeding (Fig. 3). Reduction of ARA derived eicosanoids was similar in plasma and
367 kidney (free and total) for all investigated pathways with 40-75% lower levels after STD+n3
368 compared to STD feeding. Similarly, DHA derived oxylipins were 1.5-3 fold increased in the
369 kidney, however a more pronounced elevation (7-12 fold) was observed for DHA derived

370 products from 12/15-LOX as well as CYP in plasma. In contrast, EPA derived metabolites were
371 increased to a greater extent (12-53 fold) and in several cases were below the LLOQ in STD
372 while clearly elevated in STD+n3 fed mice. These changes are overall consistent with earlier
373 studies after n3-PUFA (EPA and DHA) supplementation in mice [13, 22, 54, 66-69], rats [55, 66,
374 70-72] and humans [67, 73-75].

375 The strongest decrease was observed in kidney levels of ARA derived prostanoids, i.e. PGE₂
376 (250 vs. 62 nmol/kg) and TxB₂ (6.9 vs <1.2 nmol/kg) and similar to earlier studies with EPA and
377 DHA supplemented chow in mice [54, 66, 69]. Though respective EPA derived counterparts
378 were simultaneously increased resulting levels were considerably lower compared to their
379 corresponding ARA derived prostanoids which is in line with their lower conversion rate by COX
380 in addition to a simple PUFA competition [76]. Considering that EPA derived prostanoids are
381 less bioactive, e.g. PGE₃ possesses less affinity towards EP receptors and exhibits lower
382 potency regarding second messenger release [77] and also have been shown to cause less
383 secretion of pro-inflammatory IL-6 [78] compared to PGE₂, this clear shift in the renal prostanoid
384 pattern suggests that STD+n3 can contribute to a lower inflammatory status.

385 Regarding CYP derived metabolites a massive increase in levels of free terminal epoxy-PUFA
386 derived from EPA and DHA, i.e. 17(18)-EpETE and 19(20)-EpDPE, was observed especially in
387 plasma, concomitant with a relatively marked decrease (16 vs 5 nmol/kg in kidney and 1.3 vs
388 0.4 nM in plasma) in ARA derived 14(15)-EpETrE. Besides displacement of ARA by the n3-
389 PUFA in response to the STD+n3 feeding this profound increase in n3-PUFA derived epoxy-
390 PUFA can be explained by equal or higher conversion rates of DHA and EPA by several CYP
391 isoforms in comparison to ARA [55, 79]. This marked elevation in n3-PUFA derived terminal
392 epoxy-PUFA in plasma following STD+n3 is similar to their clear increase observed in human
393 plasma after fish oil supplementation [73, 80] supporting their ability to function as indicator for
394 n3-supplementation as has been suggested [39]. Studies regarding the physiological role of
395 epoxy-PUFA in the kidney are mainly focused on ARA derived EpETrE and comprise, e.g.

396 regulation of renal blood flow, inhibition of tubular sodium transport thereby promotion of salt
397 excretion as well as anti-inflammatory effects [81]. Treatment of renal IRI in mice with an
398 inhibitor of the soluble epoxide hydrolase have been shown to ameliorate kidney injury
399 suggesting that epoxy-PUFA have beneficial effects on kidney function in renal IRI [82].
400 Similarly, n3-PUFA derived EpETE and EpDPE might impact renal physiology, e.g. owing to
401 their higher potency regarding vasodilatory actions compared to EpETrE as well as similar anti-
402 inflammatory effects [81]. Regarding epoxy-PUFA, 19(20)-EpDPE reduced kidney fibrosis in a
403 murine unilateral ureteral obstruction (UUO) model [83]. In contrast, in renal IRI administration of
404 19(20)-EpDPE led to aggravation of kidney damage in mice while 14(15)-EpETrE as well as
405 DHA itself alleviated kidney injury in mice [84].

406 In the past two decades, a class of multiple hydroxylated PUFA were proposed which actively
407 terminate inflammation, so-called specialized pro-resolving mediators (SPM). From DHA,
408 D-series resolvins as well as protectins and maresins are formed, while EPA gives rise to E-
409 series resolvins [38]. Because of their low biological levels, these mediators are difficult to
410 analyze [40]. Consistent with earlier reports [22, 66, 70] feeding of the n3-PUFA EPA and DHA
411 led to a massive increase (2-9 fold) in the precursors of SPM, i.e. 17-HDHA, 14-HDHA and 18-
412 HEPE. For example, the 18-HEPE content in the kidney in the STD+n3 group was 9.7 nmol/kg
413 while in the control group it was below the LLOQ (1 nmol/kg). However, except for PD1 all
414 signals for SPM were below the LLOQ, i.e. in most cases below 2 nmol/kg kidney tissue. Thus, it
415 has to be concluded that a 14-day feeding strategy with high n3-PUFA content (2%) does not
416 increase the concentration of these mediators above 2 nmol/kg tissue or 0.56 nM blood. Either
417 the SPM influence physiology in these picomolar, i.e. ppt (pg/g) concentrations or one has to
418 conclude that they may not play a role in the physiological effects caused by n3-PUFA
419 supplementation.

420 The signals for free PD1 in the kidney in response to STD+n3 are in a similar range to a
421 previous report detecting PD1 after fish oil supplementation in mice undergoing renal IRI [13].

422 Analysis of total oxylipins in the kidney suggested even higher levels of PD1 (SI, Fig. S1). As
423 internationally agreed for LC-MS compound identification (e. g. for pesticides [85]), we evaluated
424 two mass transitions, i.e. m/z 359 \rightarrow 153 [13, 86-88] and 359 \rightarrow 206 [86, 89, 90] which also
425 have been previously used for PD1 detection. Particularly in the analysis of the total PD1
426 concentration in the kidney massive divergence in the area ratio of the two transitions (compared
427 to authentic PD1) results (SI Fig. S1). This clearly suggests that the signals result from isobaric
428 interferences from the sample matrix and not from PD1 (SI Fig. S1). For free oxylipins in the
429 kidney no signal was observed for m/z 359 \rightarrow 206 and thus, it is even more clear that the signal
430 of PD1 results from interfering matrix and not from the SPM. Isobaric interferences at the
431 retention time of PD1 in reversed-phase HPLC at m/z 359 \rightarrow 153 causing an apparent PD1
432 concentration have been recently also reported for plasma samples after storage [91]. By
433 contrast, the relative intensities of the signals of the PD1-isomer PDX showed identical ratios
434 between standard and samples (SI Fig. S1). Overall our data do not support the earlier
435 described renal formation of PD1 in a mouse model of AKI following feeding a slightly lower
436 dose of an n3-PUFA (1.4% n3-PUFA in chow compared to 2% in this study) [13].
437 N3-PUFA are prone to oxidation and may lead to increased oxidative stress. Indeed, in humans
438 receiving a very high dose of DHA (1.6 g/d) urinary 15-F_{2t}-IsoP, a marker of oxidative stress,
439 was increased [92]. This could be even more important in ischemic injury were enhanced
440 oxidative stress plays a key role in pathophysiology [6]. However, our data reveal, that the
441 STD+n3 group did not show elevated IsoP levels in the kidney as well as in plasma following IRI
442 compared to the STD diet (Fig. 3, SI Fig. S2). Similarly, lower levels of the autoxidation marker
443 malondialdehyde were observed in the kidney tissue of rats treated with n3-PUFA after renal IRI
444 [11, 12]
445 Altogether feeding of STD+n3 led to a maximal modulation in n3-PUFA and its lipid mediators
446 dominated by n3-PUFA derived oxylipins. Thus, the feeding strategy allows to evaluate the
447 effects of maximal dietary n3-PUFA modulation based on a western diet on acute kidney injury.

448 Despite the clear effects on oxylipins the STD+n3 diet did not result in improvement of overall
449 renal function or reduction of pro-inflammatory cytokine production or leukocyte infiltration 24 h
450 after IRI. However, there were measurable effects on protective molecular mechanisms such as
451 up-regulation of HO-1. It has been shown that HO-1 fosters renal regeneration and healing in
452 several models of renal injury [45, 93, 94]. In addition, the tubular transport function which
453 depends on intact energy metabolism of the tubuli was preserved as shown by A1M staining.
454 There was a direct correlation in previous studies between higher A1M expression and less
455 tissue edema measured by functional MRI [48]. In the current study there was also less ALT
456 elevation which is a marker for impaired liver function. It is well known that AKI causes distant
457 organ injury via enhanced leukocyte infiltration into other organs such as liver, lung, brain, gut
458 [50, 95]. Mechanisms are multifactorial such as release of cytokines and inflammatory
459 mediators, increase in oxidative stress, activation of various immune cells, neutrophil
460 extravasation, generalized endothelial injury, increased vascular permeability and tissue edema
461 formation [96]. In a mouse model of liver IRI also beneficial effects with less tissue damage has
462 been attributed to n3-PUFA treatment [97]. It is conceivable that the moderate beneficial effects
463 would improve patient outcome in longer follow-up by reducing the general inflammatory status.
464 In a previous study on renal IRI beneficial effects of dietary n3-PUFA supplementation have
465 been reported [13]. However, the severity of AKI might differ between the studies. In the study
466 by Hassan et al. creatinine elevation was only 2-3-fold which is less than in our study (6-fold)
467 and might reflect a milder AKI model. In the clinical context interesting and relevant benefits from
468 n3-PUFA diets were observed, e.g. they seemed to reduce cardiovascular mortality in patients
469 with chronic kidney disease [98]. In addition, another study showed that n3-PUFA reduced blood
470 pressure and triglyceride levels in patients with CKD stage 3-4 in a placebo-controlled
471 intervention study [99]. Since AKI has a high risk of progression to CKD there might be later
472 beneficial effects on clinical outcome which are missed by the nature of the current study design

473 with a 24 hour follow-up. Further investigations with in depth analysis of oxylipin levels also at
474 later time points of disease progression might be of interest and shall be addressed in the future.

475

476 **Conclusion**

477 In the present study supplementation of a western-style, i.e. linoleic rich diet, with the long chain
478 n3-PUFA EPA and DHA led to a pronounced modulation of the PUFA and oxylipin pattern.

479 However, in a murine model of moderate AKI pre-feeding of n3-PUFA did not attenuate renal
480 function impairment, morphological renal damage and inflammation characterized by pro-
481 inflammatory cytokine (IL-6 and MCP-1) mRNA up-regulation and neutrophil infiltration.

482 However, also beneficial effects were seen such as preservation of tubular transport function
483 indicated by enhanced alpha-1 microglobulin (A1M) expression in cortical proximal tubular
484 epithelial cells and enhanced up-regulation of tubular heme oxygenase-1 (HO-1) expression
485 which has been correlated to improved renal regeneration [45].

486 Overall it is concluded that - despite a maximal elevation of endogenous n3-PUFA and n3-PUFA
487 derived oxylipin levels - n3-PUFA supplementation did not result in attenuation of renal function
488 impairment or acute kidney injury within 24 hours in this model.

489

490

491

492 **Acknowledgement**

493 We thank Herle Chlebusch and Michaela Beese for excellent technical assistance. Our work
494 was supported by the Fonds der Chemischen Industrie to KR and the German Research
495 Foundation (DFG, Grant SCHE 1801 to NHS and GU 613/1-1 to FG)

496

497

498 .

499 **Literature**

- 500 [1] Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group,
501 KDIGO Clinical Practice Guideline for Acute Kidney Injury, *Kidney International Supplements*
502 2(1) (2012) 1-138.
- 503 [2] S. Sovik, M.S. Isachsen, K.M. Nordhuus, C.K. Tveiten, T. Eken, K. Sunde, K.G. Brurberg, S.
504 Beitland, Acute kidney injury in trauma patients admitted to the ICU: a systematic review and
505 meta-analysis, *Intensive Care Med* (2019).
- 506 [3] G. Chalikias, L. Serif, P. Kikas, A. Thomaidis, D. Stakos, D. Makrygiannis, S. Chatzikiyiakou,
507 N. Papoulidis, V. Voudris, A. Lantzouraki, M. Muller, S. Arampatzis, S. Konstantinides, D.
508 Tziakas, Long-term impact of acute kidney injury on prognosis in patients with acute myocardial
509 infarction, *Int J Cardiol* (2019).
- 510 [4] C.E. Palant, R.L. Amdur, L.S. Chawla, Long-term consequences of acute kidney injury in the
511 perioperative setting, *Curr Opin Anaesthesiol* 30(1) (2017) 100-104.
- 512 [5] L.S. Chawla, P.W. Eggers, R.A. Star, P.L. Kimmel, Acute Kidney Injury and Chronic Kidney
513 Disease as Interconnected Syndromes, *New England Journal of Medicine* 371(1) (2014) 58-66.
- 514 [6] D.N. Granger, P.R. Kvietys, Reperfusion injury and reactive oxygen species: The evolution of
515 a concept, *Redox Biology* 6 (2015) 524-551.
- 516 [7] J.V. Bonventre, L. Yang, Cellular pathophysiology of ischemic acute kidney injury, *The*
517 *Journal of clinical investigation* 121(11) (2011) 4210-4221.
- 518 [8] H.K. Eltzschig, T. Eckle, Ischemia and reperfusion—from mechanism to translation, *Nature*
519 *Medicine* 17 (2011) 1391.
- 520 [9] G.R. Kinsey, L. Li, M.D. Okusa, Inflammation in acute kidney injury, *Nephron. Experimental*
521 *nephrology* 109(4) (2008) e102-e107.
- 522 [10] D.H. Gwon, T.W. Hwang, J.-Y. Ro, Y.-J. Kang, J.Y. Jeong, D.-K. Kim, K. Lim, D.W. Kim,
523 D.E. Choi, J.-J. Kim, High Endogenous Accumulation of ω -3 Polyunsaturated Fatty Acids Protect
524 against Ischemia-Reperfusion Renal Injury through AMPK-Mediated Autophagy in Fat-1 Mice,
525 *International journal of molecular sciences* 18(10) (2017) 2081.
- 526 [11] M. Ajami, S.H. Davoodi, R. Habibey, N. Namazi, M. Soleimani, H. Pazoki-Toroudi, Effect of
527 DHA+EPA on oxidative stress and apoptosis induced by ischemia-reperfusion in rat kidneys,
528 *Fundam Clin Pharmacol* 27(6) (2013) 593-602.
- 529 [12] S.C. Ashtiyani, H. Najafi, K. Kabirinia, E. Vahedi, L. Jamebozorky, Oral omega-3 fatty acid
530 for reduction of kidney dysfunction induced by reperfusion injury in rats, *Iran J Kidney Dis* 6(4)
531 (2012) 275-83.
- 532 [13] I.R. Hassan, K. Gronert, Acute Changes in Dietary ω -3 and ω -6 Polyunsaturated Fatty
533 Acids Have a Pronounced Impact on Survival following Ischemic Renal Injury and Formation of
534 Renoprotective Docosahexaenoic Acid-Derived Protectin D1, *J Immunol* 182(5) (2009) 3223-
535 3232.
- 536 [14] M.L. Kielar, D.R. Jeyarajah, X.J. Zhou, C.Y. Lu, Docosahexaenoic acid ameliorates murine
537 ischemic acute renal failure and prevents increases in mRNA abundance for both TNF-alpha
538 and inducible nitric oxide synthase, *J Am Soc Nephrol* 14(2) (2003) 389-96.
- 539 [15] J. Torras, K. Soto, M. Riera, I. Herrero, J. Valles, J.M. Cruzado, J. Alsina, J.M. Grinyo,
540 Changes in renal hemodynamics and physiology after normothermic ischemia in animals
541 supplemented with eicosapentaenoic acid, *Transpl Int* 9 Suppl 1 (1996) S455-9.
- 542 [16] R.G. Fassett, G.C. Gobe, J.M. Peake, J.S. Coombes, Omega-3 Polyunsaturated Fatty Acids
543 in the Treatment of Kidney Disease, *American Journal of Kidney Diseases* 56(4) (2010) 728-
544 742.
- 545 [17] B. Gopinath, D.C. Harris, V.M. Flood, G. Burlutsky, P. Mitchell, Consumption of long-chain
546 n-3 PUFA, alpha-linolenic acid and fish is associated with the prevalence of chronic kidney
547 disease, *Br J Nutr* 105(9) (2011) 1361-8.

548 [18] X. Huang, B. Lindholm, P. Stenvinkel, J.J. Carrero, Dietary fat modification in patients with
549 chronic kidney disease: n-3 fatty acids and beyond, *J Nephrol* 26(6) (2013) 960-74.

550 [19] J. Hu, Z. Liu, H. Zhang, Omega-3 fatty acid supplementation as an adjunctive therapy in the
551 treatment of chronic kidney disease: a meta-analysis, *Clinics (Sao Paulo, Brazil)* 72(1) (2017)
552 58-64.

553 [20] W.S. Harris, C. Von Schacky, The Omega-3 Index: a new risk factor for death from coronary
554 heart disease?, *Prev Med* 39(1) (2004) 212-20.

555 [21] C. von Schacky, The Omega-3 Index as a risk factor for cardiovascular diseases,
556 *Prostaglandins & other lipid mediators* 96(1) (2011) 94-98.

557 [22] A.I. Ostermann, P. Waindok, M.J. Schmidt, C.-Y. Chiu, C. Smyl, N. Rohwer, K.-H. Weylandt,
558 N.H. Schebb, Modulation of the endogenous omega-3 fatty acid and oxylipin profile in vivo—A
559 comparison of the fat-1 transgenic mouse with C57BL/6 wildtype mice on an omega-3 fatty acid
560 enriched diet, *PLoS one* 12(9) (2017) e0184470.

561 [23] W.S. Harris, N.L. Tintle, M.R. Etherton, R.S. Vasan, Erythrocyte long-chain omega-3 fatty
562 acid levels are inversely associated with mortality and with incident cardiovascular disease: The
563 Framingham Heart Study, *J Clin Lipidol* 12(3) (2018) 718-727 e6.

564 [24] A.S. Abdelhamid, T.J. Brown, J.S. Brainard, P. Biswas, G.C. Thorpe, H.J. Moore, K.H.
565 Deane, F.K. AlAbdulghafoor, C.D. Summerbell, H.V. Worthington, F. Song, L. Hooper, Omega-3
566 fatty acids for the primary and secondary prevention of cardiovascular disease, *Cochrane*
567 *Database Syst Rev* 11 (2018) CD003177.

568 [25] T. Aung, J. Halsey, D. Kromhout, H.C. Gerstein, R. Marchioli, L. Tavazzi, J.M. Geleijnse, B.
569 Rauch, A. Ness, P. Galan, E.Y. Chew, J. Bosch, R. Collins, S. Lewington, J. Armitage, R.
570 Clarke, C. for the Omega-3 Treatment Trialists', Associations of Omega-3 Fatty Acid
571 Supplement Use With Cardiovascular Disease Risks: Meta-analysis of 10 Trials Involving 77 917
572 Individuals Meta-analysis of Associations of Omega-3 Fatty Acids and Cardiovascular Risk Meta-
573 analysis of Associations of Omega-3 Fatty Acids and Cardiovascular Risk, *JAMA Cardiology*
574 3(3) (2018) 225-233.

575 [26] A. Tatsioni, M. Chung, Y. Sun, B. Kupelnick, A.H. Lichtenstein, R. Perrone, P. Chew, J. Lau,
576 P.A. Bonis, Effects of fish oil supplementation on kidney transplantation: a systematic review and
577 meta-analysis of randomized, controlled trials, *J Am Soc Nephrol* 16(8) (2005) 2462-70.

578 [27] P.C. Calder, Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms
579 and clinical relevance, *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of*
580 *Lipids* 1851(4) (2015) 469-484.

581 [28] M. Gabbs, S. Leng, J.G. Devassy, M. Monirujjaman, H.M. Aukema, Advances in Our
582 Understanding of Oxylipins Derived from Dietary PUFAs, *Advances in Nutrition* 6(5) (2015) 513-
583 540.

584 [29] W. Wang, J. Zhu, F. Lyu, D. Panigrahy, K.W. Ferrara, B. Hammock, G. Zhang, ω -3
585 Polyunsaturated fatty acids-derived lipid metabolites on angiogenesis, inflammation and cancer,
586 *Prostaglandins & other lipid mediators* 113-115 (2014) 13-20.

587 [30] C. Gladine, A.I. Ostermann, J.W. Newman, N.H. Schebb, MS-based targeted metabolomics
588 of eicosanoids and other oxylipins: analytical and inter-individual variabilities, *Free Radical*
589 *Biology and Medicine* (2019).

590 [31] M.W. Buczynski, D.S. Dumlao, E.A. Dennis, Thematic Review Series: Proteomics. An
591 integrated omics analysis of eicosanoid biology, *Journal of lipid research* 50(6) (2009) 1015-
592 1038.

593 [32] J.D. Morrow, K.E. Hill, R.F. Burk, T.M. Nammour, K.F. Badr, L.J. Roberts, 2nd, A series of
594 prostaglandin F₂-like compounds are produced in vivo in humans by a non-cyclooxygenase, free
595 radical-catalyzed mechanism, *Proceedings of the National Academy of Sciences of the United*
596 *States of America* 87(23) (1990) 9383-7.

597 [33] J.-M. Galano, Y.Y. Lee, C. Oger, C. Vigor, J. Vercauteren, T. Durand, M. Giera, J.C.-Y. Lee,
598 Isoprostanes, neuroprostanes and phytoprostanes: An overview of 25years of research in
599 chemistry and biology, *Progress in lipid research* 68 (2017) 83-108.

600 [34] J.D. Imig, Eicosanoid regulation of the renal vasculature, *Am J Physiol Renal Physiol* 279(6)
601 (2000) F965-81.

602 [35] C.M. Hao, M.D. Breyer, Roles of lipid mediators in kidney injury, *Semin Nephrol* 27(3)
603 (2007) 338-51.

604 [36] C.M. Hao, M.D. Breyer, Physiological regulation of prostaglandins in the kidney, *Annu Rev*
605 *Physiol* 70 (2008) 357-77.

606 [37] F. Fan, R.J. Roman, Effect of Cytochrome P450 Metabolites of Arachidonic Acid in
607 Nephrology, *J Am Soc Nephrol* 28(10) (2017) 2845-2855.

608 [38] C.N. Serhan, Pro-resolving lipid mediators are leads for resolution physiology, *Nature* 510
609 (2014) 92.

610 [39] R.C. Murphy, Specialized pro-resolving mediators: do they circulate in plasma?, *Journal of*
611 *lipid research* 56(9) (2015) 1641-1642.

612 [40] L. Kutzner, K.M. Rund, A.I. Ostermann, N.M. Hartung, J.-M. Galano, L. Balas, T. Durand,
613 M.S. Balzer, S. David, N.H. Schebb, Development of an Optimized LC-MS Method for the
614 Detection of Specialized Pro-Resolving Mediators in Biological Samples, 10(169) (2019).

615 [41] K. Hueper, M. Gutberlet, S. Rong, D. Hartung, M. Mengel, X. Lu, H. Haller, F. Wacker, M.
616 Meier, F. Gueler, Acute kidney injury: arterial spin labeling to monitor renal perfusion impairment
617 in mice-comparison with histopathologic results and renal function, *Radiology* 270(1) (2014) 117-
618 24.

619 [42] F. Gueler, N. Shushakova, M. Mengel, K. Hueper, R. Chen, X. Liu, J.K. Park, H. Haller, G.
620 Wensvoort, S. Rong, A novel therapy to attenuate acute kidney injury and ischemic allograft
621 damage after allogenic kidney transplantation in mice, *PloS one* 10(1) (2015) e0115709.

622 [43] T.L. Blasbalg, J.R. Hibbeln, C.E. Ramsden, S.F. Majchrzak, R.R. Rawlings, Changes in
623 consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century,
624 *The American journal of clinical nutrition* 93(5) (2011) 950-962.

625 [44] K.M. Rund, A.I. Ostermann, L. Kutzner, J.-M. Galano, C. Oger, C. Vigor, S. Wecklein, N.
626 Seiwert, T. Durand, N.H. Schebb, Development of an LC-ESI(-)-MS/MS method for the
627 simultaneous quantification of 35 isoprostanes and isofurans derived from the major n3- and n6-
628 PUFAs, *Analytica chimica acta* 1037 (2018) 63-74.

629 [45] A. Thorenz, K. Derlin, C. Schröder, L. Dressler, V. Vijayan, P. Pradhan, S. Immenschuh, A.
630 Jörns, F. Echtermeyer, C. Herzog, R. Chen, S. Rong, J.H. Bräsen, C. van Kooten, T. Kirsch, C.
631 Klemann, M. Meier, A. Klos, H. Haller, B. Hensen, F. Gueler, Enhanced activation of interleukin-
632 10, heme oxygenase-1, and AKT in C5aR2-deficient mice is associated with protection from
633 ischemia reperfusion injury-induced inflammation and fibrosis, *Kidney international* 94(4) (2018)
634 741-755.

635 [46] A.I. Ostermann, M. Müller, I. Willenberg, N.H. Schebb, Determining the fatty acid
636 composition in plasma and tissues as fatty acid methyl esters using gas chromatography – a
637 comparison of different derivatization and extraction procedures, *Prostaglandins, Leukotrienes*
638 *and Essential Fatty Acids* 91(6) (2014) 235-241.

639 [47] H. Gottschall, C. Schmöcker, D. Hartmann, N. Rohwer, K. Rund, L. Kutzner, F. Nolte, A.I.
640 Ostermann, N.H. Schebb, K.H. Weylandt, Aspirin alone and combined with a statin suppresses
641 eicosanoid formation in human colon tissue, *Journal of lipid research* 59(5) (2018) 864-871.

642 [48] K. Hueper, M. Gutberlet, J.H. Brasen, M.S. Jang, A. Thorenz, R. Chen, B. Hertel, A.
643 Barrmeyer, M. Schmidbauer, M. Meier, S. von Vietinghoff, A. Khalifa, D. Hartung, H. Haller, F.
644 Wacker, S. Rong, F. Gueler, Multiparametric Functional MRI: Non-Invasive Imaging of
645 Inflammation and Edema Formation after Kidney Transplantation in Mice, *PloS one* 11(9) (2016)
646 e0162705.

647 [49] F. Gueler, J.K. Park, S. Rong, T. Kirsch, C. Lindschau, W. Zheng, M. Elger, A. Fiebeler, D.
648 Fliser, F.C. Luft, H. Haller, Statins attenuate ischemia-reperfusion injury by inducing heme
649 oxygenase-1 in infiltrating macrophages, *The American journal of pathology* 170(4) (2007) 1192-
650 9.

651 [50] K. Doi, H. Rabb, Impact of acute kidney injury on distant organ function: recent findings and
652 potential therapeutic targets, *Kidney international* 89(3) (2016) 555-64.

653 [51] M. Salvadori, G. Rosso, E. Bertoni, Update on ischemia-reperfusion injury in kidney
654 transplantation: Pathogenesis and treatment, *World J Transplant* 5(2) (2015) 52-67.

655 [52] W. Jiang, J. Teng, J. Xu, B. Shen, Y. Wang, Y. Fang, Z. Zou, J. Jin, Y. Zhuang, L. Liu, Z.
656 Luo, C. Wang, X. Ding, Dynamic Predictive Scores for Cardiac Surgery-Associated Acute
657 Kidney Injury, *J Am Heart Assoc* 5(8) (2016).

658 [53] J.E. Swanson, J. Mark Black, J.E. Kinsella, Dietary menhaden oil: effects on the rate and
659 magnitude of modification of phospholipid fatty acid composition of mouse heart and brain,
660 *British Journal of Nutrition* 59(3) (1988) 535-545.

661 [54] D. Kelton, C. Lysecki, H. Aukema, B. Anderson, J.X. Kang, D.W.L. Ma, Endogenous
662 synthesis of n-3 PUFA modifies fatty acid composition of kidney phospholipids and eicosanoid
663 levels in the fat-1 mouse, *Prostaglandins, Leukotrienes and Essential Fatty Acids* 89(4) (2013)
664 169-177.

665 [55] C. Arnold, M. Markovic, K. Blossey, G. Wallukat, R. Fischer, R. Dechend, A. Konkel, C. von
666 Schacky, F.C. Luft, D.N. Muller, M. Rothe, W.H. Schunck, Arachidonic acid-metabolizing
667 cytochrome P450 enzymes are targets of {omega}-3 fatty acids, *The Journal of biological*
668 *chemistry* 285(43) (2010) 32720-33.

669 [56] G.P. Ossani, V.C. Denninghoff, A.M. Uceda, M.L. Diaz, R. Uicich, A.J. Monserrat, Short-
670 term menhaden oil rich diet changes renal lipid profile in acute kidney injury, *Journal of oleo*
671 *science* 64(5) (2015) 497-503.

672 [57] J.C. Gigliotti, V.A. Bedito, R. Livengood, C. Oldaker, N. Nanda, J.C. Tou, Feeding
673 Different Omega-3 Polyunsaturated Fatty Acid Sources Influences Renal Fatty Acid
674 Composition, Inflammation, and Occurrence of Nephrocalcinosis in Female Sprague-Dawley
675 Rats, *Food and Nutrition Sciences* Vol.04No.09 (2013) 12.

676 [58] H.M. Aukema, T. Yamaguchi, H. Takahashi, D.J. Philbrick, B.J. Holub, Effects of dietary fish
677 oil on survival and renal fatty acid composition in murine polycystic kidney disease, *Nutrition*
678 *Research* 12(11) (1992) 1383-1392.

679 [59] J.X. Kang, J. Wang, L. Wu, Z.B. Kang, Transgenic mice: fat-1 mice convert n-6 to n-3 fatty
680 acids, *Nature* 427(6974) (2004) 504.

681 [60] P. Couture, A.J. Hulbert, Membrane fatty acid composition of tissues is related to body
682 mass of mammals, *The Journal of Membrane Biology* 148(1) (1995) 27-39.

683 [61] R.E. Druilhet, M.L. Overturf, W.M. Kirkendall, Structure of neutral glycerides and
684 phosphoglycerides of human kidney, *International Journal of Biochemistry* 6(12) (1975) 893-901.

685 [62] G. Rouser, G. Simon, G. Kritchevsky, Species variations in phospholipid class distribution of
686 organs: I. Kidney, liver and spleen, *Lipids* 4(6) (1969) 599-606.

687 [63] B.M. Anderson, D.W.L. Ma, Are all n-3 polyunsaturated fatty acids created equal?, *Lipids in*
688 *Health and Disease* 8(1) (2009) 33.

689 [64] W.S. Harris, Omega-3 long-chain PUFA and triglyceride lowering: minimum effective
690 intakes, *European Heart Journal Supplements* 3(suppl_D) (2001) D59-D61.

691 [65] T.A.B. Sanders, M.C. Hochland, A comparison of the influence on plasma lipids and platelet
692 function of supplements of ω 3 and ω 6 polyunsaturated fatty acids, *British Journal of Nutrition*
693 50(3) (1983) 521-529.

694 [66] J.G. Devassy, T. Yamaguchi, M. Monirujjaman, M. Gabbs, A. Ravandi, J. Zhou, H.M.
695 Aukema, Distinct effects of dietary flax compared to fish oil, soy protein compared to casein, and
696 sex on the renal oxylipin profile in models of polycystic kidney disease, *Prostaglandins,*
697 *Leukotrienes and Essential Fatty Acids* 123 (2017) 1-13.

698 [67] X. Zhang, N. Yang, D. Ai, Y. Zhu, Systematic metabolomic analysis of eicosanoids after
699 omega-3 polyunsaturated fatty acid supplementation by a highly specific liquid chromatography-
700 tandem mass spectrometry-based method, *J Proteome Res* 14(4) (2015) 1843-53.

701 [68] M.G. Balvers, K.C. Verhoeckx, S. Bijlsma, C.M. Rubingh, J. Meijerink, H.M. Wortelboer,
702 R.F. Witkamp, Fish oil and inflammatory status alter the n-3 to n-6 balance of the

703 endocannabinoid and oxylipin metabolomes in mouse plasma and tissues, *Metabolomics* 8(6)
704 (2012) 1130-1147.

705 [69] L.S. Gallon, U.O. Barcelli, Measurement of prostaglandin E3 and other eicosanoids in
706 biologic samples using high pressure liquid chromatography and radioimmunoassay,
707 *Prostaglandins* 31(2) (1986) 217-225.

708 [70] S. Leng, T. Winter, H.M. Aukema, Dietary ALA, EPA and DHA have distinct effects on
709 oxylipin profiles in female and male rat kidney, liver and serum, *The Journal of Nutritional*
710 *Biochemistry* 57 (2018) 228-237.

711 [71] H.M. Aukema, J. Lu, F. Borthwick, S.D. Proctor, Dietary fish oil reduces glomerular injury
712 and elevated renal hydroxyeicosatetraenoic acid levels in the JCR:LA-cp rat, a model of the
713 metabolic syndrome, *Br J Nutr* 110(1) (2013) 11-9.

714 [72] N.H. Ibrahim, Y. Jia, J.G. Devassy, T. Yamaguchi, H.M. Aukema, Renal cyclooxygenase
715 and lipoxygenase products are altered in polycystic kidneys and by dietary soy protein and fish
716 oil treatment in the Han:SPRD-Cy rat, *Molecular nutrition & food research* 58(4) (2014) 768-81.

717 [73] R. Fischer, A. Konkel, H. Mehling, K. Blosssey, A. Gapelyuk, N. Wessel, C. von Schacky, R.
718 Dechend, D.N. Muller, M. Rothe, F.C. Luft, K. Weylandt, W.H. Schunck, Dietary omega-3 fatty
719 acids modulate the eicosanoid profile in man primarily via the CYP-epoxygenase pathway,
720 *Journal of lipid research* 55(6) (2014) 1150-64.

721 [74] A.M. Zivkovic, J. Yang, K. Georgi, C. Hegedus, M.L. Nording, A. O'Sullivan, J.B. German,
722 R.J. Hogg, R.H. Weiss, C. Bay, B.D. Hammock, Serum oxylipin profiles in IgA nephropathy
723 patients reflect kidney functional alterations, *Metabolomics* 8(6) (2012) 1102-1113.

724 [75] N.H. Schebb, A.I. Ostermann, J. Yang, B.D. Hammock, A. Hahn, J.P. Schuchardt,
725 Comparison of the effects of long-chain omega-3 fatty acid supplementation on plasma levels of
726 free and esterified oxylipins, *Prostaglandins & other lipid mediators* 113-115 (2014) 21-29.

727 [76] W.L. Smith, Y. Urade, P.-J. Jakobsson, Enzymes of the Cyclooxygenase Pathways of
728 Prostanoid Biosynthesis, *Chemical reviews* 111(10) (2011) 5821-5865.

729 [77] M. Wada, C.J. DeLong, Y.H. Hong, C.J. Rieke, I. Song, R.S. Sidhu, C. Yuan, M. Warnock,
730 A.H. Schmaier, C. Yokoyama, E.M. Smyth, S.J. Wilson, G.A. FitzGerald, R.M. Garavito, D.X.
731 Sui, J.W. Regan, W.L. Smith, Enzymes and Receptors of Prostaglandin Pathways with
732 Arachidonic Acid-derived Versus Eicosapentaenoic Acid-derived Substrates and Products,
733 *Journal of Biological Chemistry* 282(31) (2007) 22254-22266.

734 [78] D. Bagga, L. Wang, R. Farias-Eisner, J.A. Glaspy, S.T. Reddy, Differential effects of
735 prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2
736 expression and IL-6 secretion, *Proceedings of the National Academy of Sciences of the United*
737 *States of America* 100(4) (2003) 1751-1756.

738 [79] J.P. Schuchardt, S. Schmidt, G. Kressel, I. Willenberg, B.D. Hammock, A. Hahn, N.H.
739 Schebb, Modulation of blood oxylipin levels by long-chain omega-3 fatty acid supplementation in
740 hyper- and normolipidemic men, *Prostaglandins, Leukotrienes and Essential Fatty Acids* 90(2)
741 (2014) 27-37.

742 [80] C. Skarke, N. Alamuddin, J.A. Lawson, X. Li, J.F. Ferguson, M.P. Reilly, G.A. FitzGerald,
743 Bioactive products formed in humans from fish oils, *Journal of lipid research* 56(9) (2015) 1808-
744 20.

745 [81] C. Westphal, A. Konkel, W.H. Schunck, Cytochrome p450 enzymes in the bioactivation of
746 polyunsaturated Fatty acids and their role in cardiovascular disease, *Adv Exp Med Biol* 851
747 (2015) 151-87.

748 [82] J.P. Lee, S.H. Yang, H.Y. Lee, B. Kim, J.Y. Cho, J.H. Paik, Y.J. Oh, D.K. Kim, C.S. Lim,
749 Y.S. Kim, Soluble epoxide hydrolase activity determines the severity of ischemia-reperfusion
750 injury in kidney, *PloS one* 7(5) (2012) e37075.

751 [83] A. Sharma, M.A. Hye Khan, S.P. Levick, K.S. Lee, B.D. Hammock, J.D. Imig, Novel Omega-
752 3 Fatty Acid Epoxygenase Metabolite Reduces Kidney Fibrosis, *Int J Mol Sci* 17(5) (2016).

753 [84] B.Q. Deng, Y. Luo, X. Kang, C.B. Li, C. Morisseau, J. Yang, K.S.S. Lee, J. Huang, D.Y. Hu,
754 M.Y. Wu, A. Peng, B.D. Hammock, J.Y. Liu, Epoxide metabolites of arachidonate and

755 docosahexaenoate function conversely in acute kidney injury involved in GSK3beta signaling,
756 Proceedings of the National Academy of Sciences of the United States of America 114(47)
757 (2017) 12608-12613.

758 [85] Guidance document on analytical quality control and method validation procedures for
759 pesticide residues and analysis in food and feed., in: E. Commission (Ed.) SANTE/11813/2017,
760 2017.

761 [86] A. Barden, E. Mas, K.D. Croft, M. Phillips, T.A. Mori, Short-term n-3 fatty acid
762 supplementation but not aspirin increases plasma proresolving mediators of inflammation,
763 Journal of lipid research 55(11) (2014) 2401-7.

764 [87] A. Toewe, L. Balas, T. Durand, G. Geisslinger, N. Ferreirós, Simultaneous determination of
765 PUFA-derived pro-resolving metabolites and pathway markers using chiral chromatography and
766 tandem mass spectrometry, Analytica chimica acta 1031 (2018) 185-194.

767 [88] J.T. English, P.C. Norris, R.R. Hodges, D.A. Dartt, C.N. Serhan, Identification and Profiling
768 of Specialized Pro-Resolving Mediators in Human Tears by Lipid Mediator Metabolomics,
769 Prostaglandins, Leukotrienes and Essential Fatty Acids 117 (2017) 17-27.

770 [89] K.A. Massey, A. Nicolaou, Lipidomics of oxidized polyunsaturated fatty acids, Free radical
771 biology & medicine 59 (2013) 45-55.

772 [90] M. Masoodi, A.A. Mir, N.A. Petasis, C.N. Serhan, A. Nicolaou, Simultaneous lipidomic
773 analysis of three families of bioactive lipid mediators leukotrienes, resolvins, protectins and
774 related hydroxy-fatty acids by liquid chromatography/electrospray ionisation tandem mass
775 spectrometry, Rapid communications in mass spectrometry : RCM 22(2) (2008) 75-83.

776 [91] H.S. Jonasdottir, H. Brouwers, R.E.M. Toes, A. Ioan-Facsinay, M. Giera, Effects of
777 anticoagulants and storage conditions on clinical oxylipid levels in human plasma, Biochimica et
778 Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1863(12) (2018) 1511-1522.

779 [92] N. Guillot, E. Caillet, M. Laville, C. Calzada, M. Lagarde, E. Vericel, Increasing intakes of the
780 long-chain omega-3 docosahexaenoic acid: effects on platelet functions and redox status in
781 healthy men, FASEB J 23(9) (2009) 2909-16.

782 [93] G.M. Goncalves, M.A. Cenedeze, C.Q. Feitoza, P.M. Wang, A.P. Bertocchi, M.J. Damiao,
783 H.S. Pinheiro, V.P. Antunes Teixeira, M.A. dos Reis, A. Pacheco-Silva, N.O. Camara, The role
784 of heme oxygenase 1 in rapamycin-induced renal dysfunction after ischemia and reperfusion
785 injury, Kidney international 70(10) (2006) 1742-9.

786 [94] J.F. Ndisang, S. Tiwari, Mechanisms by which heme oxygenase rescue renal dysfunction in
787 obesity, Redox Biol 2 (2014) 1029-37.

788 [95] J.F. Doyle, L.G. Forni, Acute kidney injury: short-term and long-term effects, Crit Care 20(1)
789 (2016) 188.

790 [96] W. Druml, Systemic consequences of acute kidney injury, Curr Opin Crit Care 20(6) (2014)
791 613-9.

792 [97] D.A. Raptis, P. Limani, J.H. Jang, U. Ungethum, C. Tschuor, R. Graf, B. Humar, P.A.
793 Clavien, GPR120 on Kupffer cells mediates hepatoprotective effects of omega3-fatty acids, J
794 Hepatol 60(3) (2014) 625-32.

795 [98] V.M. Saglimbene, G. Wong, A. van Zwieten, S.C. Palmer, M. Ruospo, P. Natale, K.
796 Campbell, A. Teixeira-Pinto, J.C. Craig, G.F.M. Strippoli, Effects of omega-3 polyunsaturated
797 fatty acid intake in patients with chronic kidney disease: Systematic review and meta-analysis of
798 randomized controlled trials, Clin Nutr (2019).

799 [99] T.A. Mori, V. Burke, I. Puddey, A. Irish, C.A. Cowpland, L. Beilin, G. Dogra, G.F. Watts, The
800 effects of [omega]3 fatty acids and coenzyme Q10 on blood pressure and heart rate in chronic
801 kidney disease: a randomized controlled trial, J Hypertens 27(9) (2009) 1863-72.

802

803

804 **Figure Captions**

805 **Fig. 1:** Simplified overview of the biosynthesis of arachidonic acid (20:4n6, ARA) from linoleic
806 acid (18:2n6, LA) and eicosapentaenoic acid (20:5n3, EPA) and docosahexaenoic acid (20:6n3,
807 DHA) from α -linolenic acid (18:3n3, ALA) and selected pathways for their oxygenation within the
808 ARA cascade.

809 COX – cyclooxygenase, LOX – lipoxygenase, CYP – cytochrome P450 monooxygenase, Tx –
810 thromboxane; HETE – hydroxyeicosatetraenoic acid; HEPE – hydroxyeicosapentaenoic acid;
811 HDHA – hydroxydocosahexaenoic acid; EpETrE – epoxyeicosatrienoic acid; EpETE –
812 epoxyeicosatetraenoic acid; EpDPE – epoxydocosapentaenoic acid; IsoP – isoprostanes;
813 NeuroP – neuroprostanes; Rv – resolvin; Lx – lipoxin; PD – protectin; MaR - maresin

814
815 **Fig. 2:** Fatty acid composition in **(A)** kidney tissue and **(C)** whole blood 24 h after renal IRI in
816 mice was shifted towards higher levels of n3-PUFA, while n6-PUFA decreased after 14 days of
817 feeding a sunflower oil based diet enriched with EPA and DHA (STD+n3) compared to the same
818 diet without EPA and DHA (STD). Consistently relative levels of EPA and DHA of all detected FA
819 in **(B)** kidney tissue and **(D)** whole blood were significantly higher in mice on the STD+n3 diet.
820 Shown are mean \pm SEM, n=14, **p<0.01, ***p<0.001.

821
822 **Fig. 3:** Concentration of free oxylipins in **(A)** plasma and **(B)** kidney tissue as well as **(C)** total,
823 i.e. free and esterified oxylipins in kidney tissue. Shown are concentrations of selected (iso-)
824 prostanoids, 5-LOX, 12/15-LOX, CYP4 and CYP2 products of ARA, EPA and DHA as well as
825 18-HEPE and PD1. Except for the sham group all patterns are determined in mice 24 h after
826 renal IRI on a STD or STD+n3 diet. Feeding of the STD+n3 led to an increase of EPA and DHA
827 derived lipid mediators while ARA derived oxylipins were decreased. Shown are mean \pm SEM
828 (sham n=5, (A) STD: n=19, STD+n3: n=18; (B) STD: n=13, STD+n3: n=14; (C) STD: n=7,
829 STD+n3: n=7). The lower limit of quantification (LLOQ) for those oxylipins is indicated by a
830 dashed line in case it was not exceeded in >50% of the samples per group. Statistical
831 differences were determined between STD and STD+n3.

832
833 **Fig. 4:** Acute kidney injury and inflammation. Renal damage after IRI was comparable in STD
834 and STD+n3 fed mice **(A, D, G;** STD: n=18, STD+n3: n=21). Neutrophil infiltration to the outer
835 medulla was present in both groups without differences **(B, E, H;** STD: n=18, STD+n3: n=21).
836 Renal function was significantly impaired after IRI in both groups **(I;** STD: n=19, STD+n3: n=21,
837 sham n=5). Relative expression of **(C)** IL-6 and **(F)** MCP-1 mRNA in kidney tissue was
838 significantly up-regulated at 24 h after IRI in both groups without differences (STD: n=7,
839 STD+n3: n=7). Shown are mean \pm SEM.

840
841 **Fig. 5:** Tubular transport and tubular regeneration. A1M expression was significantly higher after
842 STD+n3 diet which indicates preserved tubular transport function **(A, C, F,** STD: n=18, STD+n3:

843 n=14). In addition, HO-1 expression was significantly upregulated in the STD+n3 fed group (**B**,
844 **D**, **G**; STD: n=7, STD+n3 n=7) which correlates with tubular regeneration. Noteworthy, the
845 elevation of the liver enzyme ALT was attenuated (**E**) by the STD+n3 diet and could point
846 towards reduced distant organ injury (STD: n=19, STD+n3 n=21, sham n=5). Shown are mean \pm
847 SEM, * $p < 0.05$.

848