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## Protective effects of n-6 fatty acids-enriched diet on intestinal ischaemia/reperfusion injury involve lipoxin A4 and its receptor

Thomas Gobbetti, Simon Ducheix, Pauline Le Faouder, Teresa Pérez-Berezo, Fabien Riols, Jérôme Boué, Justine Bertrand-Michel, Marc Dubourdeau, Hervé Guillou, Mauro Perretti, et al.

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1 **Protective effects of n-6 fatty acids-enriched diet on intestinal ischemia/reperfusion injury**  
2 **involve lipoxin A4 and its receptor.**

3 **Running title:** Effects of n-6 diet on ischemia/reperfusion injury

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# Accepted Article

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24

25 **Abbreviations:**

26 Acetonitrile (ACN), arachidonic acid (AA), bovine serum albumin (BSA), cyclooxygenase  
27 (COX), dihydroxy-eicosatetraenoic acid (DiHETE), docosahexaenoic acid (DHA),  
28 eicosapentaenoic acid (EPA), epoxyeicosatrienoic acid (EET), formic acid (FA), formyl peptide  
29 receptor (FPR), gas chromatography (GC), Hank's balanced salt solution (HBSS),  
30 hydroxydocosahexaenoic acid (HDoHE), hydroxyeicosapentaenoic acid (HEPE),  
31 hydroxyeicosatetraenoic acid (HETE), ischemia/reperfusion (I/R), chemokine (C-X-C motif)  
32 ligand 1 (CXCL1), leukotriene B<sub>4</sub> (LTB<sub>4</sub>), leukotriene B<sub>4</sub> deuterated (LTB<sub>4</sub>-d<sub>4</sub>), leukotriene B<sub>5</sub>  
33 (LTB<sub>5</sub>), limit of detection (LOD), limit of quantification (LOQ), lipoxin A<sub>4</sub> (LxA<sub>4</sub>), lipoxin A<sub>4</sub>  
34 deuterated (LxA<sub>4</sub>-d<sub>5</sub>), lipoxin B<sub>4</sub> (LxB<sub>4</sub>), lipoxygenase (LOX), liquid chromatography/tandem  
35 mass spectrometry (LC-MS/MS), 7(S)-maresin (7-MaR1), methanol (MeOH), chemokine ligand  
36 2 (CCL2), myeloperoxidase (MPO), neonatal necrotizing enterocolitis (NEC),  
37 oxoeicosatetraenoic acid (oxo-ETE), phosphate-buffered saline (PBS), polyunsaturated fatty  
38 acids (PUFA), prostaglandin A<sub>1</sub> (PGA<sub>1</sub>), 8-iso prostaglandin A<sub>2</sub> (8-iso PGA<sub>2</sub>), prostaglandin E<sub>2</sub>  
39 (PGE<sub>2</sub>), prostaglandin E<sub>3</sub> (PGE<sub>3</sub>), 6-keto-prostaglandin F<sub>1α</sub> (6kPGF<sub>1α</sub>), 10(S),17(S)-protectin  
40 (PDx), resolvin D1 (RvD1), resolvin D2 (RvD2), superior mesenteric artery (SMA),  
41 thromboxane B<sub>2</sub> (TXB<sub>2</sub>), ultra high pressure liquid chromatography (UHPLC).

42

43 **Summary**

44 **Background and purpose:** Long-term intake of dietary fatty acids is known to predispose to  
45 chronic inflammation, but their potential influence on acute intestinal ischemia/reperfusion (I/R)  
46 injury is unknown. The aim of this study was to determine the consequences of a diet rich in n-3  
47 or n-6 polyunsaturated fatty acids (PUFA) on intestinal I/R-induced damage.

48 **Experimental approach:** Mice were fed 3 different isocaloric diets: a balanced diet used as a  
49 control, and two different PUFA-enriched diets, providing either high n-3 or high n-6 intake.  
50 Intestinal injury was evaluated after intestinal I/R. PUFA metabolite quantification was  
51 performed in intestinal tissues by liquid chromatography tandem mass spectrometry.

52 **Key results:** In control diet-fed mice, intestinal I/R caused inflammation and increased  
53 cyclooxygenase (COX) and lipoxygenase (LOX)-derived metabolites, compared to sham-  
54 operated animals. Lipoxin A<sub>4</sub> (LxA<sub>4</sub>) was significantly and selectively increased after ischemia.  
55 Animals fed a high n-3 diet did not display a different inflammatory profile following intestinal  
56 I/R, compared to control diet-fed animals. In contrast, intestinal inflammation was decreased in  
57 I/R group fed a high n-6 diet and level of LxA<sub>4</sub> was increased post-ischemia, compared to  
58 control diet-fed mice. Blockade of formyl-peptide receptor-2, the LxA<sub>4</sub> receptor, abrogated n-6  
59 rich diet-associated anti-inflammatory effects.

60 **Conclusions and Implications:** This study indicates that high n-6, but not n-3, PUFAs leads to  
61 significant protection against intestinal I/R-induced damage and demonstrates that LxA<sub>4</sub>  
62 endogenous production can be influenced by diet.

63

64

## 65 Introduction

66 There is growing evidence, which indicates bioactive lipid mediators derived from n-3 or  
67 n-6 polyunsaturated fatty acid (PUFA) play a pivotal role in initiating and sustaining the  
68 inflammatory response or engaging pro-resolving/anti-inflammatory pathways in several  
69 cardiovascular and inflammatory pathologies. These include atherosclerosis, thrombosis,  
70 arrhythmia, ischemia reperfusion (I/R) injury (De Caterina, 2011). The recent development of  
71 new techniques of high sensitivity liquid chromatography-tandem mass spectrometry (LC-  
72 MS/MS) allows a better comprehension of disease-associated lipidic metaboloma detecting  
73 simultaneously the presence of a number of PUFA metabolites in tissues. In our previous study,  
74 we characterized, by LC-MS/MS, the tissue profile of n-6 and n-3 PUFA metabolites in an  
75 experimental model of murine intestinal ischemia reperfusion (I/R) (Gobbetti *et al.*, 2013). We  
76 identified temporal and quantitative differences in n-3 and n-6 PUFA metabolite production,  
77 which correlated with inflammatory damage. However, it is unclear whether modulating the n-  
78 3/n-6 ratio composition in membranes could be used as therapeutic tool to modify the resistance  
79 of the gut to I/R injury.

80 Acute mesenteric ischemia is a life-threatening pathological event associated with a  
81 number of diseases including vessel occlusion, hernias, septic shock, major cardiovascular  
82 surgery, necrotizing enterocolitis and small bowel transplantation (Cerqueira *et al.*, 2005;  
83 Eltzschig *et al.*, 2011; Vollmar *et al.*, 2011). Intestinal ischemia remains a clinical challenge due  
84 to difficulty in diagnosis and especially to the lack of established pharmacological treatments.  
85 Therefore, the need for new therapeutic strategies is urgent. The intestinal mucosa is particularly  
86 prone to I/R injury due to the anatomical and physiological characteristic of the villus  
87 microcirculation (Vollmar *et al.*, 2011). The temporary interruption of the blood leads to a lack

88 of oxygen supply to the tissue (ischemia), which in turn causes cellular dysfunction, protease and  
89 phospholipases activation ([Gobbetti et al., 2012](#); [Otamiri et al., 1987](#); [Vollmar et al., 2011](#)).

90 Paradoxically, the restoration of blood flow and the consequent tissue re-oxygenation  
91 (reperfusion) aggravates the local (epithelial/endothelial damage) and systemic inflammatory  
92 response, leading to bacterial translocation and multiple organ failure ([Cerqueira et al., 2005](#)).

93 The main objective of this study was to identify if the modification of n-6/n-3PUFA  
94 status induced by the modification of PUFA metabolite biosynthesis associated with diet changes  
95 might improve intestinal resistance to ischemia reperfusion injury. We performed a dietary  
96 intervention by exposing mice to a diet rich in n-3, n-6 PUFA or to a balanced diet ([Ducheix et](#)  
97 [al., 2013](#); [Kelavkar et al., 2006](#)) and we evaluated the importance of n-3 and n-6 PUFA  
98 metabolites on intestinal I/R injury. Dietary intervention significantly changed the abundance of  
99 hepatic PUFA and intestinal PUFA metabolites. Intestinal inflammation was decreased in the  
100 group fed with a high n-6 diet and the level of LxA<sub>4</sub> after ischemia was increased compared to  
101 the group fed with a balanced diet. Early systemic pharmacological blockade of LxA<sub>4</sub> receptor,  
102 formyl-peptide receptor-2 (Fpr2), abrogated the anti-inflammatory effects attained by the n-6  
103 rich diet. This study indicates that early mobilization of LxA<sub>4</sub> leads to significant protection  
104 against intestinal I/R-induced damage.

105

## 106 MATERIALS AND METHODS

### 107 Animals

108 C57 BL/6 male mice (3 weeks-old) were purchased from Janvier (Le Genest Saint Isle, France).  
109 Animals were maintained in ventilated cages (4 mice per cage) in a specific pathogen free room  
110 at 20–24°C and relative humidity (40%–70%) with a 12 hours light/dark cycle and given free  
111 access to food and water. Animal Care and ethic Committee of US006/CREFE (CEEA-122)  
112 approved the whole study protocol (permit No. MP/01/64/09/12). In total 160 mice were  
113 included in this study. All animal experiments were conducted in accordance with Guide for the  
114 Care and Use of Laboratory Animals of the European council, and were reported in accordance  
115 with the ARRIVE guidelines (Kilkenny *et al.*, 2010).

116

### 117 Chemicals

118 *N*-tert-butoxycarbonyl-L-Phe-D-Leu-L-Phe-D-Leu-L-Phe (Boc2; Bachem, Bubendorf  
119 Switzerland), was dissolved in dimethyl sulfoxide (DMSO 1%) / sodium chloride (NaCl) 0.9%.  
120 6-keto-prostaglandin  $F_{1\alpha}$  (6kPGF $_{1\alpha}$ ), thromboxane B $_2$  (TXB $_2$ ), prostaglandin E $_2$  (PGE $_2$ ),  
121 prostaglandin A $_1$  (PGA $_1$ ), 8-iso prostaglandin A $_2$  (8-iso PGA $_2$ ), prostaglandin E $_3$  (PGE $_3$ ), lipoxin  
122 A $_4$  (LxA $_4$ ), lipoxin B $_4$  (LxB $_4$ ), lipoxin A $_4$  deuterated (LxA $_4$ -d5), resolvin D1 (RvD1), resolvin D2  
123 (RvD2), 7(S)-maresin (7-MaR1), leukotriene B $_4$  (LTB $_4$ ), leukotriene B $_5$  (LTB $_5$ ), leukotriene B $_4$   
124 deuterated (LTB $_4$ -d4), 10(S),17(S)-protectin (PDx), 18-hydroxyeicosapentaenoic acid (18-  
125 HEPE), dihydroxy-eicosatetraenoic acid (5,6-DiHETE), 15-hydroxyeicosatetraenoic acid (15-  
126 HETE) and 12-HETE, 8-HETE, 5-HETE, 5-HETE-d8, 17-hydroxy-docosahexaenoic acid (17-  
127 HDoHE) and 14-HDoHE, 14,15-epoxyeicosatrienoic acid (14,15-EET) and 11,12-EET, 8,9-  
128 EET, 5,6- EET, 5-oxoeicosatetraenoic acid (5-oxo-ETE) were purchased from Cayman



129 Chemicals (Ann Arbor, MI, USA). Molecular target nomenclature conformed to BJP's Guide to  
130 Receptors and Channels (Alexander *et al.*, 2011).

131

### 132 **Study design and surgical procedure**

133 One hundred and twenty mice were randomly assigned to one of 3 isocaloric diets containing 5%  
134 fat (w/w) for 9 weeks. A balanced diet providing both n-3 and n-6 fatty acid was used as a  
135 control, against two different PUFA-enriched diets, providing either high n-3 or high n-6 intake.

136 Mice were weighed once a week during the diet and the weight gain determined. After 9 weeks,  
137 a randomized complete block design has been used to split the experiment into a number of 5  
138 “mini-experiments”. The days of the surgery each diet group was split in 4 different

139 experimental groups: (i) sham ischemia, (ii) ischemia (iii) sham ischemia/reperfusion (sham I/R)  
140 and (iiii) ischemia/reperfusion (I/R) (n=10 per group). For each surgery day 2 animals of the 3  
141 different diet conditions have been subject to the surgical protocol (24 animals/day for 5 days).

142 Mice were anaesthetized by an intraperitoneal injection of sodium pentobarbital 50 mg/kg.  
143 Animals were kept under anaesthesia during the all period of surgery. The adequacy of  
144 anesthesia was monitored with a toe pinch testing to check the animal's reaction. Following

145 abdominal laparotomy, the small bowel was retracted to the left and the superior mesenteric  
146 artery (SMA) was temporarily occluded using a microvascular clip (Harvard Apparatus, Les  
147 Ulis, France) to cause ischemia. Small bowel and SMA were replaced in mice abdomen and the

148 incision protected with gauze soak with saline solution (NaCl 0.9%). After 50 minutes the clip  
149 was gently removed allowing reperfusion. Mice of the ischemia groups were sacrificed  
150 immediately after the ischemic period. Mice of I/R groups were sacrificed 5 hours after

151 reperfusion. Sham-operated animals, in which abdominal laparotomy and artery isolation were

152 performed without occlusion of the vessel, served as controls and were sacrificed just after the  
153 corresponding ischemic (sham ischemia) or I/R (sham I/R) time. After the surgical procedure,  
154 two-layer sutures closed the midline incision of the abdominal wall. During the surgical  
155 procedure mice were placed on a warming plate (32°C). An independent experiment was  
156 performed to determine the effect of Fpr2 endogenous activation during intestinal ischemia  
157 reperfusion. Eighty mice were fed with the diet providing high n-6 intake. After 9 weeks,  
158 animals were randomized in 4 different sub-groups of 20 animals: sham ischemia, sham I/R,  
159 ischemia and I/R as previously described. Ten mice per group were treated by intravenous  
160 injection of the tail vein with Boc2 (500 µg/kg). Animals were euthanized at the end of the study  
161 for the purpose of sample collection by a lethal overdose of pentobarbital i.p. followed by  
162 cervical dislocation

163

#### 164 **Fatty composition of diets**

165 The three diets were isocaloric and contained 5% fat (w/w) and were designed as  
166 previously described (Ducheix *et al.*, 2013). Pellets were prepared by UPAE (INRA, Jouy en  
167 Josas). Oils used for experimental diets preparation were grape seed and colza oils (50/50) for  
168 the Control diet (Ctrl), grape seed oil (n-6), and colza oil / fish oils (80/20) for a long chain n-3  
169 fatty acid enriched diet (n-3). The EPA enriched fish oil that was used in this study was obtained  
170 from Polaris (Quimper, France). The full composition of the diet is given in table 1. The fatty  
171 acid composition expressed in percentage of total polyunsaturated fatty acids is provided in table  
172 2. Pellets were stored under vacuum at -20°C. The diets were changed twice a week in each  
173 animal cage to avoid oxidative degradation of lipids.

174

175 **Assessment of inflammation**

176 Specimens of the ileum were embedded in paraffin. Sections (5 µm) were stained with  
177 hematoxylin and eosin. Microscopic histological damage score was performed blinded to the  
178 investigator and was based on a semi quantitative scoring system in which the following features  
179 were graded: extent of destruction of normal mucosal architecture, presence and degree of  
180 cellular infiltration, extent of muscle thickening, presence or absence of crypt abscesses, and  
181 presence or absence of goblet cell depletion. The scores for each feature were summed with a  
182 maximum possible score of 11 as previously described (Cattaruzza *et al.*, 2006).

183 Myeloperoxidase (MPO) activity was assessed in the ileum tissue and was used as an  
184 index of granulocyte infiltration as previously described (Motta *et al.*, 2012). Chemokine (C-X-C  
185 motif) ligand 1 (CXCL1) and chemokine ligand 2 (CCL2) were measured in intestinal tissue by  
186 Cytometric Bead Array (BD Biosciences) as previously described (Motta *et al.*, 2012).

187

188 **Immunohistochemistry**

189 Intestines were cryoprotected, cut into 5 µm sections in a cryostat, and mounted on a  
190 Superfrost slide (Thermo Fisher Scientific). Slides were washed in phosphate buffered saline  
191 (PBS), 0.5% Triton X-100, and 1% bovine serum albumin (BSA) solution (Sigma) and incubated  
192 overnight at 4°C with the primary antibody directed against Fpr2 (1/100, Santa Cruz, Heidelberg,  
193 Germany) along with anti-cytokeratin 18 (1/500, Santa Cruz), anti-CD45 (1/500, R&D systems,  
194 Lille, France) or anti-Ly-6B.2 (1/400, Bio-Rad AbD Serotec GmbH, Colmar, France). After  
195 washes, slides were incubated with appropriate secondary antibody (Alexa Fluor 488 and 555;  
196 Invitrogen, Cergy Pontoise, France) for 2 hours. Slides were mounted with Prolong<sup>®</sup> containing  
197 DAPI (Invitrogen). As control procedures, reactions were performed with omission of primary or

198 secondary antibodies. Images were acquired using Zeiss LSM-710 confocal microscopes with  
199 63X objective in the inverted configuration (Carl Zeiss MicroImaging Inc., Göttingen,  
200 Germany). Images of stained and control slides were collected and processed identically. The  
201 quantification of fluorescence was determined using Metamorph software (Molecular Devices).

202

### 203 **Real-time PCR analysis**

204 Total RNA from small intestine was isolated with TRIzol reagent (Invitrogen Paisley,  
205 UK) and 1 $\mu$ g of the total RNA was then reverse-transcribed with random hexamer  
206 oligonucleotides and SuperScript III (Invitrogen), according to the manufacturer's instructions.  
207 Amplification was performed with a LightCycler 480 using a SYBR Green I Master kit (Qiagen  
208 Ltd, UK) and one of the following mouse primer: *fpr2* (QT00171514, Qiagen), *alox12b*  
209 (QT00173551), *alox15* (QT00111034) or *alox5* (QT00258622). Relative expression of the target  
210 gene was normalized to expression of the *Hprt* gene, using the  $\Delta\Delta C_t$  method ([Boue \*et al.\*, 2011](#)).

211

### 212 **Hepatic and intestinal fatty acid profiling**

213 Hepatic fatty acids were analyzed as described previously ([Zadravec \*et al.\*, 2010](#)). Briefly,  
214 following homogenization of tissue samples in methanol/5 mM EGTA (2:1, v/v), lipids  
215 corresponding to an equivalent of 2 mg of tissue were extracted in the presence of glyceryl  
216 triheptadecanoate as an internal standard. The lipid extract in 1ml of heptane was  
217 transmethylated with 1 mL of BF<sub>3</sub> in methanol (MeOH,1:20, v/v) for 150 min at 100°C,  
218 evaporated to dryness, and the FAMES were extracted with hexane/water (3:1). The organic  
219 phase was evaporated to dryness and dissolved in 50  $\mu$ L ethyl acetate. One microliter of FAME  
220 was analyzed by gas-liquid chromatography on a 5890 Hewlett-Packard system (Hewlett-

221 Packard, Palo Alto, CA, USA) using a Famewax fused-silica capillary column (30 mm X 0.32  
222 mm i.d., 0.25 µm film thickness; Restek, Belfast, UK). Oven temperature was programmed from  
223 110 to 220°C at a rate of 2°C/min, and the carrier gas was hydrogen (0.5 bar). The injector and  
224 the detector were at 225 and 245°C, respectively.

225

### 226 **Intestinal lipid extraction**

227 Mouse small intestines were crushed with a FastPrep®-24 Instrument (MP biomedical) in 500  
228 µL of Hank's balanced salt solution (HBSS, Invitrogen) and 5 µL of internal standard mixture  
229 (LxA<sub>4</sub>-d<sub>5</sub>, LTB<sub>4</sub>-d<sub>4</sub> and 5-HETE-d<sub>8</sub> at 400 ng/mL in MeOH). After 2 crush cycles (6.5 m/s, 30  
230 s), 10µL is withdrawn for protein quantification and 300 µL of cold methanol was added.  
231 Samples were centrifuged at 3000 rpm for 15 min at 4°C. Supernatants were collected,  
232 completed to 2 mL in H<sub>2</sub>O and submitted to solid-phase extraction using HRX-50 mg 96-well  
233 (Macherey Nagel, Hoerd, France). Briefly, after plate conditioning, the sample was loaded at  
234 flow rate of 0.1 mL/min. After complete loading, the plate was washed with H<sub>2</sub>O/MeOH (90:10,  
235 2 mL) and lipid mediators were eluted with MeOH (2 mL). Solvent was evaporated under  
236 nitrogen and samples were dissolved with MeOH and stored at -80 °C for liquid  
237 chromatography/tandem mass spectrometry measurements.

238

### 239 **Liquid chromatography/tandem mass spectrometry (LC-MS/MS) measurements**

240 By this technique we performed the quantification of 6kPGF<sub>1α</sub>, TXB<sub>2</sub>, PGE<sub>2</sub>, PGA<sub>1</sub>, 8-isoPGA<sub>2</sub>,  
241 PGE<sub>3</sub>, LxA<sub>4</sub>, LxB<sub>4</sub>, RvD1, RvD2, 7-MaR1, LTB<sub>4</sub>, LTB<sub>5</sub>, PDx, 18-HEPE, 5,6-DiHETE, 15-  
242 HETE, 12-HETE, 8-HETE, 5-HETE, 17-HDoHE, 14-HDoHE, 14,15-EET, 11,12-EET, 8,9-EET,  
243 5,6-EET and 5-oxo-EET in mouse intestinal tissue (Le Faouder *et al.*, 2013). To simultaneously

244 separate 27 lipids of interest and 3 deuterated internal standards, LC-MS/MS analysis was  
245 performed on ultra high performance liquid chromatography system (UHPLC, Agilent LC1290  
246 Infinity) coupled to Agilent 6460 triple quadrupole MS (Agilent Technologies) equipped with  
247 electro-spray ionization operating in negative mode. Reverse-phase UHPLC was performed  
248 using ZorBAX SB-C18 column (Agilent Technologies) with a gradient elution. The mobile  
249 phases consisted of water, acetonitrile (CAN) and formic acid (FA) (75:25:0.1;v/v/v) (A) and  
250 ACN, FA (100:0.1, v/v) (B). The linear gradient was as follows: 0% B at 0 min, 85% B at 8.5  
251 min, 100% B at 9.5 min, 100% B at 10.5 min and 0% B at 12 min. The flow rate was 0.35  
252 mL/min. The autosampler was set at 5°C and the injection volume was 5 µL. Data were acquired  
253 in Multiple Reaction Monitoring (MRM) mode with optimized conditions. Peak detection,  
254 integration and quantitative analysis were done using Mass Hunter Quantitative analysis  
255 software (Agilent Technologies). For each standard, calibration curves were built using 10  
256 solutions at concentration ranging from 0.95 ng/mL to 500 ng/mL. A linear regression with a  
257 weight factor of 1/X was applied for each compound. The limit of detection (LOD) and the limit  
258 of quantification (LOQ) were determined for the 27 compounds using signal to noise ratio (S/N).  
259 The LOD corresponded to the lowest concentration leading to a signal to noise over 3 and LOQ  
260 corresponded to the lowest concentration leading to a signal to noise over 10. All values under  
261 the LOQ were not considered. Importantly, blank samples were evaluated, and their injection  
262 showed no interference (no peak detected), during the analysis. Hierarchical clustering and heat-  
263 map were obtained with R ([www.r-project.org](http://www.r-project.org)). PUFA metabolite quantities were transformed to  
264 z-scores and clustered based on 1-Pearson correlation coefficient as distance and the Ward  
265 algorithm as agglomeration criterion.

266

267 **Statistical analysis**

268 Data are presented as mean  $\pm$  SEM. Analyses were performed using GraphPad Prism 5 software.

269 All data were normally distributed. Between-group comparisons were performed by Student's

270 unpaired 2-tailed t-test. Multiple comparisons within groups I/R were performed by repeated-

271 measures 1-way ANOVA followed by Tukey's procedure. Statistical significance was accepted

272 at  $P \leq 0.05$ .

273

274 **Results:**

275 **Effect of dietary intervention on the abundance of fatty acids.**

276 In order to investigate the effect of the dietary intervention on the lipid composition,  
277 intestinal and hepatic fatty acids (FA) were profiled by gas chromatography (GC) analysis.  
278 Compared to the control group, the ratio between arachidonic (AA) and docosahexaenoic (DHA)  
279 acid increased 3-fold in the liver of animals receiving the n-6 diet (Figure 1). Conversely,  
280 animals receiving the n-3 diet had a significantly decreased ratio. Even if the total intestinal FA  
281 content was the same the dietary intervention had a major impact on the relative FA composition  
282 of the gut (Table 3). In the intestine of mice fed with n-6 diet, linoleic acid (LA, C18:2 n-6),  
283 C20:2 n-6, arachidonic acid (AA, C20:4 n-6) were significantly increased compared to the  
284 control diet group (Table 3). In the intestine of mice fed on n-3 diet,  $\alpha$ -linolenic acid (ALA,  
285 C18:3 n-3), eicosapentaenoic acid (EPA, C20:5 n-3), C22:5 n-3 and docosahexaenoic acid  
286 (DHA, C22:6 n-3) were significantly increased compared to the control diet. On the other hand,  
287 in this group C20:2 n-6, C20:3 n-6 and AA were significantly decreased. The ratio between  
288 arachidonic acid (AA) and docosahexaenoic acid (DHA) mirrored the changes in the relative  
289 abundance of intestinal polyunsaturated fatty acid. During dietary intervention weight gain was  
290 weekly assessed and it was found to be similar regardless of diet (Supplementary figure 1)

291

292

293 **Long-term n-6 diet prevents intestinal ischemia reperfusion injury**

294 In animals fed the control diet, 50 minutes of ischemia followed by 5 hours of reperfusion  
295 resulted in acute inflammation. This was characterized by microscopic damage: fragments of  
296 mucosa and red blood cells were found in the lumen. Villi were completely denuded or severely



297 damaged, together with loss of crypt architecture. Myeloperoxidase (MPO) activity and  
298 chemokine (C-X-C motif) ligand 1 (CXCL1)/ chemokine ligand 2 (CCL2) expressions were also  
299 markedly increased in I/R *versus* sham operation in control diet animals (Figure 2 A-E). These  
300 inflammatory markers were unchanged in animals receiving a high n-3 diet and then subjected to  
301 ischemia-reperfusion. Conversely, the microscopic damage score, MPO activity and  
302 CXCL1/CCL2 intestinal expression were significantly decreased in the IR group fed with a high  
303 n-6 diet (Figure 2 A-E), compared to control diet group. Taken together these data demonstrate  
304 that the administration of a long-term n-6 diet, but not of n-3 diet, protects the gut from the pro-  
305 inflammatory damaging consequences of I/R injury.

306

### 307 **Effects of diet on the production of intestinal n-6 and n-3 metabolites after ischemia or** 308 **ischemia followed by reperfusion**

309 We questioned how much the dietary fatty acids might influence the eicosanoid and  
310 docosanoid profiles likely to influence I/R induced damage. RvD1, RvD2, 7-MaR1, 14,15-EET,  
311 11,12-EET, 8,9-EET and 5,6-EET were not detectable and PGA<sub>1</sub>, 8-isoPGA<sub>2</sub> and 5,6-DiHETE  
312 were not quantifiable in intestinal tissue. PUFA metabolites hierarchical clustering was used to  
313 reveal the main differences (Figure 3A). The columns represented the average of PUFA  
314 metabolite quantity (pg/mg of proteins) of the different group of mice fed Ctrl, n-3 or n-6 diet.  
315 PUFA metabolites formed 4 different clusters. The first cluster was composed by product of  
316 arachidonic acid metabolism by cyclooxygenase (COX)- (PGE<sub>2</sub>, 6kPGF<sub>1α</sub>, TXB<sub>2</sub>) and  
317 lipoxygenase (LOX)-derived metabolites (LTB<sub>4</sub>, LxB<sub>4</sub>, 5/8/15/12-HETE). The principal  
318 characteristic of this cluster was that the quantity of eicosanoids was maximal following  
319 ischemia/reperfusion in groups of mice submitted to control diet (Figure 3A). 8/15/12-HETE,

320 PGE<sub>2</sub>, 6kPGF<sub>1α</sub>, TXB<sub>2</sub>, LTB<sub>4</sub> and LxB<sub>4</sub> were significantly increased only in control diet group  
321 submitted to I/R compared to sham I/R (Supplementary Figure 2). 5-HETE was significantly  
322 increased in control diet group submitted to ischemia compared to sham ischemia  
323 (Supplementary Figure 2). The second cluster was represented only by LxA<sub>4</sub>; its quantity was  
324 maximal in group of mice submitted to n-6 diet and to ischemia (Figure 3 A). 5oxo-ETE and  
325 15dPGj<sub>2</sub> were forming the third cluster characterized by a decrease of these two metabolites by  
326 ischemia independently of the diet. The last cluster regrouped all n-3 PUFA metabolites. They  
327 were increased in animals submitted to n-3 diet, independently of ischemia or  
328 ischemia/reperfusion (Figure 3A).

329 Due to the protective effect of n-6 diet on ischemia reperfusion injury, we focused our  
330 attention on LxA<sub>4</sub>. In mice receiving the control diet, the anti-inflammatory mediator LxA<sub>4</sub> was  
331 significantly increased compared to sham after ischemia (Figure 3B). In mice submitted to n-6  
332 diet, after ischemia, LxA<sub>4</sub> was significantly increased compared to sham and also compared to  
333 mice submitted to control diet and ischemia (Figure 3B). Moreover, in this group of mice we  
334 quantified a significant increase in *alox5*, *alox12* and *alox15* mRNA expression compared to  
335 mice receiving the control diet after ischemia (Supplementary Figure 3). These findings suggest  
336 that diet supplementation is able to change the PUFA metabolites quantity in colonic tissue after  
337 ischemia or ischemia/reperfusion by an increase of lipoxygenase expression. Interestingly, after  
338 ischemia, the increased LxA<sub>4</sub> in n-6 diet vs. control diet suggests a possible protective role for  
339 this anti-inflammatory lipid in the protection offered by this diet.

340

#### 341 **Fpr2 is expressed in mouse small intestine**

342 Since the tissue levels of LxA<sub>4</sub> were modified in some of the groups studied, we also

343 investigated the expression of the LxA<sub>4</sub> receptor Fpr2 in the same tissues. Ischemia did not  
344 modify *fpr2* expression compared to sham (Figure 4A). We observed an increase of *fpr2* mRNA  
345 after I/R in control and n-3 diet groups compared to corresponding sham (Figure 4A). Following  
346 I/R, the expression of *fpr2* mRNA was not different in mice that received the n-6 diet, compared  
347 to sham (Figure 4A). By immunohistochemistry, we observed that Fpr2 was expressed in the  
348 mucosa of murine small intestine (Figure 4B). Fpr2-immunoreactivity co-localized with  
349 cytokeratin 18, Ly-6B.2 and partially with CD45-immunoreactivity meaning that Fpr2 was  
350 expressed on epithelial cells, neutrophils and on 44% of nucleated hematopoietic cells,  
351 respectively (Figure 4B). In those different cell types, no difference of Fpr2 fluorescence  
352 intensity was observed (Supplementary Figure 4A) between the different groups or diets. In  
353 correlation with MPO activity (Figure 2B), Ly-6B.2 expression was increased following  
354 ischemia/reperfusion in control and n-3 diet groups compared to I/R n-6 diet group  
355 (Supplementary Figure 4B). Thus, in these two groups, the overexpression of *fpr2* could be the  
356 consequence of the infiltration of granulocytes expressing Fpr2. Pictures of immunohistochemistry  
357 control procedures are shown in supplementary figure 4C.

358  
359 **Pharmacological blockade of Fpr2 abrogated the anti-inflammatory effects obtained by the**  
360 **n-6 rich diet.**

361 LxA<sub>4</sub> is an effector of endogenous anti-inflammation acting *via* Fpr2. In mice receiving a  
362 high n-6 diet, administration of the Fpr pan antagonist, Boc2 (500 µg/kg before ischemia i.v.)  
363 abolished the inhibition of microscopic damage score, MPO activity, CXCL1 and CCL2  
364 expression induced by n-6 diet following I/R (Figure 5A). Administration of Fpr antagonist  
365 abrogated also the inhibition of 15-HETE, PGE<sub>2</sub>, 6kPGF<sub>1α</sub> and TXB<sub>2</sub> induced by the n-6

366 enriched diet (Figure 5B). Administration of Boc2 had no effect on the increase of LxA4 induced  
367 by ischemia in n-6 diet group (Figure 5C). These findings show that the blockade of the LxA<sub>4</sub>  
368 receptor (Fpr2) annulled the anti-inflammatory effects obtained by the n-6 rich diet and that n-6  
369 supplementation-induced protection after I/R is indeed mediated by FPR2 activation.

370

371 **Discussion**

372 In the present study we evaluated the effect of dietary intervention based on different n-6  
373 and n-3 PUFA intake, on injury associated with intestinal I/R. Our data indicate that n-6 PUFA  
374 afforded significant protection against I/R-induced tissue injuries, while n-3 PUFA diet had no  
375 effect on intestinal damage as determined in these settings. Specifically, in mice subjected to  
376 intestinal I/R, n-6 PUFA supplementation in diet decreased mucosal damage, granulocyte  
377 recruitment and chemokine expression, compared to animals fed a control balanced diet.  
378 Protection against I/R injury was associated with an increase of *alox5*, *alox12* and *alox15* mRNA  
379 expression and consequently of LxA<sub>4</sub> production following ischemia in intestinal tissues from n-  
380 6 enriched fatty acid-treated group compared to control diet-fed group. *In vivo* experiments  
381 demonstrated that the LxA<sub>4</sub> receptor Fpr2 was involved in the protective effects associated with  
382 n-6 PUFA-enriched diet. Collectively, these data make us propose that early during ischemia, an  
383 endogenous protective pathway mediated by LxA<sub>4</sub> and its receptor could be triggered by changes  
384 in dietary fatty acid.

385 Our results further confirmed the essential role that AA-derived lipid mediators could  
386 play in resolving inflammatory response *in vivo* (Serhan *et al.*, 1988). This notion has been well  
387 developed by a number of studies, and AA is now considered not only as a precursor of pro-  
388 inflammatory bioactive lipids, but also of potent anti-inflammatory mediators named Lipoxins  
389 ([Dufton \*et al.\*, 2010](#); [Morris \*et al.\*, 2006](#); [Schwab \*et al.\*, 2006](#); [Wallace, 2006](#)). Indeed, in our  
390 experimental conditions, we observed that ischemia and ischemia followed by reperfusion led to  
391 an increase of pro-inflammatory bioactive lipids or pathway marker derived from AA  
392 metabolism by COX (PGE<sub>2</sub>, 6kPGF<sub>1α</sub>, TxB<sub>2</sub>) and LOX (5-, 8-, 12-, 15-HETE, LTB<sub>4</sub>).  
393 Simultaneously, potent anti-inflammatory mediators derived from the AA metabolism, LxA<sub>4</sub> and

394 LxB<sub>4</sub> were also increased. These two lipoxins are differentially expressed in intestinal tissues  
395 over time, LxA<sub>4</sub> being produced from the ischemic period and LxB<sub>4</sub> (his positional isomer)  
396 increased only after 5 hours reperfusion. In our study, increased level of LxA<sub>4</sub> was significantly  
397 higher in n-6 diet group after ischemia compared to control diet group. However, levels LxB<sub>4</sub>  
398 (whose receptor(s) is yet to be discovered), were the same in both groups. We have recently  
399 reported a mechanism activated by neutrophil/platelet aggregates to increased circulating levels  
400 of LxA<sub>4</sub> at the end of 30 min mesenteric ischemia (Brancaleone *et al.*, 2013). This is in  
401 agreement with our current data and supports the notion that LxA<sub>4</sub> is an early metabolite of  
402 ischemic conditions. The effect of LXA<sub>4</sub>/Fpr2 system in the protective effects of n-6 diet  
403 suggests a minor role for LxB<sub>4</sub> in our experimental conditions.

404  
405 LxA<sub>4</sub> was identified by Serhan and colleagues in 1984 ([Serhan \*et al.\*, 1984](#)) as a short-  
406 lived lipid produced principally by transcellular synthesis ([Serhan \*et al.\*, 1984](#)). LxA<sub>4</sub> is  
407 described as a potent endogenous anti-inflammatory bioactive lipid mediator (Serhan *et al.*,  
408 2005). Acting through its receptor, Fpr2 ([Lavigne \*et al.\*, 2002](#); [Perretti \*et al.\*, 2002](#); [Ye \*et al.\*,](#)  
409 2009), a G protein-coupled receptor belonging to the formyl peptide receptor family, LxA<sub>4</sub> is  
410 able to halt leukocyte migration (Chiang *et al.*, 2006; Perretti *et al.*, 2009) and promote  
411 macrophage phagocytosis of infectious agents and apoptotic neutrophils ([Godson \*et al.\*, 2000](#);  
412 [MADERNA \*et al.\*, 2005](#)). In agreement with our results, several studies have demonstrated a  
413 protective role for pharmacologically delivered lipoxins or stable lipoxin analogues in I/R  
414 ([BANNENBERG \*et al.\*, 2004](#); [Sun \*et al.\*, 2009](#); [Wu \*et al.\*, 2012](#)).

415 Cell-cell interaction is one of the routes by which lipoxins are endogenously  
416 biosynthesized by cooperation between 15- and 5-lipoxygenases (LOX) ([Serhan \*et al.\*, 2008](#)).

417 Primarily described in granulocytes and later in airway epithelial cells and dendritic cells,  
418 metabolism of AA by 15-LOX produces 15S-hydroperoxyeicosatetraenoic acid or the reduced  
419 alcohol form 15S-hydroxyeicosatetraenoic acid (15S-HETE) ([Levy et al., 1993](#); [Serhan, 1989](#);  
420 [Serhan et al., 2008](#)). These metabolites are further transformed into epoxytetraene intermediates  
421 by the action of leukocyte 5-LOX yielding LxA<sub>4</sub> and LxB<sub>4</sub> ([Levy et al., 1993](#); [Serhan, 1989](#);  
422 [Serhan et al., 2008](#)). In the intestine, resident tissue cells such as intestinal epithelial cells  
423 expressed 15-LOX and could further contribute to LxA<sub>4</sub> generation by the production of 15S-  
424 H(p)ETE ([McMahon et al., 2001](#)). Indeed, LxA<sub>4</sub> is synthesized in the human gut by colonic  
425 mucosa ([Mangino et al., 2006](#)). Interestingly, in pathological conditions such as ulcerative  
426 colitis, LxA<sub>4</sub> endogenous production is significantly reduced compared to control patients  
427 ([Mangino et al., 2006](#)). This decrease was coupled to a lower level of 15-LOX in the mucosa of  
428 ulcerative colitis patients, compared to tissues from healthy controls ([Mangino et al., 2006](#)). In  
429 our experimental conditions, where *alox5* and *alox15* expression and LxA<sub>4</sub> were increased after  
430 ischemia before granulocyte infiltration, epithelial cells could be the principal source of 15S-  
431 H(p)ETE or 15s-HETE metabolized into LxA<sub>4</sub> by 5-LOX bearing cells (polymorphonuclear  
432 leukocytes, macrophages, and mast cells that physiologically populate the gut). LxA<sub>4</sub> acts on a  
433 specific target receptor termed formyl-peptide receptor-2/lipoxin A<sub>4</sub> receptor (FPR2/ALX) in  
434 humans and Fpr2/3 in mice ([Perretti et al., 2002](#); [Ye et al., 2009](#)). FPR2 transduces the pro  
435 resolving properties of LXA<sub>4</sub>, but also the glucocorticoid regulated protein Annexin A1 and  
436 humanin ([Dufton et al., 2010](#)). Increasing evidence suggests that members of the FPR family and  
437 in particular FPR2 might have an important role in the pathophysiology of I/R injury ([Gavins,](#)  
438 [2010](#)). In a recent study, the use of *fpr2/3*-deficient mice highlighted the importance of this  
439 receptor in I/R; in the absence of Fpr2/3, an aberrant inflammatory response characterized by



440 prolonged neutrophil adhesion and infiltration ([Brancaleone et al., 2013](#)). Furthermore,  
441 exogenous delivery of LxA<sub>4</sub> attenuated I/R-mediated inflammation in wild type but not in Fpr2/3  
442 deficient animals ([Brancaleone et al., 2013](#)). In mice receiving a high n-6 diet, administration of  
443 the Fpr2 antagonist before ischemia reverted the protective anti-inflammatory effect of this diet.  
444 Our data indicate that the protective association between n-6 supplementation and the prevention  
445 of post-ischemic damage was due, at least in part, to the interaction between LxA<sub>4</sub> (produced in  
446 higher amount in the n-6 group) and its receptor, Fpr2. Human FPR2/ALX was first identified on  
447 neutrophils and monocytes ([Fiore et al., 1992](#); [Takano et al., 1997](#)). Here, we observed that the  
448 murine receptor was expressed also in intestinal epithelial cells, confirming a previous *in vitro*  
449 study that had identified Fpr2 in intestinal epithelial cell lines ([Goh et al., 2001](#)). Interestingly, *in*  
450 *vitro* studies have identified Fpr2 expression preferentially on the basolateral side of intestinal  
451 epithelial cells ([Kucharzik et al., 2003](#)). LxA<sub>4</sub> produced on the serosal side of the epithelium by  
452 leukocyte-epithelial cell interaction could rapidly signal by activating the receptor on the  
453 basolateral side, resulting in potential attenuation of inflammation. LxA<sub>4</sub> promotes anti-  
454 inflammatory signals not only by regulating innate immune cells, but also by acting on intestinal  
455 epithelial cells. Upon stimulation with bacteria (*Salmonella typhimurium*) ([Gewirtz et al., 1998](#)),  
456 TNF $\alpha$  ([Goh et al., 2001](#); [Gronert et al., 1998](#)) or LPS ([Kure et al., 2010](#)), human intestinal  
457 epithelial cells release the pro-inflammatory chemokine interleukin-8: this response could be  
458 inhibited by cell exposure to LxA<sub>4</sub>. In accordance with these data, we observed that after I/R the  
459 n-6-enriched diet was associated with a decrease in CXCL1, the murine equivalent of human  
460 interleukin-8. Other pro-inflammatory signals, such as the transcription factor NF- $\kappa$ B can be  
461 modulated by LxA<sub>4</sub> in epithelial cells ([Gewirtz et al., 1998](#)). Such inhibition is also downstream  
462 of Fpr2 engagement by LxA<sub>4</sub> ([Gewirtz et al., 2002](#)). There is conflicting data regarding how



463 much n-6 (i.e. linoleic acid, LA) should be consumed in a healthy diet. Some claim that high LA  
464 intake promotes inflammation through accumulation of tissue arachidonic acid (AA) and  
465 subsequent production of pro-inflammatory lipid mediators. Human studies indicate that high LA  
466 in the diet or circulation is not associated with higher *in vivo* or *ex vivo* pro-inflammatory  
467 responses. Several studies have shown that individuals consuming the highest level of LA have  
468 the lowest inflammatory status (Fritsche, 2008). In our experimental animal study we did not  
469 detect any “pro-inflammatory phenotype” of the n-6 diet group that underwent a sham operation  
470 compared to the n-3 or control diet. Conversely, the n-6 but not the n-3 enriched diet displayed  
471 anti-inflammatory properties indicating that the consumption of high n-6 diets may work in  
472 specific intestinal scenarios. Exploiting this n-6 dependent endogenous anti-inflammatory loop  
473 described here could be of therapeutic impact in diseases in which intestinal ischemia contributes  
474 to the pathogenesis, such as neonatal necrotizing enterocolitis (NEC) (Berman *et al.*, 2011). It  
475 has been shown that the type and composition of infant diet can regulate the outcome of this  
476 pathology. In a clinical study, Carlson *et al.* found a lower incidence of NEC in infants fed  
477 formula containing higher amounts of PUFA compared with controls (Carlson *et al.*, 1998). In  
478 the rat NEC model, supplementation of the formula with AA have been shown to reduce the  
479 degree of intestinal injury (Lu *et al.*, 2007). Thus in this pathology, delivery of LxA<sub>4</sub>, shown to  
480 be the most important AA metabolite in our model, could be even more effective than AA-based  
481 treatment.

482 In conclusion these data prompt us to propose that the endogenous anti-inflammatory circuit  
483 activated by a selective Fpr2 agonist, LxA<sub>4</sub>, could be harnessed by boosting n-6 PUFA diet  
484 supplementation as a viable therapeutic approach for the prevention of the ischemia reperfusion  
485 injuries

486

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502 Marc Dubourdeau: interpretation of data; redaction of the manuscript

503 Hervé Guillou: conception and design; interpretation of data; redaction of the manuscript

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505 Nathalie Vergnolle: conception and design; redaction of the manuscript

506 Nicolas Cenac: conception and design; acquisition, analysis and interpretation of data; redaction  
507 of the manuscript

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509 Thomas Gobbetti and Nicolas Cenac take responsibility for the integrity of the work as a whole,  
510 from inception to published article

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698



699 **Table 1: Composition in % of the 3 isocaloric diets**

700

	Control	n-6	n-3
Cellulose	2	2	2
Casein	22	22	22
Starch	43.6	43.6	43.6
Methionine	0.2	0.2	0.2
Sucrose	21.8	21.8	21.8
Minerals	4.5	4.5	4.5
Vitamins	1.0	1.0	1.0
Grape Seed Oil	2.5	5	0
Colza Oil	2.5	0	4
Fish Oil	0	0	1

701  
702 **Table 2: Composition in % of polyunsaturated fatty acid of the 3 isocaloric diets containing**

703 **5% fat (w/w)**

	Control	n-6	n-3
C12:0	0.0	0.0	0.0
C14:0	0.0	0.0	0.0
C16:0	6.0	6.5	4.5
C18:0	2.5	4.2	0.7
C18:1n-9	38.8	18.4	47.4
C18:2n-6	47.4	70.0	19.9
C18:3n-3	5.2	1.0	7.6
C20:5n-3	0.0	0.0	17.0
C22:6n-3	0.0	0.0	3.0
n-6/n-3 ratio	10	70	0.72

704

705

706 **Table 3: Concentration of fatty acid (nmol/mg of protein) in mouse intestine.** Mice were  
 707 submitted to 9 weeks of control, n-3 or n-6 diet before fatty acid (FA) metabolite extraction. Data  
 708 are express as mean  $\pm$  SEM, n=10. \*p<0.05 vs. control diet group. SAFA: saturated fatty acid;  
 709 MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; LA: linoleic acid; ALA:  
 710  $\alpha$ -linolenic acid; AA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic  
 711 acid.

	Diet		
	Control	n-3	n-6
10:0	0.00	0.00	0.00
12:0	0.00	0.00	0.00
14:0	0.00	5.39 $\pm$ 2.57	1.02 $\pm$ 1.02
15:0	0.00	0.00	0.00
16:0	286.85 $\pm$ 43.95	349.08 $\pm$ 75.12	329 $\pm$ 79.41
18:0	185.97 $\pm$ 23.68	147.24 $\pm$ 21.21	127.53 $\pm$ 42.45
20:0	0.00	0.00	0.00
21:0	0.00	0.00	0.00
22:0	0.00	0.00	0.00
14:1 n-5	0.00	0.00	0.00
15:1 n-5	0.00	0.00	0.00
16:1 n-9	0.00	0.00	0.00
16:1 n-7	150.83 $\pm$ 13.09	157.22 $\pm$ 38.09	146.05 $\pm$ 14.37
17:1 n-7	0.00	0.00	0.00
18:1 n-9	413.22 $\pm$ 68.93	450.11 $\pm$ 49.71	445.18 $\pm$ 65.70
18:1 n-7	100.73 $\pm$ 17.17	93.00 $\pm$ 11.58	94.35 $\pm$ 19.73
20:1 n-9	0.00	0.00	0.00
22:1 n-9	0.00	0.00	0.00
24:1 n-9	0.00	0.00	0.00
18:2 n-6 (LA)	84.56 $\pm$ 18.38	69.76 $\pm$ 10.07	209.05 $\pm$ 23.64 *
18:3 n-6	0.00	0.00	0.00
18:3 n-3 (ALA)	0.00	15.65 $\pm$ 1.65 *	0.00
20:2 n-6	3.41 $\pm$ 1.50	0.00 *	6.97 $\pm$ 0.92 *
20:3 n-3	0.00	0.00	0.00
20:3 n-6	18.29 $\pm$ 1.03	8.56 $\pm$ 0.86 *	13.47 $\pm$ 1.34
20:4 n-6 (AA)	39.95 $\pm$ 2.34	13.81 $\pm$ 1.19 *	60.37 $\pm$ 5.70 *
20:5 n-3 (EPA)	0.00	10.78 $\pm$ 1.86 *	0.00
22:5 n-3	0.00	5.69 $\pm$ 0.78 *	0.00
22:2 n-6	0.00	0.00	0.00
22:4 n-6	0.00	0.00	0.00
22:5 n-6	0.00	0.00	0.00
22:6 n-3 (DHA)	1.88 $\pm$ 1.21	19.87 $\pm$ 3.14 *	0.93 $\pm$ 0.52
<b>20:4 n-6 / 22:6 n-3</b>	<b>9.51 <math>\pm</math> 0.75</b>	<b>0.73 <math>\pm</math> 0.07 *</b>	<b>44.09 <math>\pm</math> 11.34 *</b>
<b>Total SAFA</b>	<b>472.82 <math>\pm</math> 67.64</b>	<b>501.71 <math>\pm</math> 98.91</b>	<b>457.98 <math>\pm</math> 122.88</b>
<b>Total MUFA</b>	<b>664.78 <math>\pm</math> 99.19</b>	<b>700.33 <math>\pm</math> 99.38</b>	<b>685.58 <math>\pm</math> 99.80</b>
<b>Total PUFA</b>	<b>148.08 <math>\pm</math> 24.46</b>	<b>144.50 <math>\pm</math> 19.93</b>	<b>290.78 <math>\pm</math> 32.30 *</b>
<b>Total FA</b>	<b>1284 <math>\pm</math> 151</b>	<b>1345 <math>\pm</math> 162</b>	<b>1432 <math>\pm</math> 114</b>



712 **Figure legends**

713 **Figure 1: Effect of dietary intervention on the hepatic fatty acids composition.** Total  
714 arachidonic acid (C20:4 n-6) and docosahexaenoic acid (C22:6 n-3) ratio in liver of mice  
715 submitted to control, n-3 or n-6 PUFA diet for 9 weeks. Values are mean  $\pm$  SEM, n= 6-8 per  
716 group. \*\* p<0.01, \*\*\* p<0.001, significantly different from control diet group.

717 **Figure 2: Inflammation of the intestine induced by I/R.** (A) representative hematoxylin and  
718 eosin-stained histological sections of small intestine from sham-operated mouse or mouse  
719 submitted to I/R and receiving control, n-3 or n-6 PUFA diet. Different inflammatory  
720 parameters were followed: microscopic damage scores (B), myeloperoxidase activity (MPO) (C)  
721 in sham (white bar) or I/R (black bar) mice exposed to control, n-3 or n-6 PUFA diet for 9  
722 weeks. (D-E) CXCL1 and CCL2 expressions were quantified in I/R mice *versus* corresponding  
723 sham, exposed to control (white bar), n-3 (black bar) or n-6 (gray bar) PUFA diet. Values are  
724 mean  $\pm$  SEM, n= 6-8 per group. \* p <0.05, \*\* p<0.01, \*\*\* p<0.001, significantly different from  
725 sham; + p <0.05, ++ p<0.01, significantly different from control diet group.

726 **Figure 3: Quantification of PUFA metabolites in intestine of mice exposed to I/R and to the**  
727 **three different diets.** (A) Heat-map and clustering of quantified PUFA metabolites by liquid  
728 chromatography-tandem mass spectrometry. The heatmap represents a color coding of average z-  
729 scores obtained for the different PUFA metabolites (in row) and the groups of mice (in column).  
730 A red color indicates a quantity greater than the mean of the row, a green color indicates a  
731 quantity lower than the mean and black color a quantity close to the mean. The dendrogram  
732 reflects the correlation of the PUFA metabolites. The dotted red line represents the clusters. (B)  
733 Synthesis of lipoxin A<sub>4</sub> (LxA<sub>4</sub>) was measured by liquid chromatography-tandem mass  
734 spectrometry in sham ischemia (white bar), sham I/R animals (stripped bar) and following 50

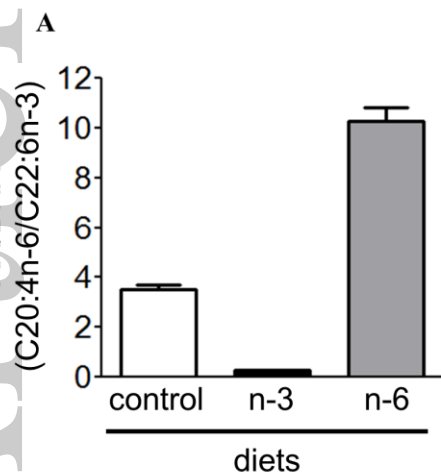
735 minutes of ischemia (gray bar) or 50 minutes of ischemia and 5 hours of reperfusion (I/R, black  
736 bar). Data are expressed in pg/mg protein and represent means  $\pm$  SEM of 6 to 8 animals per  
737 group. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , significantly different from corresponding sham; ++  $p < 0.01$ ,  
738 significantly different from ischemia control diet group

739 **Figure 4: Expression of FPR2 in mouse small intestine.** (A) FPR2 mRNA expression was  
740 quantified in I/R mice *versus* corresponding sham, exposed to control (white bar), n-3 (black bar)  
741 or n-6 (gray bar) PUFA diet. (B) Pictures of FPR2, cytokeratin 18, CD45 and Ly-6B.2  
742 immunoreactivity detected in the small intestine of mice exposed to sham and to n-6 PUFA diet  
743 (scale: 50  $\mu$ m). Values are mean  $\pm$  SEM, n= 6-8 per group. \*  $p < 0.05$ , \*\*  $p < 0.01$ , significantly  
744 different from corresponding ischemia group.

745 **Figure 5: Role of FPR2 antagonist in n-6 PUFA diet anti-inflammatory effects.** (A) Different  
746 inflammatory parameters were followed: myeloperoxidase activity, microscopic damage scores,  
747 chemokine (C-X-C motif) ligand 1 (CXCL1) expression and chemokine ligand 2 (CCL2)  
748 expression, in sham (grey bar) or I/R (black bar) mice treated with FPR2 antagonist (BOC-2, 500  
749  $\mu$ g/kg before ischemia i.v.) and exposed to n-6 PUFA diet for 9 weeks. (B) N-6 PUFA derived  
750 metabolites in sham (grey bar) or I/R (black bar) mice treated with FPR2 antagonist (BOC-2, 500  
751  $\mu$ g/kg before ischemia i.v.) and exposed to n-6 PUFA diet for 9 weeks. Data are expressed as  
752 percentage of inhibition of I/R induced effect in control diet group. Values are mean  $\pm$  SEM, n=  
753 6-8 per group. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , significantly different from sham; +  $p < 0.05$ ,  
754 ++  $p < 0.01$ , significantly different from I/R vehicle group.

755  
756 **Disclosures:** The authors have no potential conflicts (financial, professional, or personal) that are  
757 relevant to the manuscript.

Figure 1

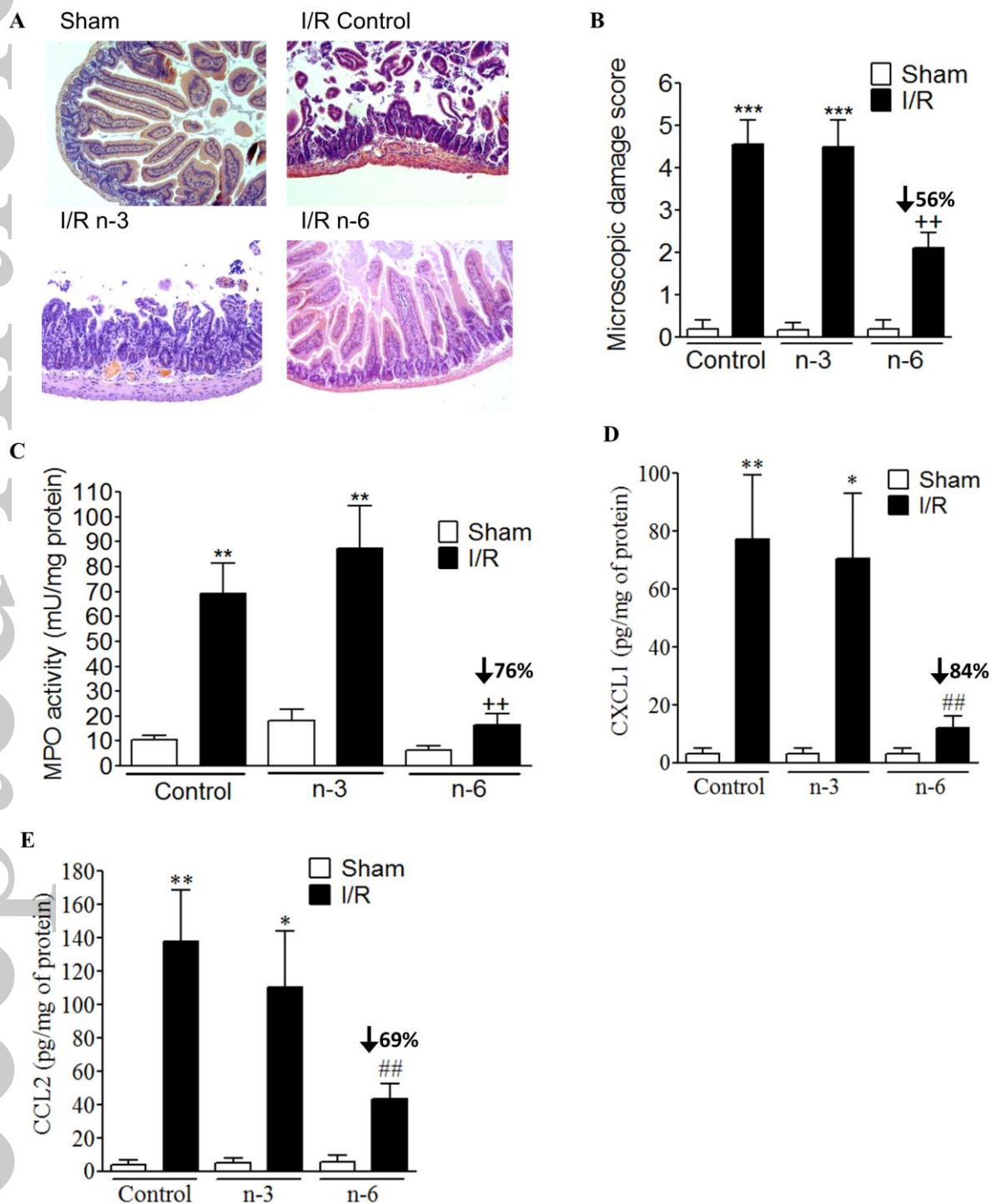


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Figure 2

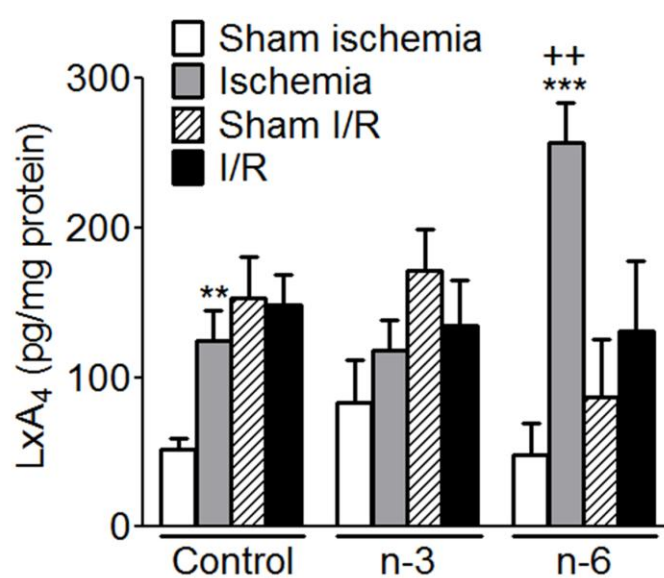
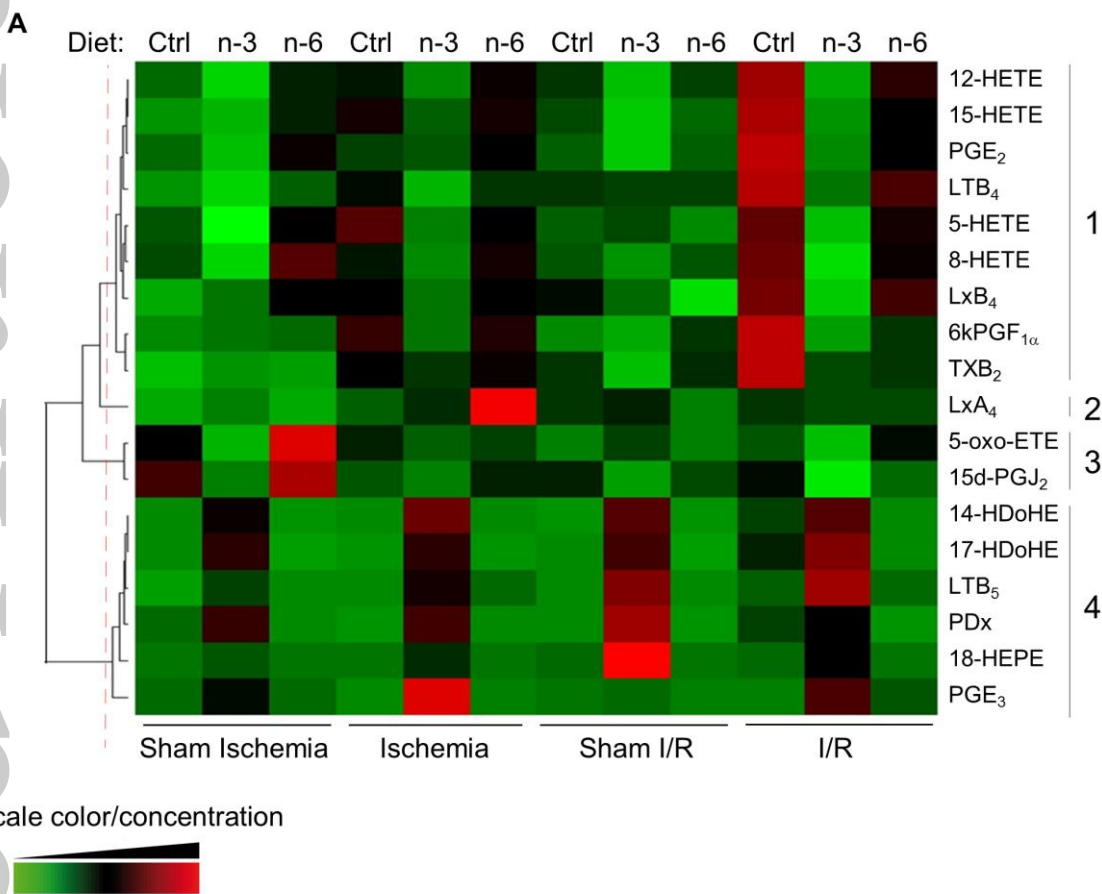


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Figure 3



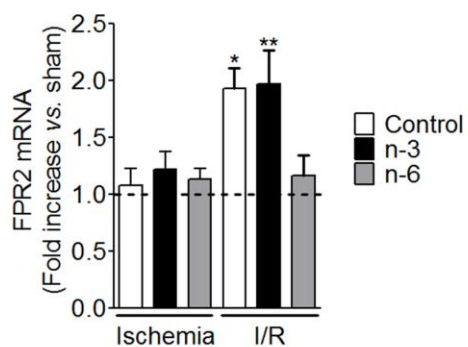
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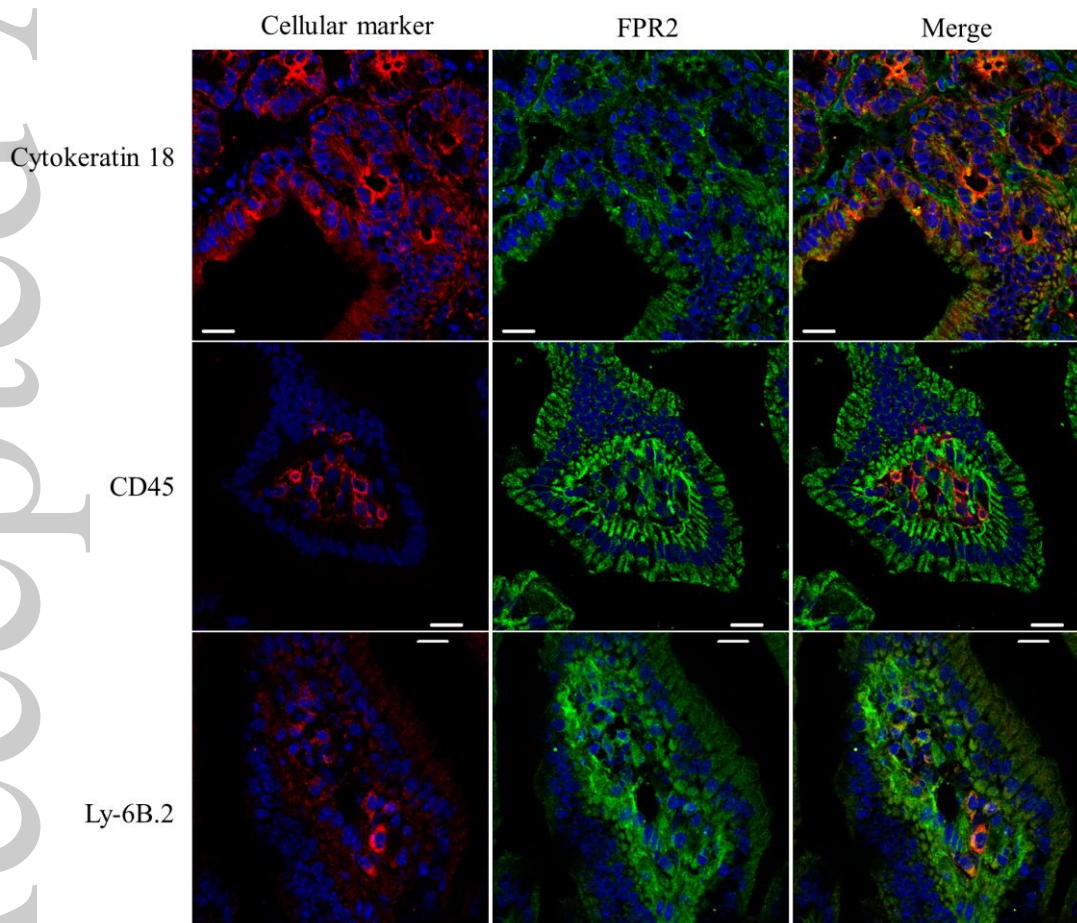
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Figure 4

A



B



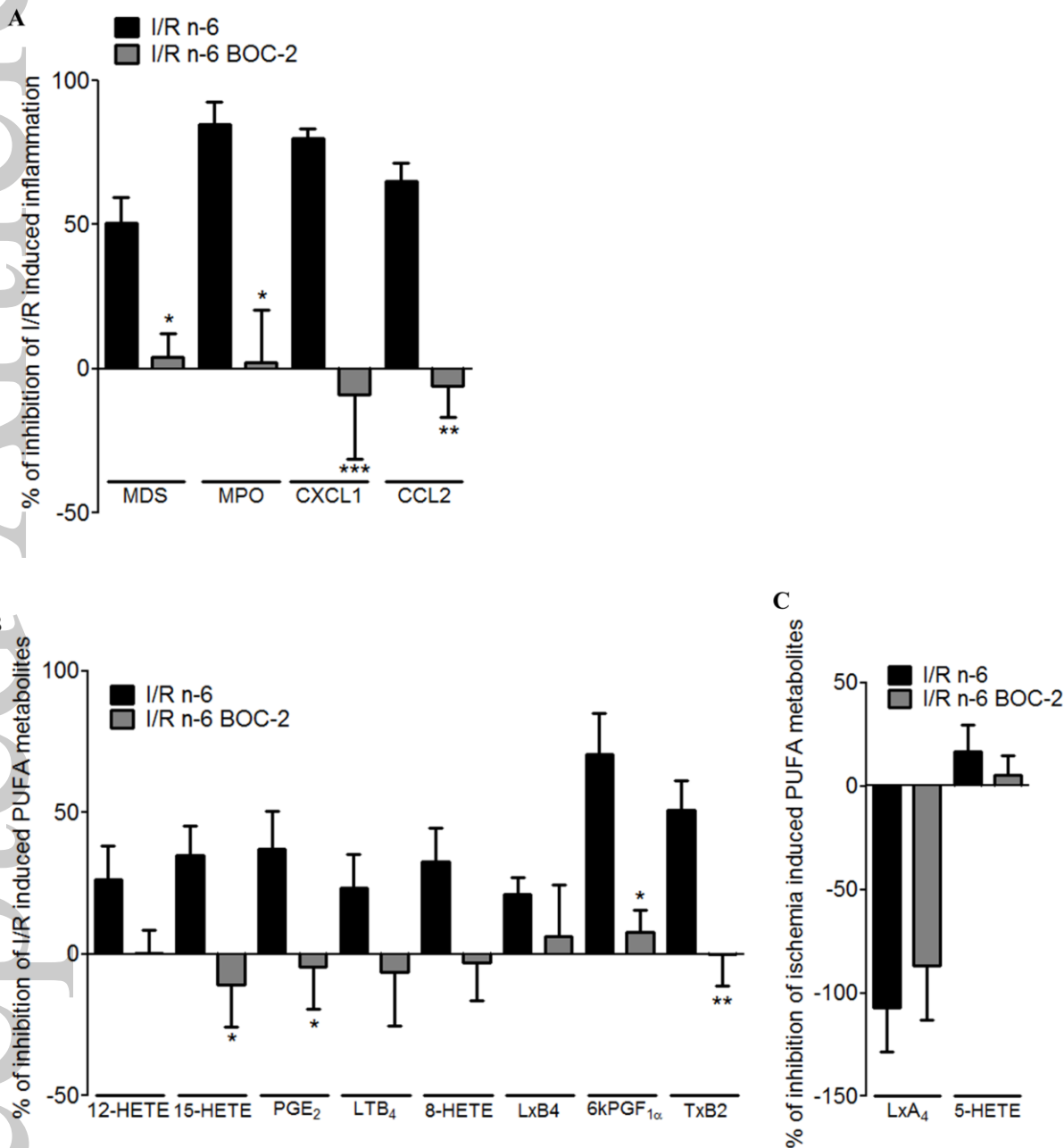
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Figure 5



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