SUPPLEMENTAL DIGITAL CONTENT 1

Anti-inflammatory Effects of Omega-3 Polyunsaturated Fatty Acids and Soluble Epoxide Hydrolase Inhibitors in Angiotensin-II Dependent Hypertension

Arzu Ulu, MSc[*]; Todd R Harris, PhD[*]; Christophe Morisseau, PhD[*]; Christina Miyabe, BS[*]; Hiromi Inoue, DVM[#]; Gertrud Schuster, PhD[§], Hua Dong, PhD[*]; Ana-Maria Iosif, PhD[£], Jun-Yan Liu, PhD[*]; Robert H Weiss, MD[#¥]; Nipavan Chiamvimonvat, MD[ϕ¥]; John D Imig, PhD[†]; Bruce D Hammock, PhD[*]

[*]Department of Entomology and UC Davis Comprehensive Cancer Center, University of California, Davis; [#]Division of Nephrology, [ϕ] Division of Cardiovascular Medicine, Department of Internal Medicine, [§]Department of Nutrition, [£]Division of Biostatistics, Department of Public Health Sciences, School of Medicine, University of California, Davis, USA; [¥]U.S. Department of Veterans' Affairs Medical Center, Sacramento, California, USA; [†]Medical College of Wisconsin, Milwaukee, WI, USA.

Corresponding author:
Dr Bruce D. Hammock
E-mail: bdhammock@ucdavis.edu
Telephone: 530-752-7519
Fax: 530-752-1537

Short title: Fish oil and sEH inhibitors in hypertension

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Sections:

A) Fatty acid analysis of the diets

To quantify the fatty acid composition, lipids of the powered diets were extracted from weighed aliquots, mixed with internal standards, transesterified and analyzed using gas chromatography with mass spectral detection. Specifically, ~50 mg aliquot of diets were weighed into a pre-tarred 2mL safe lock tubes, lipids were extracted with 400 µL isopropanol, followed by 500 µL cyclohexane and phase separation with 550 µL 0.1 M ammonium acetate. Samples were centrifuged at 3500rpm for 5min at room temperature. A 10 µL extract aliquot was transferred to a 2 mL glass amber vial containing 3 µM SS-15:1n5-triacylglceride (NuChek Prep; Elisian, MN) derivatization standard in 1:9 toluene/methanol (v/v). These samples were enriched with 100µL 0.5M anhydrous sodium methoxide (Sigma-Aldrich; St. Louis, MO), incubated 1hr at 60^{o}C, enriched with 100µL 3N methanolic hydrochloric acid (Sigma-Aldrich; St. Louis, MO), and incubated 30 min at 60^{o}C. Reaction mixtures were neutralized by addition of 400µL of 0.25M $KHCO_3$:0.5M K_2CO_3, and fatty acid methyl esters were extracted by vortexing with 400µL hexane, with phase separation being assisted by 5min centrifugation at 3500 rpm. A 100µL aliquot of the hexane extract was spiked with 10µL hexane containing 44µM C23:0 as an internal standard. Analyte residues in 1µL sample aliquots were separated on a 30m x 0.25mm, 0.25µm DB-225 ms column and detected on an Agilent 5973 mass selective detector with selected ion monitoring responses calibrated against a 7 point calibration curve used to quantify the results. All reported results are corrected for surrogate loss.

TABLE S1A. Composition of mouse diets administered in the present study.

	Corn oil diet	**ω-3 rich diet***
Components	**(% weight)**	
Cornstarch	40	40
Casein	20	20
Dextrose	15	15
Sucrose	10	10
Cellulose	5	5
Mineral mix	3.5	3.5
Vitamin mix	1	1
Cysteine	0.3	0.3
Choline bitartrate	0.2	0.2
Corn oil (Mazola)	5	3.5
EPA	-	0.75
DHA	-	0.75

***Preparation:** Briefly, dextrose, mineral mixture, vitamin mixture, cysteine and choline bitartrate were combined and mixed for 30 min, then cornstarch, casein, sucrose and cellulose were added to this mixture. The oil ingredients those are corn oil, 90% pure EPA and 90% pure DHA (Larodan Fine Chemicals, Sweden) were weighed together in a glass beaker and added to the dry ingredients of the diet. The diet lacking these omega-3 fatty acids (corn oil diet) was prepared using the same ingredients except for the EPA and DHA, which was replaced by the corn oil. Animals were started on corresponding diets one week before the induction of hypertension. Daily food consumption was recorded.

TABLE S1B. Percentage fatty acid composition of total fat for the corn oil, ω-3 rich and standard diets.

Fatty Acids	C: D	Corn oil diet (%)	ω-3 rich diet (%)	Standard diet* (Harlan-Teklad) (%)
Saturated fats				
Palmitic	C16:0	9.53	7.58	7.64
Magaric	C17:0	0.04	0.03	0.00
Stearic	C18:0	1.17	0.93	1.5
Arachidic	C20:0	0.23	0.19	0.01
Behenic	C22:0	0.06	0.05	0.03
Lignoceric	C24:0	0.07	0.06	0.00
Monounsaturated fats				
trans -7-hexa-deanoic	C16:1n7t	0.02	0.02	N/A
Palmitoleic	C16:1n7	0.06	0.07	0.07
cis-10-Heptadecenoic	C17:1n7	0.02	0.02	N/A
Oleic	C18:1n9	26.97	20.90	12.59
cis-Vaccenic	C18:1n7	0.18	0.15	N/A
Gadoleic	C20:1n9	0.08	0.07	0.17
n-6 polyunsaturated fats				
Linoleic	C18:2n6	60.92	46.35	31.35
Conjugated linoleic	9c,11t-CLA	0.01	0.01	N/A
Eicosadienoic	C20:2n6	0.00	0.01	0.00
Arachidonic	C20:4n6	0.00	0.22	0.00
Docosapentaenoic	C22:5n6	0.00	0.07	0.00
n-3 polyunsaturated fats				
α- linolenic	C18:3n3	0.63	0.53	2.76
Eicosatetraenoic	C20:4n3	0.00	0.06	N/A
Eicosapentaenoic	C20:5n3	0.01	11.53	0.00
Docosapentaenoic	C22:5n3	0.00	0.19	0.00
Docosahexaenoic	C22:6n3	0.00	10.98	0.00
Σ saturated fatty acids		11.10	8.84	9.59
Σ monounsaturated fatty acids		27.33	21.22	12.84
Σ (n-6) polyunsaturated fatty acids		60.93	46.66	31.35
Σ (n-3) polyunsaturated fatty acids		0.64	23.28	2.76
Σ (n-6)/ Σ (n-3)		95.4	2	11.4

*****2018 Teklad Global 18% Protein Rodent Diet, Harlan Laboratories, Hayward, CA. Fatty analysis data are reprinted from Arendash et al.[9] The fatty acid analysis of the corn oil and ω-3 rich diet, but not the standard diet (reprinted from the analysis of Arendash et al) was performed by us. Of note, while the corn oil diet was a purified diet and used in its powder form, the standard diet was a commercially available regular rodent chow in pellet form, which might vary with changes in the ingredients and processing by the manufacturer.
N/A: not available

B) Measurement of blood pressure

To avoid the investigator bias, we blinded the operator (A.U.) by placing cards that block the treatment information associated with each cage during the blood pressure recordings. Also, each cage and associated blocking card was given a number. Measurements were saved by the cage number and the number identifying each animal (for example; Cage 1-mouse 1), which also provided blinded data analysis. For each animal, we used the exact same method to analyze the data. Briefly, we exclude the first 3 (acclimation), the highest and the lowest value among the 20 cycles and last we eliminate the readings associated with animal movement. Then, we average the remaining blood pressure readings to obtain the final systolic blood pressure for each animal. Animal movement results in inaccurate measurements, because the software recognizes the movements as a reference point on the blood pressure curve.

C) Oxylipin extraction and analysis

Fifty mg of murine kidney was homogenized in 400 µL ice-cold methanol containing 0.1% glacial acetic acid and 0.1% BHT and added with 10 µL anti-oxidant solution (0.2 mg/ mL BHT (butyl hydroxytoluene) and EDTA in 1:1 ratio methanol and water) and 10 µL internal standard. Then, tissue homogenates were centrifuged at 10,000 rpm at 4°C for 10 min. Collected supernatants were extracted using SPE cartridges (Waters Oasis HLB C18 cartridge), then eluted by ethyl acetate, and thoroughly dried using a vacuum centrifuge. The samples were then re-dissolved in 50 µL additional standard solution (instrumental standard) in methanol and quantified using an LC/MS/MS technique.[1]

TABLE S2A. HPLC solvent gradient to separate ARA, LA, EPA and DHA metabolites.

Gradient[a] (min)	Aqueous phase [b]	Organic Phase [c]
0	65	35
0.25	65	35
1	55	45
3	45	55
8.5	34	66
12.5	28	72
15	18	82
16.5	5	95
18	5	95
18.1	65	35
21.5	65	35

[a]0.25 ml/min flow rate, [b] Distilled Water 99.9, Acetic Acid 0.1, volume %,
[c] ACN 80, Methanol 20, Acetic Acid 0.1, volume %.

TABLE S2B. Retention time, selected reaction monitoring (SRM) and internal standard information used to quantify all analytes.

Peak Name	Precursor ion (m/z)	Product ion (m/z)	Surrogate Internal Standards	Retention time (min)
6-keto-PGF1α	369.3	163.2	d4-6-keto-PGF1a	3.54
d4-6-keto-PGF1 α	373.3	167.1	Instrumental standard (IS)*	3.56
Resolvin E1	349.3	195	d4-PGE2	3.58
d4-TXB2	373.3	173.2	IS	4.28
TXB2	369.2	169.1	d4-TXB2	4.29
PGE3	349.3	269.2	d4-PGE2	4.37
PGD3	349.3	269.2	d4-PGE2	4.61
PGF2 α	353.2	309.2	d4-PGE2	4.75
d4-PGE2	355.2	275.3	IS	4.97
PGE2	351.2	271.3	d4-PGE2	4.99
PGD2	351.2	271.3	d4-PGE2	5.29
17,18-DiHETE	335.3	247.2	d11-14,15-DHET	8.34
14,15-DiHETE	335.3	207.2	d11-14,15-DHET	8.84
Instrumental Standard (IS)	339.2	214.3	None	8.86
11,12-DiHETE	335.2	167.1	d11-14,15-DHET	9.01
12,13-DiHOME	313.2	183.2	d11-14,15-DHET	9.18
8,9-DiHETE	335.2	127.1	d11-14,15-DHET	9.31
9,10-DiHOME	313.2	201.2	d11-14,15-DHET	9.54
d11-14,15-DHET	348.2	207.1	IS	10.0
19,20-DiHDPE	361.2	273.2	d11-14,15-DHET	10.12
14,15-DHET	337.2	207.1	d11-14,15-DHET	10.13
16,17-DiHDPE	361.2	233.2	d11-14,15-DHET	10.64
11,12-DHET	337.2	167.1	d11-14,15-DHET	10.79
13,14-DiHDPE	361.2	193.2	d11-14,15-DHET	10.86
10,11-DiHDPE	361.2	153.2	d11-14,15-DHET	11.17
8,9- DHET	337.2	127.1	d11-14,15-DHET	11.31
d6-20-HETE	325.2	281.2	d11-14,15-DHET	11.95
20-HETE	319.2	275.1	d6-20-HETE	12.03
15-HEPE	317.2	219.2	d8-12-HETE	12.05
8-HEPE	317.2	155.2	d8-12-HETE	12.32
12-HEPE	317.2	179.2	d8-12-HETE	12.51
5-HEPE	317.2	115.1	d8-12-HETE	12.92
d4-9-HODE	299.2	172.3	IS	13.13
13-HODE	295.2	195.2	d4-9-HODE	13.17
9-HODE	295.2	171.1	d4-9-HODE	13.24
15-HETE	319.2	219.2	d8-12-HETE	13.82
17(18)-EpETE	317.2	215.2	d11-11(12)-EET	13.99
11-HETE	319.2	167.2	d8-12-HETE	14.32
d8-12-HETE	327.2	184.2	IS	14.56
9-oxo-ODE	293.2	185.1	d4-9-HODE	14.57
15-oxo-ETE	317.2	113.1	d11-11(12)-EET	14.57
14(15)-EpETE	317.2	207.2	d11-11(12)-EET	14.66
8-HETE	319.2	155.2	d8-5-HETE	14.68
12-HETE	319.2	179.2	d8-12-HETE	14.75
11(12)-EpETE	317.2	167.2	d11-11(12)-EET	14.83
8(9)-EpETE	317.2	127.2	d11-11(12)-EET	14.98

9-HETE	319.2	167.2	d8-12-HETE	15.05
15(S)-HETrE	321.2	221.2	d8-12-HETE	15.12
d8-5-HETE	327.2	116.1	IS	15.13
12-oxo-ETE	317.2	153.1	d11-11(12)-EET	15.28
5-HETE	319.2	115.2	d8-5-HETE	15.31
19(20)-EpDPE	343.2	241.2	d11-11(12)-EET	15.98
12(13)-EpOME	295.2	195.2	d4-9(10)-EpOME	16.06
d4-9(10)-EpOME	299.2	172.2	IS	16.16
14(15)-EET	319.2	219.3	d11-11(12)-EET	16.24
9(10)-EpOME	295.2	171.1	d4-9(10)-EpOME	16.26
16(17)-EpDPE	343.2	233.2	d11-11(12)-EET	16.46
13(14)-EpDPE	343.2	193.2	d11-11(12)-EET	16.55
10(11)-EpDPE	343.2	153.2	d11-11(12)-EET	16.66
d11-11(12)-EET	330.2	167.2	IS	16.68
11(12)-EET	319.2	167.2	d11-11(12)-EET	16.81
8(9)-EET	319.2	167.2	d11-11(12)-EET	16.98

*1-Adamantan-1-yl-3-decyl-urea is used as an additional (instrumental) standard. IS was used to quantify the surrogate internal standards and to calculate extraction recovery after SPE extraction of the oxylipins from the kidney. All the analytes were quantified using the deuterated surrogate standards.

D) qRT-PCR gene expression assays

Kidneys were perfused with cold PBS, and immediately snap frozen in the Trizol reagent (Invitrogen, Carlsbad, CA) and then stored at -80°C until processing. Total RNA was extracted from 10 µg of the renal cortex using a pure link Micro total RNA purification kit (Invitrogen, Carlsbad, CA). Quantity and quality of the RNA samples were assayed by a spectrophotometer and then 1 µg DNase treated total RNA was converted to cDNA using a high-capacity cDNA reverse transcriptase kit (Applied Biosystems, Foster City, CA). In the resultant cDNA samples, fold change in mRNA expression was determined using TaqMan gene expression assays. The following TaqMan gene expression assays (Applied Biosystems, Foster City, CA) were employed: *Ephx2* (Mm00514706_m1), *Ptgs-2* (prostaglandin endoperoxide synthase 2, Mm00478374_m1), *Alox5* (arachidonate 5-lipoxygenase, Mm01182747_m1*), *Scnn1a* (epithelial sodium channel, Mm00803386_m1) and *Ace-2* (angiotensinI converting enzyme-2, Mm01159003_m1). Experiments were run on the same day in duplicate along with a house keeping gene, mouse GAPDH (glyceraldehyde 3-phosphate dehydrogenase message). Fold change in gene expression was normalized to the endogenous control, GAPDH, and to the calibrator samples (controls).

E) Analysis of the SBP data using mixed-effects model with three between subjects factors

We performed additional statistical analyses of the SBP data to examine the individual effects of the given treatments. To this end, we analyzed the SBP data using Ang-II, ω-3 rich diet and TPPU as three between subjects factors and time (after day 1) as repeated measures in a mixed-effects model. While Ang-II and ω-3 rich diet were analyzed at two levels as present and absent (+/-), TPPU was analyzed at three levels as absent, low dose and high dose (- / low/ high). We performed full factorial analysis of the fixed effects that are 'Ang-II', 'ω-3 rich diet', 'TPPU' and 'time', while including 'subjects' as random effects. This analysis revealed statistically significant main effects of Ang-II ($F= 51.5$, $P < 0.01$), TPPU ($F= 18.7$, $P < 0.01$), ω-3 rich diet

(F= 10.8, $P < 0.01$) and an interaction for ω-3 rich diet and TPPU (F= 4.07, $P = 0.045$). The findings obtained from the mixed effects model with three between subjects factors are consistent with the mixed effects model analysis with 'group' as between subjects factor (see Results). It is worth noting that there is a discrepancy in the interpretation of the results when the data are analyzed with mixed-effects model with 'group' as one between subjects factor versus with mixed-effects model with three between subjects factor. Statistical analysis using three between subjects factors allowed examining individual effects of each treatment and therefore provides evidence on how much each treatment (e.g. Ang-II, ω-3 rich diet, TPPU) contributes to the changes in blood pressure. The mixed effects model with only 'group' as between subjects factor allowed making group contrasts while relying on pairwise comparisons.

Mixed-effects models with residuals diagnostics:

We used residuals analysis of the SBP data which showed as expected that the treated groups had indistinguishable values on day 1. This was followed by a sharp increase in SBP in the Ang-II treated group and then a plateau. To capture these salient features of the data and separate out the main effects of ω-3 rich diet and TPPU over the course of the study, we fitted a linear mixed-effects core model allowing for a constant trajectory for the control group, a different value for the Ang-II treated groups on day 1, and different effects for Ang-II, ω-3 rich diet, and TPPU after day 1. To this core model we added and tested terms for an additional Ang-II effect after the third day (to evaluate further changes in SBP after the first increase in SBP), for TPPU (to evaluate the benefit of a higher dose versus a lower one) and for the interaction between TPPU and ω-3 rich diet. None of these terms were significant, and they were not retained in the model. Table S3 summarizes the results of the mixed-effects models with residuals diagnostics used to analyze the blood pressure data. The results revealed that Ang-II infused mice experienced an increase in blood pressure after day 1 (estimate = 20.14, SE = 4.05, $p < 0.0001$). After the first day, mice receiving Ang-II and TPPU experienced a reduction in blood pressure of about 11 arbitrary units, which was statistically significant ($p < 0.01$), and those receiving the ω-3 rich diet also experienced a 4.5 unit reduction in blood pressure ($p = 0.24$).

TABLE S3. Summary (parameter estimates and standard errors) of the random-effects models assessing the relationship between Ang-II, ω-3 rich diet, and TPPU and blood pressure.

	Estimate* (SEM)	***P* value**
Model Term		
Intercept (average blood pressure for control mice)	2.64 (3.29)	0 .43
Estimated difference between Ang-II treated mice and controls	7.11 (7.79)	0.37
Ang-II effect after day 1	20.14 (4.05)	< 0.0001
ω-3 rich diet effect after day 1	-4.51 (3.83)	0.24
TPPU effect after day 1	-11.25 (3.89)	0.004

*****Arbitrary units.

F) Examination of the effects of each treatment on blood pressure at day11

In order to test our specific hypotheses regarding group differences in SBP a one way ANOVA of SBP at day 11 was conducted, which revealed a significant effect of group ($F = 4.65$, $P = 0.002$). Pairwise comparisons using Student-Newman Keuls post hoc tests showed that at day 11 SBP was significantly lower in all treatment groups compared to Ang-II, but also in A-ω3-TL compared to A-ω3 ($P = 0.05$), a trend for lower SBP in A-ω3-TH compared to A-ω3 ($P = 0.08$) and in A-TH compared to A-ω3 ($P = 0.06$).

Figure S1 Examination of the area under the systolic blood pressure (SBP) – time curve across all the groups from days 1-11 and from days 7-11.
Panel A: Area under the SBP-time curve corrected to the baseline readings with respect to control group average trajectory was calculated by trapezoidal rule as described before.[11] Differences in the AUC values among the groups were examined by one-way ANOVA. $P < 0.05$ was considered statistically significant. Data are mean ± SEM. Controls, n=5; group A, n=8; group A-ω3, n=8; group A-ω3-TL, n=7; group A-ω3-TH, n=8; group A-TH, n=8. * compared with controls, $^{\Phi}$ compared with group A. **Panel B:** Regression lines of the group means of the AUCs in A-ω3-TH and A-ω3-TL relative to the group A-ω3. Slopes of each regression line were compared to test if group A-ω3-TH and A-ω3-TL differ from each other. A *t*-test revealed that neither slope was different than zero or from each other.

Baseline blood pressure values were established for each group of mice based on average blood pressure taken for 3 days before treatment. Baseline blood pressures (mean ± SEM) were as follows: Controls, 105 ± 4 mmHg; group A, 99 ± 2 mmHg; group A-ω3, 102 ± 2.5 mmHg; group A-ω3-TL, 125 ± 8 mmHg, group A-ω3-TH, 104 ± 2 mmHg; group A-TH, 107 ± 2.5 mmHg.

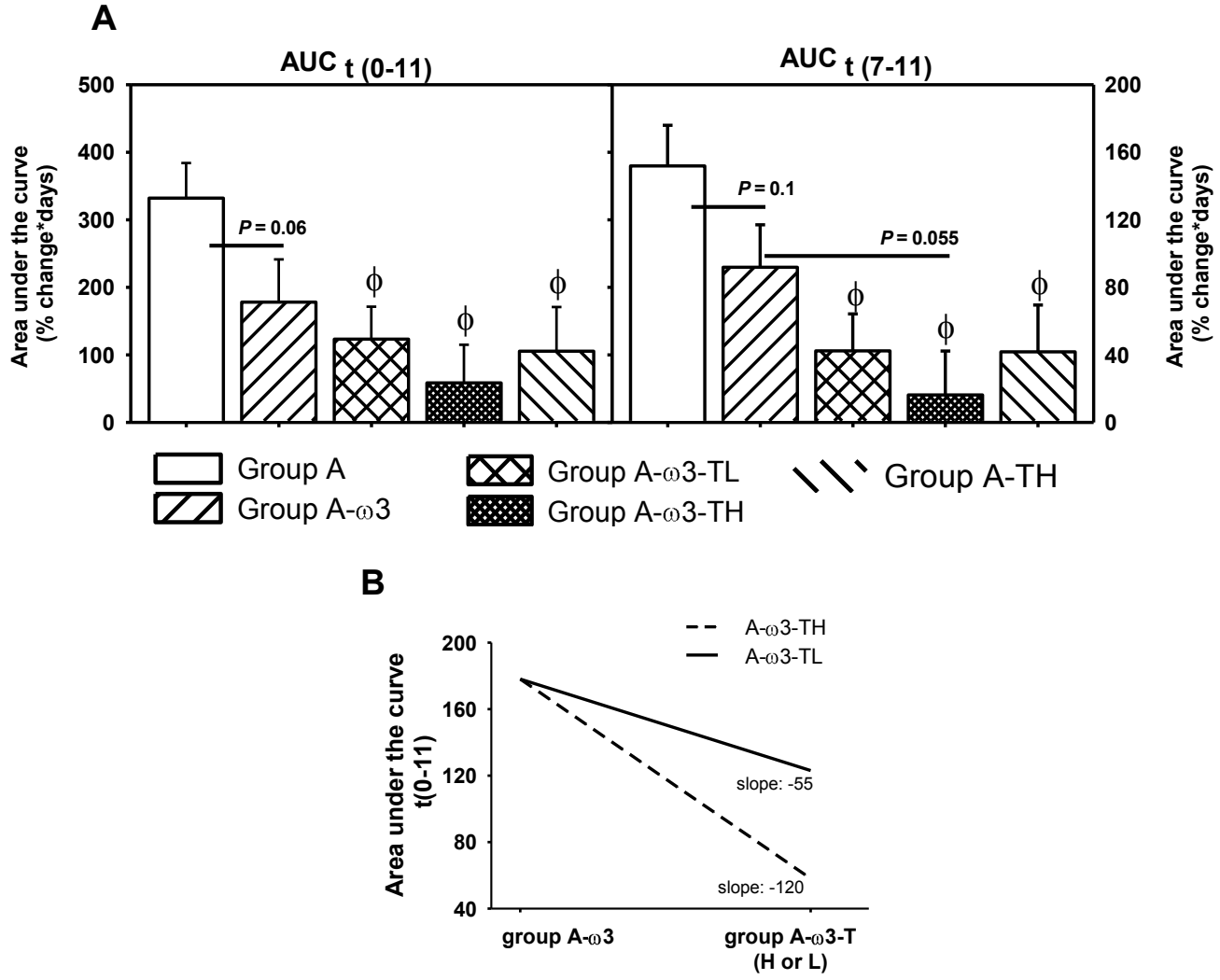


Figure S2A Time course of systolic blood pressure in mice that are on a standard diet.

A different set of mice (Swiss Webster, 8 weeks old) were fed a standard rodent chow (2018 Teklad Global 18% Protein Rodent Diet, Harlan Laboratories, Hayward, CA) throughout the whole experiment. Systolic blood pressure was measured using tail cuff blood pressure system as described in the methods section. Briefly, baseline blood pressure readings were collected following acclimation of the animals to the tail cuff blood pressure measurements. Then, TPPU was administered in the drinking water at 0.2 mg/kg dose. Blood pressure was recorded 1 and 4 days after the administration of TPPU. During this time TPPU did not alter control blood pressure levels until after Ang-II was administered. Five days after the TPPU administration, hypertension was induced by subcutaneous implantation of osmotic mini-pumps delivering Ang-II at 1 mg/kg/day. During the time of Ang-II infusion, animals continued to receive TPPU in the drinking water at 0.2 mg/kg.

The arrow heads indicate the day on which the treatment started (TPPU and Ang-II).

Group A', Ang-II alone and group A'-TL, Ang-II + TPPU (0.2 mg/kg). Data are mean ± SEM.

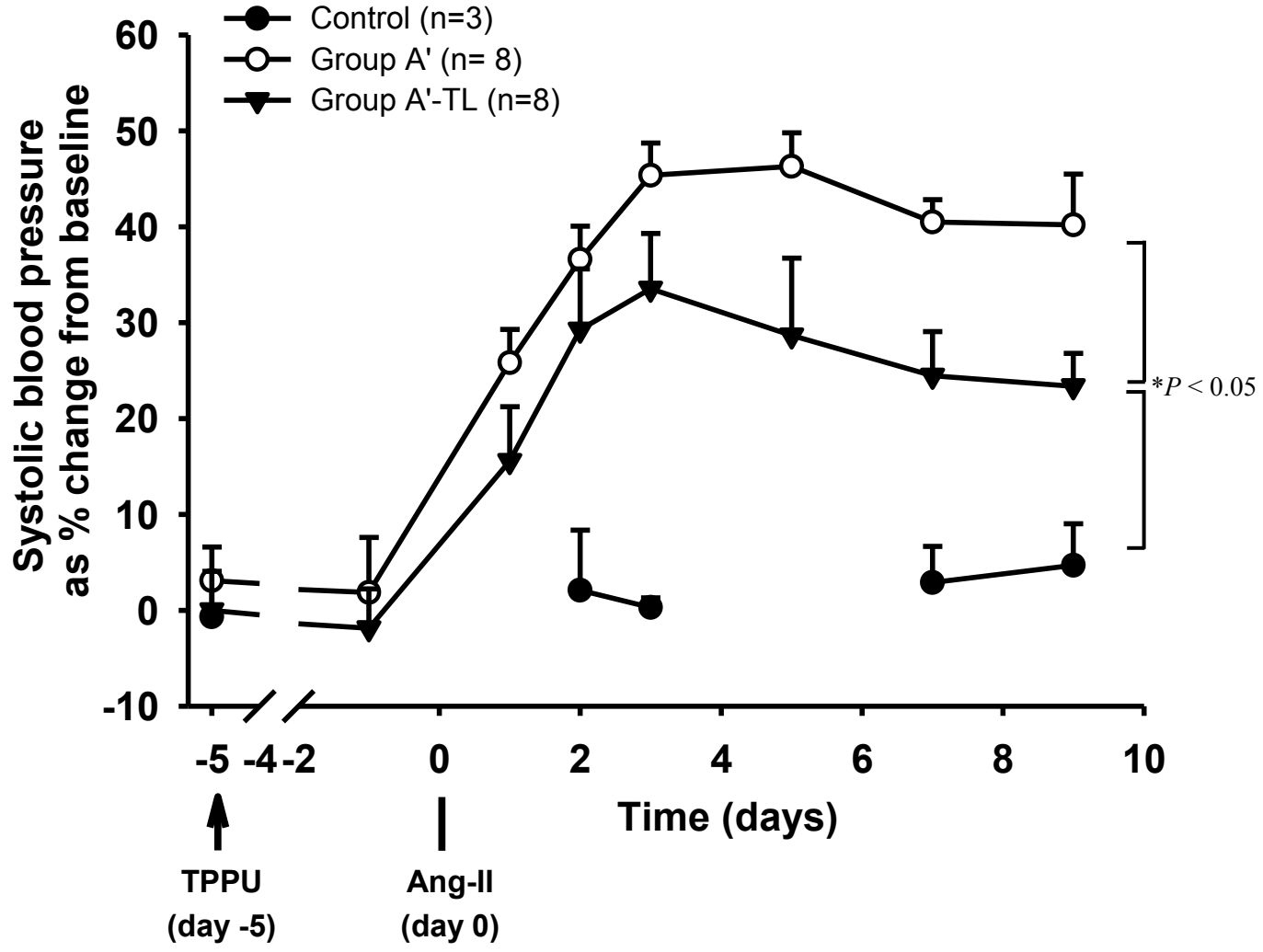
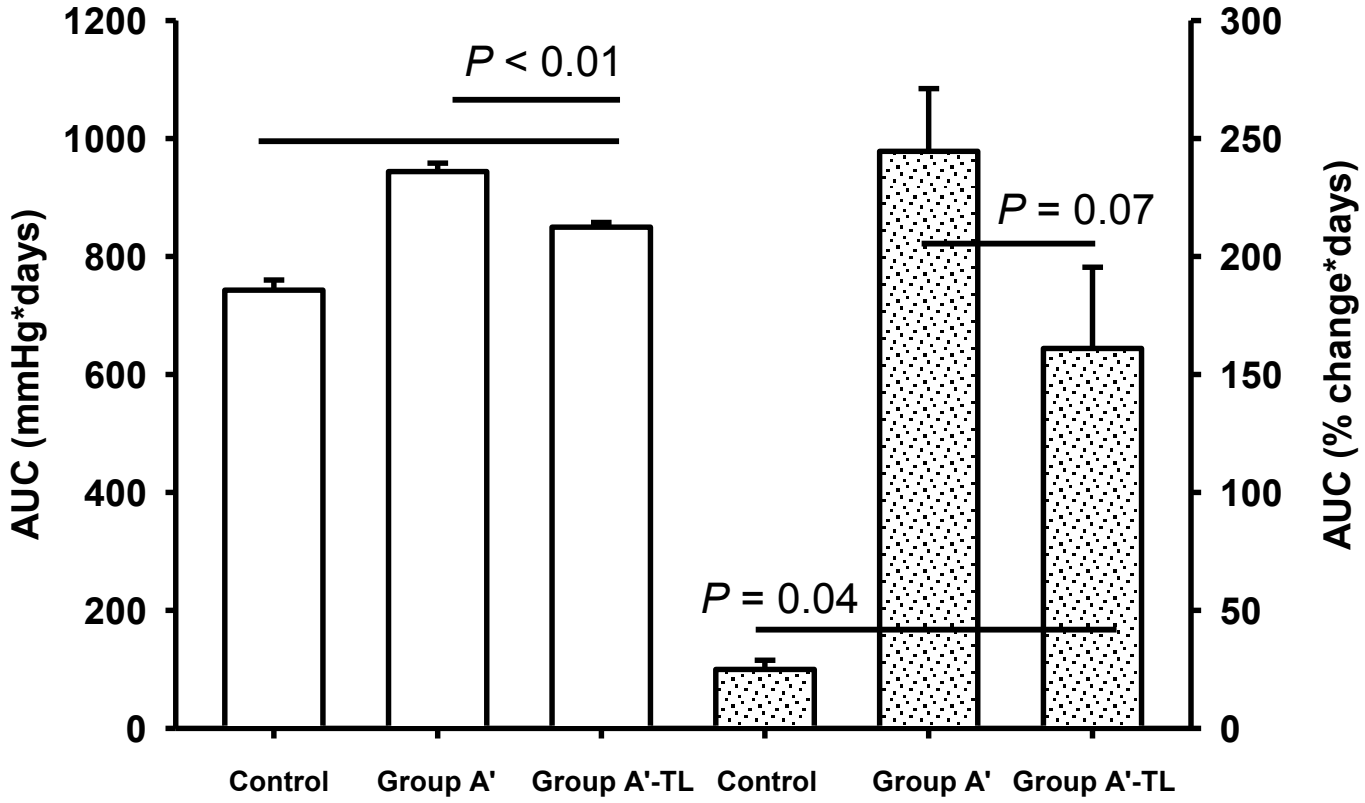


Figure S2B Area under the systolic blood pressure (SBP) – time curve from days 3-9 from data in Figure S2A.

Area under the SBP-time curve was calculated by trapezoidal rule as described before.[11] The area was calculated from blood pressure data obtained between days 3 and 9. Note that control AUC lack data on day 5. Group A, Ang-II alone and group A-TL, Ang-II + TPPU (0.2 mg/kg). Data are mean ± SEM.

While AUC values calculated using the SBP values recorded between days 3 and 9 differed significantly between group A' and group A'-TL, AUC values calculated from values of % change from baseline did not differ between these two groups (P =0.07).



G) Assessment of Renal Function and Histology

1. Methods

At the end of the experiment, renal injury was assessed by quantitative histology and by measurements of the plasma levels of creatinine, Ang-II, and the urinary levels of albumin. Multiple histological sections of each kidney were evaluated in a blinded fashion and scored by the previously described method based on multiple criteria including cellularity and hypertrophy of the glomerulus, mesangial expansion, interstitial inflammatory cell infiltration and arteriolar thickening. [2, 3] Plasma creatinine was measured as described above. Urinary albumin, which is considered as a biomarker for renal injury, was quantified using a commercially available kit (Albuwell M, Exocell Inc, PA). For this measurement, mice were housed in metabolic cages (one animal per cage) designed to collect urine free of food or feces. Urine was collected on dry ice for the last 24 hours of the experiment. Samples were stored at -80°C until analysis. Plasma levels of Angiotensin-II were measured using an ELISA kit (Phoenix Pharmaceuticals, Burlingame, CA) according to the manufacturer's instructions.

2. Results

We determined the plasma creatinine and urinary albumin concentrations as markers of renal function ($P > 0.05$). No significant differences were observed in the plasma creatinine levels (controls: 0.25 ± 0.06 mg/dl, group A- ω3-TL: < 0.2 mg/dl and group A-ω3-TH: 0.2 mg/dl) and in the urinary albumin among the selected groups (controls: 15 ± 4.5 μg/day, group A- ω3-TL: 20 ± 5 μg/day and group A- ω3-TH: 10 ± 3 μg/day). Last, we measured plasma Ang-II levels (Figure S3). Results showed an approximately 3 fold increase in plasma Ang-II levels when animals were infused with Ang-II alone at 1 mg/kg/day. Interestingly, group A-ω3-TL had significantly lower plasma levels of Ang-II compared with group A ($P < 0.05$, Kruskal Wallis one-way ANOVA).

To substantiate these results, we examined the changes in renal tissue with given treatments using glomerular injury scoring and general histology. When H & E stained sections were scored blindly by the method described in Imig *et al*[3], no significant differences were found among the groups (Figure S4A). Accordingly, there was also no change in tubules or interstitial tissue among the groups (Figure S4B). Overall, we did not observe any changes in the kidney that might be driven by HTN. Since Swiss Webster mice are resistant to hypertension driven renal damage[4, 5] and particularly resistant to Ang II driven damage in the absence of salt[2,3, 6-8], extensive histological changes in the kidney were not anticipated.

Figure S3 Plasma Angiotensin-II levels were decreased with the ω-3 rich diet and TPPU treatment.

Blood was collected at the end of the Ang-II infusion. Plasma (200 µl) was used to determine Ang-II levels. Inadequate amount of plasma was available from groups A-ω3 and A-TH, and thus Ang-II levels could not be determined. No significant differences were observed between sham operated controls (n=5) and group A (n=8). However, in groups A-ω3-TL (n=7), plasma Ang-II levels were significantly lower than group A ($P < 0.05$). Data are mean ± SEM. * compared with group A.

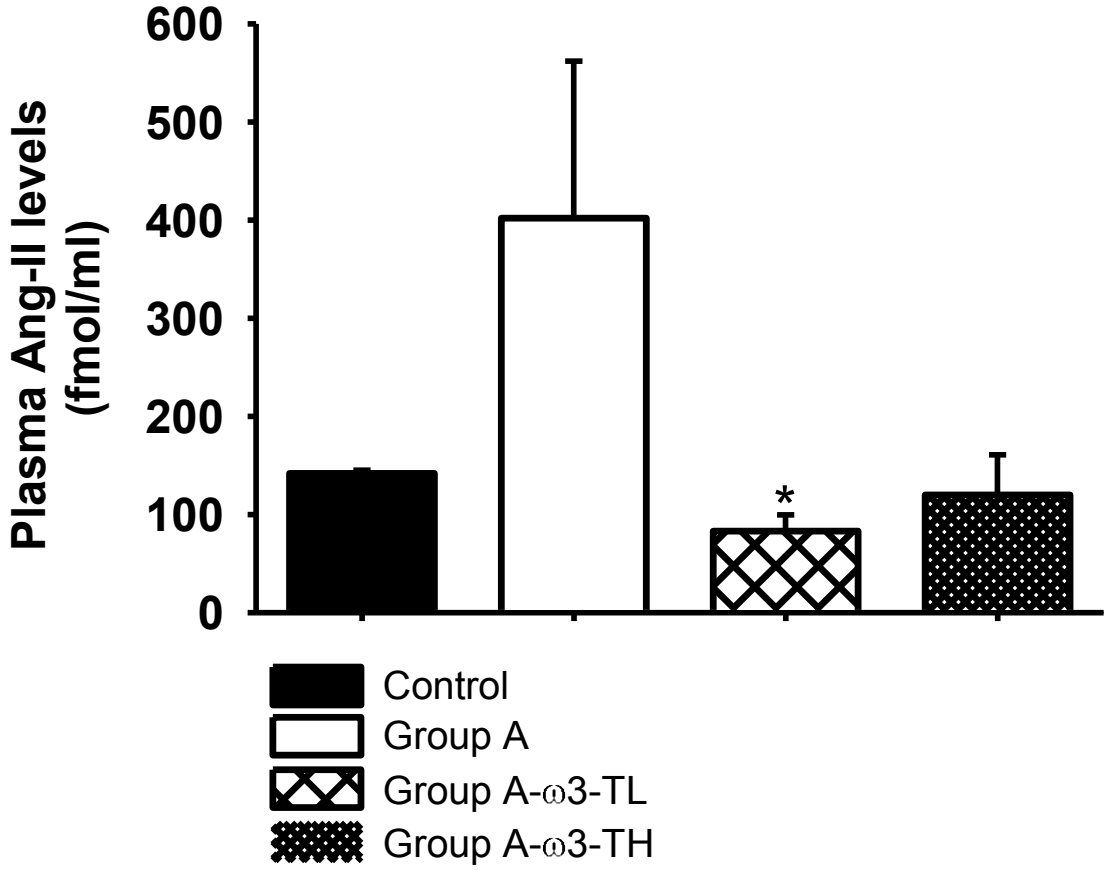


Figure S4A No significant differences in glomerular injury scores were observed among any groups following histological examination.

Kidneys were harvested and immediately fixed in 10% buffered formalin solution at the end of Ang-II infusion. The 3-4 µm kidney sections stained with hematoxylin & eosin (H&E) were prepared for light microscopic evaluation. Quantitative assessment was performed for glomeruli in each H&E stained kidney section and scoring was completed at the Medical College of Georgia (Dr John Imig). A score was given to each glomerulus by a blinded observer. Glomerular injury was evaluated by criteria including 1) hypercellularity and hypertrophy of the mesangial cells within the glomerulus, 2) apparent (open/ obvious) glomerular capillary loops and mesangial expansion. Based on a scale of 0-4 (0 being the best and 4 being the worst). Then, scores that are obtained from 250-400 glomeruli in the cortex from each kidney were averaged for each slide and added to the appropriate treatment group. Data are mean ± standard error. Sham operated controls, n=5; group A, n=5; group A-ω3, n=4; group A-ω3-TH, n=4; and group A-TH, n=3. As expected, Swiss Webster mice are generally resistant to Ang-II driven renal damage, particularly in the absence of salt.

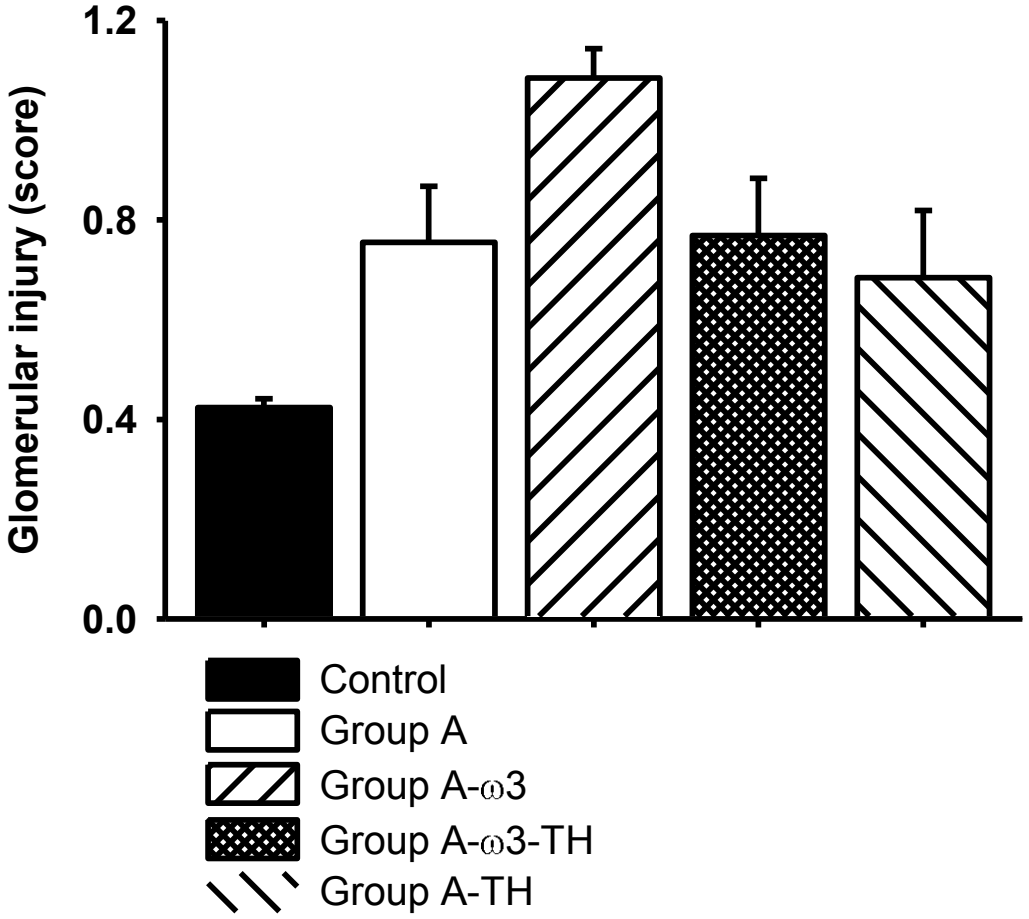
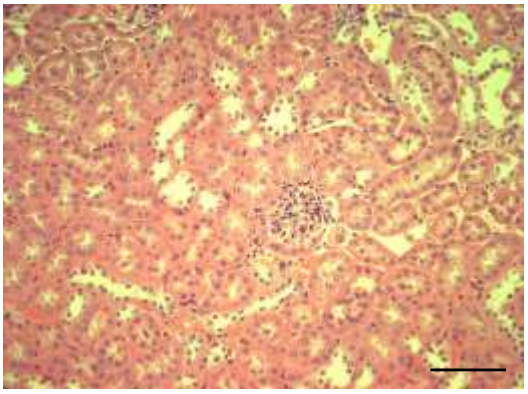
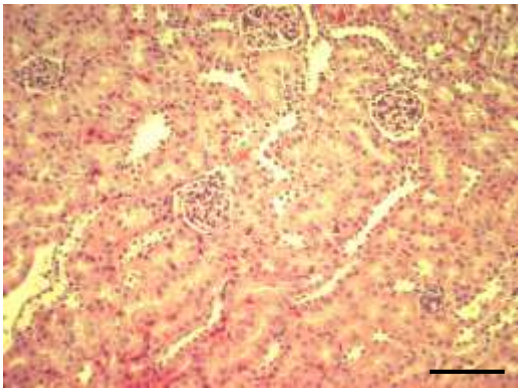


Figure S4B No significant differences in renal injury were observed among any groups following histological examination.

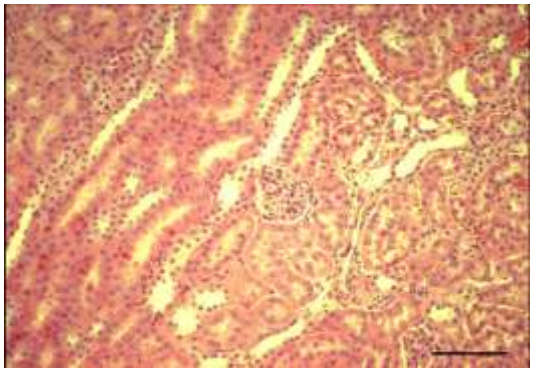
H & E stained kidney sections were examined for inflammation, degeneration of tubular epithelial cells and presence of tubular casts, and arteriolar thickening. We found no significant changes across the groups. Control (n=5); group A (n=5); group A-ω3 (n=4); group A-ω3-TH (n=4); and group A-TH (n=3). Bar = 100 μm.



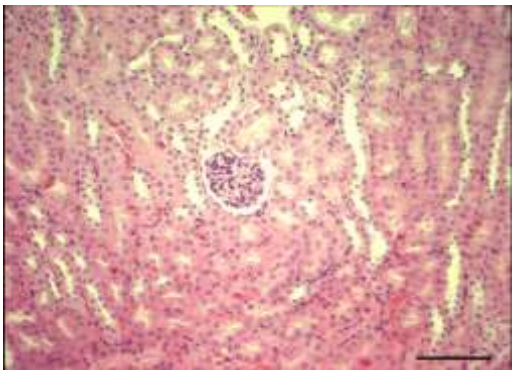
A. Control



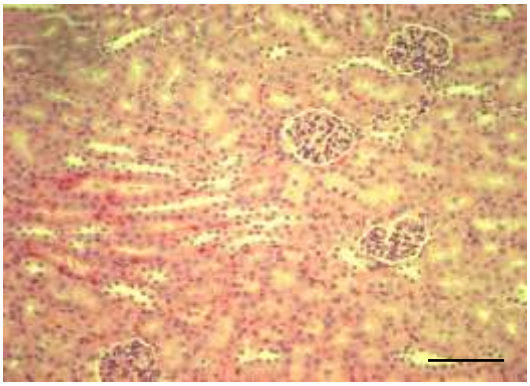
B. Group A



C. Group A-ω3



D. Group A-ω3-TH



E. Group A-TH

H) Measurement of plasma creatinine

Creatinine was purchased from Fisher Scientific (Pittsburgh, PA). The internal standard, creatinine-d3 was purchased from C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada). Thawed plasma (30 µl) was mixed with 30 µL internal standard solution (1000 nM in methanol) on a Vortex Mixer at 3,000 rpm for half min and then vortexed with 30 µl methanol at 5,000 rpm for 1 min. The mixed solution was centrifuged at 13,200 rpm for 5 min. The supernatant (25 µL) was mixed with equal volume of 12-(3-cyclohexanyl-ureido)-dodecanoic acid (CUDA, 200 nM in methanol) for analysis. Plasma creatinine was measured using an Agilent 1200 Series HPLC (Agilent Technologies, Inc. Santa Clara, CA) coupled to an Applied Biosystems 4000 QTRAP hybrid, triple-quadrupole MS instrument (Applied Biosystems, Foster City, CA). The instrument was equipped with a linear ion trap and a Turbo V ion source and was operated in positive MRM mode. Plasma creatinine was separated using a 100 mm X 4.6 mm Luna-C18 (2) 3 µm column (Phenomenex, Torrance, CA) held at 40 ºC. Analytes were eluted by using the mixture of water/acetonitrile/acetic acid (850/150/1, v/v) at a flow rate of 0.4 mL/min for 5 minutes. The injection volume was 10 µL and the samples were kept at 10 ºC in the auto sampler. The mass spectrometers were set with a positive electrospray mode with following parameters. CUR= 20 psi, GS1=25 psi, GS2=25 psi, IS=4500 V, CAD= HIGH, TEM=600^{o}C, ihe=ON, DP=20 V. The collision energies used for CAD (15 to 30 eV) varied according to molecular species and was individually maximized for each compound. The parent and daughter ions for monitoring creatinine, creatinine-d3, and CUDA are 114>86, 117.1>89.1, and 341.2>216.2, respectively.

TABLE S4. Renal epoxide metabolites of ARA, LA, EPA and DHA produced by P450s.

		Renal levels (ng/g tissue)					
	Epoxide Metabolites	**Controls (n=5)**	**Group A (n=8)**	**Group A-ω3 (n=8)**	**Group A-ω3-TL (n=7)**	**Group A-ω3-TH (n=14)**	**Group A-TH (n=8)**
ARA	8,9-EET	19 ± 4.4	5.5 ± 0.9*	3.6 ± 0.3*	5.3 ± 1*	5 ± 0.5*	7.6 ± 0.6*
	11,12-EET	14 ± 0.5	3 ± 0.2*	1.8 ± 0.1$^{*\,\phi}$	3 ± 0.5$^{*\,¥}$	2.7 ± 0.3$^{*\,¥\,\#}$	3.8 ± 0.3$^{*\,¥}$
	14,15-EET	4.5 ± 0.6	2 ± 0.2*	1.5 ± 0.3*	1.2 ± 0.2$^{*\,\phi\,\#}$	1.7 ± 0.2$^{*\,\#}$	2.7 ± 0.2$^{*\,¥}$
LA	9,10-EpOME	11 ± 0.6	2.2 ± 0.2*	1.3 ± 0.1*	8 ± 1$^{*\,\phi\,¥\,\#\,\$}$	5 ± 1$^{*\,\phi\,¥\,\#}$	2.8 ± 0.4*
	12, 13-EpOME	10 ± 0.7	2 ± 0.2*	1.3 ± 0.2*	7 ± 1$^{*\,\phi\,¥\,\#}$	6 ± 1$^{*\,\phi\,¥\,\#}$	3 ± 0.3*
EPA	8,9-EpETE	1.2 ± 0.05	0.3 ± 0.1	1 ± 0.3	4 ± 1$^{*\,\phi\,¥\,\#}$	2.9 ± 0.6$^{\phi\,¥\,\#}$	0.4 ± 0.2
	11,12-EpETE[a]	0.5 ± 0.1	< 0.1	1 ± 0.1	3 ± 1	2.2 ± 0.5	< 0.1
	14,15-EpETE[a]	1.4 ± 0.2	< 0.1	1.1 ± 0.04	1 ± 0.2	1 ± 0.1	< 0.1
	17,18-EpETE[a]	2 ± 0.2	< 0.1	2.3 ± 0.2	9.5 ± 1	8 ± 1	0.9 ± 0.1
DHA	10,11-EpDPE	6 ± 1	1.1 ± 0.1*	2.8 ± 0.2$^{*\,\phi}$	4.5 ± 0.8$^{\phi\,¥\,\#}$	3.9 ± 0.4$^{*\,\phi\,\#}$	1.5 ± 0.2*
	13,14-EpDPE	3.5 ± 0.6	0.7 ± 0.1*	2 ± 0.1$^{*\,\phi}$	3 ± 0.4$^{\phi\,\#}$	3 ± 0.4$^{\phi\,¥\,\#}$	1 ± 0.1$^{*\,¥}$
	16,17-EpDPE	4 ± 0.6	0.7 ± 0.1*	1.9 ± 0.1*	4.7 ± 0.7$^{\phi\,¥\,\#}$	4.9 ± 0.7$^{\phi\,¥\,\#}$	1.9 ± 0.2*
	19,20-EpDPE	9 ± 1	8 ± 1	20 ± 1$^{*\,\phi}$	32 ± 3$^{*\,\phi\,¥\,\#}$	37 ± 2$^{*\,\phi\,¥\,\#}$	20 ± 1$^{*\,\phi}$

$P < 0.05$ *compared with controls, $^{\phi}$ compared with group A, $^{¥}$ compared with group A-ω3, $^{\$}$ compared with group A-ω3-TH, $^{\#}$ compared with group A-TH. Data are mean ± standard error.

[a] Post hoc tests were not performed for these analytes, because at least one group has samples below 0.1 ng/g tissue concentration.

TABLE S5. Diols produced by sEH from the ARA, LA, EPA and DHA epoxides in the kidney.

		Renal levels (ng/g tissue)					
	Diol metabolites	**Controls (n=5)**	**Group A (n=8)**	**Group A-ω3 (n=8)**	**Group A-ω3-TL (n=7)**	**Group A-ω3-TH (n=14)**	**Group A-TH (n=8)**
ARA	8,9-DHET	0.9 ± 0.1	0.4 ± 0.1*	0.3 ± 0.1*	0.5 ± 0.1*	0.6 ± 0.1$^{*\,¥}$	0.7 ± 0.1$^{ϕ\,¥}$
	11,12-DHET	1.5 ± 0.4	0.9 ± 0.2*	0.6 ± 0.1*	1 ± 0.2	0.9 ± 0.1*	1.1 ± 0.1
	14,15-DHET	3.6 ± 0.5	2.7 ± 0.2	1.8 ± 0.1*	2.4 ± 0.2*	1.9 ± 0.1$^{*\,\#}$	3 ± 0.2$^{¥}$
LA	9,10-DiHOME	6 ± 1	2 ± 0.2*	1 ± 0.2*	2.5 ± 0.3*	1.8 ± 0.3*	3 ± 0.3*
	12, 13-DiHOME	19.5 ± 3.5	5.2 ± 0.5*	2.8 ± 0.3*	8 ± 1$^{*\,¥}$	6 ± 1*	5.6 ± 0.8*
EPA	8,9-DiHETE [a]	0.5 ± 0.1	< 0.1	0.6 ± 0.1	1.7 ± 0.2	1 ± 0.1	< 0.1
	11,12-DiHETE [a]	0.3 ± 0.1	< 0.1	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0. 1	< 0.1
	14,15-DiHETE	2 ± 0.6	0.2 ± 0.02*	2.3 ± 0.3^{ϕ}	4 ± 0.3$^{*\,ϕ\,¥\,\#\,\$}$	2.7 ± 0.3$^{ϕ\,\#}$	0.3 ± 0.02$^{*\,¥}$
	17,18-DiHETE	4.6 ± 0.7	0.7 ± 0.1*	8 ± 1$^{*\,ϕ}$	7 ± 0.8$^{ϕ\,\#\,\$}$	4 ± 0.4$^{ϕ\,¥\,\#}$	0.7 ± 0.1$^{*\,¥}$
DHA	10,11-DiHDPE	2 ± 0.3	0.6 ± 0.1*	1.6 ± 0.1^{ϕ}	2.3 ± 0.4$^{ϕ\,\#}$	1.7 ± 0.2$^{ϕ\,\#}$	0.7 ± 0.1$^{*\,¥}$
	13,14-DiHDPE	3.5 ± 0.5	1.3 ± 0.1	3.4 ± 0.2	10 ± 3$^{ϕ\,\#}$	6 ± 2$^{*\,\#}$	1.3 ± 0.1
	16,17-DiHDPE	28 ± 4	9.4 ± 1*	16 ± 1$^{*\,ϕ}$	16 ± 2$^{*\,ϕ\,\$}$	11 ± 1$^{*\,¥}$	10 ± 1*
	19,20-DiHDPE	69.5 ± 19	25 ± 3*	58 ± 3^{ϕ}	48.5 ± 6$^{*\,ϕ\,\#\,\$}$	24 ± 2$^{*\,¥\,\#}$	13 ± 1$^{*\,ϕ\,¥}$

$P < 0.05$ *compared with controls, ^{ϕ} compared with group A, $^{¥}$ compared with group A-ω3, $^{\$}$ compared with group A-ω3-TH, $^{\#}$ compared with group A-TH. Data are mean ± standard error.

[a] Post hoc tests were not performed for these analytes, because at least one group has samples below 0.1 ng/g tissue concentration.

TABLE S6. Renal levels of the epoxide and diol metabolites of of the ARA, LA, EPA and DHA in mice fed a standard diet (Harlan-Teklad).

	Tissue levels (ng/g tissue)		
Epoxide metabolites[a]	**Control**	**Group A'**	**Group A'-TL**
Sum EETs	38 ± 4	42 ± 3	37 ± 4
Sum EpOMEs	22 ± 2	15 ± 1	14 ± 3*
Sum EpETEs	3 ± 0.2	2 ± 0.1*	1.5 ± 0.2*
Sum EpDPEs	15 ± 2	20 ± 1	31 ± 5* $^{\phi}$
Diol metabolites[a]			
Sum DHETs	6 ± 0.4	7 ± 0.2	8 ± 0.4* $^{\phi}$
Sum DiHOMEs	37 ± 6	41 ± 2	46 ± 9 $^{\phi}$
Sum DiHETEs	6 ± 0.5	2 ± 0.1*	2 ± 0.1*
Sum DiHDPEs	89 ± 10	80 ± 2	72 ± 10

[a]Sum of ARA metabolites includes the 8, 9-, 11, 12- and 14, 15-fatty acid regioisomers. Sum of LA metabolites includes the 9, 10- and 12, 13- fatty acid regioisomers. Sum of EPA metabolites include the 8, 9-, 11, 12-, 14, 15- and 17, 18-fatty acid regioisomers, and sum of DHA metabolites includes the 10, 11-, 13, 14-, 16, 17- and 19, 20- regioisomers. Control (sham operated animals), n=3, group A' (Ang-II alone), n=6, and group A'-TL (Ang-II + TPPU 0.2 mg/kg), n=6. Statistically significant differences ($P < 0.05$) were determined by one-way ANOVA followed by pairwise comparisons. Data are mean ± SEM. ***** compared with controls, **Φ** compared with Group A'.

TABLE S7A. Renal inflammatory status across treatment groups reflected by the tissue levels of major pro- and anti-inflammatory ARA metabolites generated from COX-2 and LOX enzymes.

		Renal levels (ng/g tissue)					
	ARA metabolites	**Controls (n=5)**	**Group A (n=8)**	**Group A-ω3 (n=8)**	**Group A-ω3-TL (n=7)**	**Group A-ω3-TH (n=14)**	**Group A-TH (n=8)**
ARA COX-2	TXB_2	1.4 ± 0.1	3.9 ± 0.9*	1.5 ± 0.2$^{\phi}$	1.2 ± 0.3$^{\phi\,\#}$	1.4 ± 0.2$^{\phi\,\#}$	6 ± 0.7$^{*\,\phi\,¥}$
	PGF2α	5 ± 1.6	45.6 ± 6.4*	14 ± 1.45$^{\phi}$	5.7 ± 1.5$^{\phi\,\#}$	9.2 ± 7$^{\phi\,\#}$	69.5 ± 9.5$^{*\,\phi\,¥}$
ARA LOX	5-HETE [a]	< 0.1	8.4 ± 1.6	7.8 ± 1	2.5 ± 0.3	5 ± 1	13.5 ± 2
	8-HETE [a]	< 0.1	1.6 ± 0.2	1 ± 0.2	< 0.1	1 ± 0.1	2.8 ± 0.2
	9-HETE [a]	< 0.1	35 ± 8	17 ± 2	6.8 ± 2	13 ± 4	50 ± 6
	11-HETE	24 ± 5	36 ± 8	17 ± 2$^{\phi}$	7 ± 2$^{*\,\phi\,\#}$	13 ± 4$^{\phi\,\#}$	50 ± 6$^{*\,\phi\,¥}$
	12-HETE	34 ± 12	70 ± 13	45 ± 5	13 ± 4$^{\phi\,\#}$	22 ± 4$^{\phi\,\#}$	113 ± 40$^{*\,\phi\,¥}$
	15-HETE	6 ± 2	37 ± 7*	27 ± 3*	5 ± 1$^{\phi\,¥\,\#\,\$}$	18 ± 4$^{\phi\,\#}$	57 ± 5.5$^{*\,\phi\,¥}$
	15(S)-HETrE [a]	< 0.1	6.4 ± 1.3	5 ± 0.6	2.5 ± 0.3	3.7 ± 0.5	8.9 ± 0.75
	20-HETE	3.8 ± 0.3	5.3 ± 1.2	5 ± 1.2	12.4 ± 3$^{*\,\phi\,¥\,\#}$	8.5 ± 1*	5.5 ± 0.9

$P < 0.05$ *compared with controls, $^{\phi}$ compared with group A, $^{¥}$ compared with group A-ω3, $^{\$}$ compared with group A-ω3-TH, $^{\#}$ compared with group A-TH. Data are mean ± standard error.

[a] Post hoc tests were not performed for these analytes, because at least one group has samples below 0.1 ng/g tissue concentration.

TABLE S7B. COX-2 and LOX products of EPA in the kidney.

		Renal levels (ng/g tissue)					
	EPA metabolites	**Controls (n=5)**	**Group A (n=8)**	**Group A-ω3 (n=8)**	**Group A-ω3-TL (n=7)**	**Group A-ω3-TH (n=14)**	**Group A-TH (n=8)**
COX-2	Resolvin E1	1 ± 0.4	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	PGE3 [a]	< 0.1	< 0.1	4.5 ± 0.9	2.2 ± 0.6	5 ± 2.5	< 0.1
	PGD3 [a]	0.9 ± 0.3	0.4 ± 0.1	2 ± 0.8	< 0.1	1 ± 0.3	0.3 ± 0.1
LOX	5-HEPE [a]	4 ± 0.2	< 0.1	10 ± 2	14 ±2	9.5 ± 0.6	< 0.1
	8-HEPE [a]	1 ± 0.8	< 0.1	1.7 ± 0.3	1 ± 0.1	1.1 ± 0.1	< 0.1
	12-HEPE	45 ± 7	4.6 ± 2	170 ± 16$^{*\,\phi}$	72.5 ± 15$^{\phi\,¥\,\#}$	63 ± 12.5$^{\phi\,¥\,\#}$	7 ± 5$^{¥}$
	15-HEPE	2 ± 0.4	0.4 ± 0.1	9.6 ± 0.7$^{*\,\phi}$	18 ± 2$^{*\,\phi\,¥\,\#\,\$}$	10 ± 2$^{*\,\phi\,\#}$	0.9 ± 0.3$^{¥}$

$P < 0.05$ *compared with controls, $^{\phi}$ compared with group A, $^{¥}$ compared with group A-ω3, $^{\$}$ compared with group A-ω3-TH, $^{\#}$ compared with group A-TH. Data are mean ± standard error.

[a] Post hoc tests were not performed for these analytes, because at least one group has samples below 0.1 ng/g tissue concentration.

TABLE S8. Other enzymatic products derived from arachidonic and linoleic acid in the kidney.

Among those metabolites, 9-HODE and 13- HODE showed a similar trend to that of HETEs. Consequently, there was a strong correlation (R = 0.9) between these two HODE metabolites and the 15-HETE, which have been shown to activate PPAR-gamma.[10] The LA oxidation products also changed similarly to 9- HODE and 13- HODE across all the groups.

	Renal levels (ng/g tissue)					
Oxidation products	**Controls (n=5)**	**Group A (n=8)**	**Group A-ω3 (n=8)**	**Group A-ω3-TL (n=7)**	**Group A-ω3-TH (n=14)**	**Group A-TH (n=8)**
9-HODE	19 ± 4	86 ± 18*	44 ± 6$^{\phi}$	14 ± 1$^{\phi ¥ \#}$	33 ± 7$^{\phi \#}$	76 ± 5$^{* ¥}$
13-HODE	32 ± 6	95 ± 16*	56 ± 7.5$^{\phi}$	22 ± 1.4$^{\phi ¥ \#}$	41 ± 7$^{\phi \#}$	89 ± 9$^{* ¥}$
9-oxo-ODE	53 ± 16	4 ± 0.6*	2.5 ± 0.6*	5.5 ± 0.6*	4 ± 0.8*	4 ± 0.5*
12-oxo-ETE	14.3 ± 3	22 ± 2	13 ± 1	9.3 ± 1$^{\phi \#}$	8 ± 1$^{\phi \#}$	35.5 ± 10$^{* \phi ¥}$
15-oxo-ETE	1.5 ± 0.3	3.6 ± 0.4*	1.8 ± 0.3$^{\phi}$	0.8 ± 0.1$^{\phi ¥ \#}$	1.1 ± 0.2$^{\phi ¥ \#}$	4 ± 0.5$^{* ¥}$

$P < 0.05$ *compared with controls, $^{\phi}$ compared with group A, $^{¥}$ compared with group A-ω3, $^{\$}$ compared with group A-ω3-TH, $^{\#}$ compared with group A-TH. Data are mean ± standard error.

TABLE S9. Tissue levels of the major pro-inflammatory cytokines in the kidney.

Cytokines	Renal levels (pg/mg protein)					
	Controls (n=5)	**Group A (n=8)**	**Group A-ω3 (n=8)**	**Group A-ω3-TL (n=7)**	**Group A-ω3-TH (n=14)**	**Group A-TH (n=8)**
MCP-1	5.4 ± 0.9	66 ± 16*	29 ± 3*$^{\phi}$	5.7 ± 0.45$^{\phi\,¥\,\#}$	18 ± 4$^{\phi}$	30 ± 3*$^{\phi}$
TNF- α	43 ± 6	40 ± 11	40 ± 16	41 ± 5	35 ± 4$^{¥}$	30 ± 4$^{¥}$
IL-1β	4.7 ± 0.8	4 ± 0.5	2.5 ± 0.65	< 0.1	1 ±0.4	2 ±0.4*
IL-6	0.3 ± 0.1	0.2 ± 0.02	0.1 ±0.03	< 0.05	0.4 ±0.1	0.2 ±0.02

$P < 0.05$ *compared with controls, $^{\phi}$ compared with group A, $^{¥}$ compared with group A-ω3, $^{\$}$ compared with group A-ω3-TH, $^{\#}$ compared with group A-TH. Data are mean ± standard error.

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