

Sample size calculation

The primary outcome measured was n-3 fatty acid content in membrane phospholipid in ileal tissue of rats receiving the control diet versus the EPA+DHA diet. Secondary outcomes are oxylipins and cytokines. Preliminary data from the only other study assessing fatty acid content in membrane phospholipid in colonic mucosa show that rats receiving an intensive CPT-11 regimen had a 16% decrease in n-3 fatty acids in animals treated with CPT-11 (2.10% +/- 0.26%) compared to healthy control animals (3.31% +/- 0.43%) (26). Based on a 16% difference in n-3 fatty acids of membrane phospholipid in colonic mucosa and a 0.43% standard deviation, it was estimated that a sample size of 3 per group will be required in the animal study using colon tissue to achieve statistical power of 90% with an alpha level of 0.05. Thus, it is estimated that n=3 will achieve statistical power of 90% with an alpha level of 0.05 in the present study.

Supplementary Table 1 Targeted list of deuterated oxylipins in ileum tissue, surrogate deuterated internal standards, and detector response factors

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)	Retention Time (min)	Internal Standard	Catalogue Number (Cayman Chemical Co.)
TXB2-d4	373.3	173.2	9	7.0	ISTD1	319030
PGF _{2α} -d4	357.3	197.2	21	7.8	ISTD2	316010
PGE2-d4	355.2	275.3	10	8.0	ISTD3	314010
PGD2-d4	355.2	275.3	10	8.3	ISTD4	312010
LTB4-d4	339.2	197.2	10	13	SITD5	29629
9-HODE-d4	299.2	172	13	16.6	ISTD6	338410
9(10)-DiHOME-d4	317.3	203.2	18	14.2	ISTD7	10009993
6-k-PGF _{1α} -d4	373.3	167.2	19	5.4	ISTD8	315210
5-HETE-d8	327.3	116.1	7	17.9	ISTD9	334230
20-HETE-d6	325.2	281.2	9	15.7	ISTD10	390030
15-HETE-d8	327.3	182.2	10	16.6	ISTD11	31746
13(S)-HODE-d4	299.3	198.2	16	16.4	ISTD12	338610
12(S)-HETE-d8	327.3	184	8	17.2	ISTD13	334570
11(12)-DiHET-d11	348.3	167.2	16	15.0	ISTD14	10007975
(±)8(9)-DiHET-d11	348.3	185.1	10	15.5	ISTD15	10009998
(±)14(15)-DiHET-d11	348.3	207.1	10	14.4	ISTD16	10008040
(±)12(13)-DiHOME-d4	317.3	185.2	6	13.6	ISTD17	10009994

Supplementary Table 2 Oxylipin mass transitions, surrogate deuterated internal standards, and detector response factors

Oxylipin (n=29)	Precursor r (m/z)	Product Ion (m/z)	Collision n Energy (V)	Retention Time (min)	Internal Standard
Derived from AA					
PGD ₂	351.2	271.3	9	8.4	PGE ₂ -d ₄
PGE ₂	351.2	271.3	9	8.0	PGD ₂ -d ₄
6-keto-PGF _{1α}	369.2	163.2	22	5.5	6-k-PGF _{1α} -d ₄
PGF _{2α}	353.2	309.2	10	7.8	PGF _{2α} -d ₄
TXB ₂	369.2	169	9	6.9	TXB ₂ -d ₄
LTB ₄	335.5	195	10	14	LTB ₄ -d ₄
8,9-DiHET (8,9-DiHETrE)	337.2	127.1	16	15.7	(±)8(9)-DiHET-d ₁₁
11,12-DiHET (11,12-DiHETrE)	337.2	167.2	12	15.1	11(12)-DiHET-d ₁₁
14(15)-DiHET (14,15-DiHETrE)	337.2	207.1	8	14.5	(±)14(15)-DiHET-d ₁₁
5-HETE	319.2	115	7	18.0	5-HETE-d ₈
8-HETE	319.2	155.2	5	17.4	5-HETE-d ₈
11-HETE	319.2	167.2	6	17.1	5-HETE-d ₈
12-HETE	319.2	179.2	8	17.3	12(S)-HETE-d ₈
15-HETE	319.2	219.2	4	16.7	15-HETE-d ₈
20-HETE	319.2	245.3	9	15.8	20-HETE-d ₆
LXA ₄	351.4	115	7	10.5	LipoxinA ₄ -d ₅
Derived from LA					
9-HODE	295.2	171.1	10	16.7	9-HODE-d ₄
13-HODE	295.2	195.2	10	16.5	13(s)-HODE-d ₄
9,10-DiHOME	313.2	201.2	15	14.2	9(10)-DiHOME-d ₄
12,13-DiHOME	313.2	183.2	13	13.7	(±)12(13)-DiHOME-d ₄

Derived from EPA					
15-HEPE	317.2	219.2	6	15.7	15-HETE-d8
18-HEPE	317.2	215.3	6	15.3	20-HETE-d6
17(18)-DiHETE	335.3	247.2	8	12.5	(±)14(15)-DiHET-d11
RsVE1	349.3	195	13	6	Resolvin D1-d5
Derived from DHA					
4-HDHA (4-HDoHE)	343.2	100.9	12	18.2	5-HETE-d8
8-HDHA (8-HDoHE)	343.2	109.2	4	17.6	5-HETE-d8
13-HDHA (13-HDoHE)	343.2	221	8	16.9	15-HETE-d8
14-HDHA (14-HDoHE)	343.2	205	9	17.0	15-HETE-d8
16-HDHA (16-HDoHE)	343.2	233.1	4	16.7	15-HETE-d8
17-HDHA (17-HDoHE)	343.2	245.2	9	16.7	15-HETE-d8
20-HDHA (20-HDoHE)	343.2	241.1	7	16.4	20-HETE-d6
Protectin_D1	359.2	153.2	8	11.9	LTB4-d4
RsVD1	375.3	141	5	9.3	Resolvin D1-d5
Maresin-1	359.3	123	11	13.3	Resolvin D1-d5

Supplementary Table 3 Oxylin mass transitions below detection or quantitation limit

Oxylin	Precursor (m/z)	Ion	Product (m/z)	Ion	Internal Standard
RvE1	375.3		141		Resolvin D1-d5
MaR1	359.3		123		Resolvin D1-d5
RvD1	349.4		195		Resolvin D1-d5

Supplementary Table 4 Handling of outliers for 13-HODE and sum of LA-derived oxylin

#55 outlier retained (ng/mg protein)	#55 outlier removed (ng/mg protein)
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	3701 (outlier)	1039.9 (imputed mean)
Average	1705±682.3	1040±151.6
CNT D2		
Average	2239±861.3	1574±243.6
TOTAL LA for CNT D2		
	#88 outlier retained	#88 outlier removed
	(ng/ mg protein)	(ng/ mg protein)
	1619 (outlier)	479.2 (imputed mean)
Average	764±297	479±83.0
for EPA+DHA D4		
Average	976±310	691±103
TOTAL LA		
for EPA+DHA D2		
ng/ mg protein	#88 + #55 outlier retained	#88 + #55 outlier removed
	<i>P</i> Diet = 0.245	<i>P</i> Diet = 0.088
	<i>P</i> Day = 0.019	<i>P</i> Day = 0.006
	<i>P</i> Diet*Day =0.231	<i>P</i> Diet*Day =0.038

For 13-HODE, a linoleic acid-derived oxylipin, rat #55 from CNT D2 (3701 ng/mg protein) was more than three standard deviations from the aggregated mean of rats fed the Control diet (all timepoints). Thus, the outlier was replaced with 1040 ng/mg protein, the mean of the CNT D2 group. Rat #88 of 13-HODE from EPA+DHA D4 group was an outlier (1619 ng/mg protein) determined by being more than three standard deviations from the aggregated mean of rats fed the EPA+DHA diet (all timepoints) and was replaced with 479.2 ng/mg protein, the mean of the EPA+DHA D4 group. 13-HODE results are not presented in Table 2. However, values for total linoleic acid derived oxylipins contains 13-HODE. The interpretation of the present findings do not change whether or not these outliers are included.