SUPPLEMENTARY INFORMATION

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SUPPLEMENTARY TABLES

Supplementary Table 1: Macronutrient composition and energy content in the experimental diets.

	ND	HFD	HFD+F
Lipids (% diet, wt/wt)	2.9	31.8	32.4
Carbohydrates (% diet, wt/wt)	64.3	47.2	47.6
Proteins (% diet, wt/wt)	16.9	11.7	11.7
Energy density (kJ/g)	13.0	20.1	20.1
Supplement:			
Epax 1050 TG (g/100g)	0.0	0.0	5.3

(mol %)	ND	HFD	HFD+F
12:0	0.15	0.26	-
14:0	0.14	1.22	-
16:0	12.52	13.42	12.05
16:1 (n-9)	-	-	-
16:1 (n-7)	0.32	0.26	0.31
18:0	6.00	3.34	3.84
18:1 (trans)	0.55	0.42	0.45
18:1 (n-9)	25.64	31.00	28.44
18:1 (n-7)	1.59	0.65	0.87
18:2 (n-6)	46.62	47.22	41.10
18:3 (n-6)	-	-	-
20:0	0.42	0.43	0.50
18:3 (n-3)	4.89	1.24	1.24
20:1 (n-9)	0.31	0.26	0.47
20:2 (n-6)	-	-	0.14
20:3 (n-6)	-	-	-
20:4 (n-6)	-	-	0.47
20:5 (n-3)	-	-	1.65
22:4 (n-6)	-	-	0.12
22:5 (n-6)	-	-	0.48
22:5 (n-3)	-	-	0.34
22:6 (n-3)	0.45	-	6.39
SFA	23.71	19.48	18.14
MUFA	27.92	32.26	30.18
n-6 PUFA	46.86	47.34	42.35
n-3 PUFA	0.97	0.49	8.87

Supplementary Table 2: Fatty acid composition of dietary lipids

Fatty acid composition in total dietary lipids was determined using gas chromatography. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid. -, <0.1 %.

Gene name	Gene Sequence 5'-3'		
36B4 F	TCCAGGCTTTGGGCATCA		
36B4 R	CTTTATCAGCTGCACATCACTCAGA		
Fsp27 F	ATCAGAACAGCGCAAGAAGA		
Fsp27 R	CAGCTTGTACAGGTCGAAGG		
Cd36 F	ATGGGCTGTGATCGGAACTG		
Cd36 R	TTTGCCACGTCATCTGGGTTT		
Alpl F	GCCCTCTCCAAGACATATA		
Alpl R	CCATGATCACGTCGATATCC		
Bmp2 F	GGGACCCGCTGTCTTCTAGT		
Bmp2 R	TCAACTCAAATTCGCTGAGGAC		
Collal F	GGTGAACAGGGGTTCCTGG		
Collal R	TTCGCACCAGGTTGCCATC		
Adipoq F	GACGTTACTACAACTGAAGAGC		
Adipoq R	CATTCTTTTCCTGATACTGGTC		
Cebpa F	AAGCCAAGAAGTCGGTGGA		
Cebpa R	CAGTTCACGGCTCAGCTGTTC		
Π1β F	GCAACTGTTCCTGAACTCAACT		
Π1β R	ATCTTTTGGGGTCCGTCAACT		
Tnfa F	CCCTCACACTCAGATCATCTTCT		
Tnfa R	GCTACGACGTGGGCTACAG		
<i>p53 F</i>	TCTTATCCGGGTGGAAGGAAA		
<i>p53 R</i>	GGCGAAAAGTCTGCCTGTCTT		
p16 F	GGGTTTTCTTGGTGAAGTTCG		
p16 R	TTGCCCATCATCATCACCT		
Sod2 F	CAGACCTGCCTTACGACTATGG		
Sod2 R	CTCGGTGGCGTTGAGATTGTT		
Hmox1 F	AGGTACACATCCAAGCCGAGA		
Hmox1 R	CATCACCAGCTTAAAGCCTTCT		

Supplementary Table 3: List of mouse primers

Ppary2 F	GGGTCAGCTCTTGTGAATGG
Ppary2 R	CTGATGCACTGCCTATGAGC
Oc F	TGCGCTCTGTCTCTCTGACC
Oc R	CTGTGACATCCATACTTGCAGG
<i>p21 F</i>	CCTGGTGATGTCCGACCTG
<i>p21 R</i>	CCATGAGCGCATCGCAATC
Trap F	CAGCTCCCTAGAAGATGGATTCAT
Trap R	GTCAGGAGTGGGAGCCATATG
Rankl F	AGCCGAGACTACGGCAAGTA
Rankl R	AAAGTACAGGAACAGAGCGATG
Rela F	ACTGCCGGGATGGCTACTAT
Rela R	TCTGGATTCGCTGGCTAATGG
Opg F	CCTTGCCCTGACCACTCTTAT
Opg R	CACACACTCGGTTGTGGGT
Ctsk F	AGGCAGCTAAATGCAGAGGGTACA
Ctsk R	AGCTTGCATCGATGGACACAGAGA
Ctnnb1 F	CCCAGTCCTTCACGCAAGAG
Ctnnb1 R	CATCTAGCGTCTCAGGGAACA
Vegfa F	GTACCTCCACCATGCCAAGTG
Vegfa R	TGGGACTTCTGCTCTCCTTCTG
Vcam F	GGCTCCAGACATTTACCCAGTT
Vcam R	CATGAGCTGGTCACCCTTGAA
Fas F	CTGCACCCTGACCCAGAATAC
Fas R	ACAGCCAGGAGAATCGCAGTA
Fasgl F	CAGTCCACCCCTGAAAAAAA
Fasgl R	CCTTGAGTTGGACTTGCCTGTT
1110 F	CTGGACAACATACTGCTAACCG
1110 R	GGGCATCACTTCTACCAGGTAA
ll1rn F	GCTCATTGCTGGGTACTTACAA
Il1rn R	CCAGACTTGGCACAAGACAGG

Primary antibodies	Catalog #	Company	Dilution
Phospho-Akt Ser473_Rabbit	#4058 (193H12)	Cell Signaling	1:1000
Phospho-Akt Thr308_Rabbit	#13038 (D25E6)	Cell Signaling	1:1000
Total AKT_Rabbit	#9272	Cell Signaling	1:1000
β-actin_Rabbit	#4970 (13E5)	Cell Signaling	1:1000
Phospho-NF-kB p65_Rabbit	#3031	Cell Signaling	1:1000
NF-kB p65_Rabbit	#8242 (D14E12)	Cell Signaling	1:1000
Secondary HRP-			
conjugated antibodies			
Anti-rabbit IgG, HRP-linked	#7074	Cell Signaling	1:5000
Antibody			

Supplementary Table 4. List of primary and secondary antibodies used for western blot



Supplementary Fig. 1 legend: Metabolic parameters of investigated mice.

(**a-d**) Omega-3 PUFAs improve metabolic parameters in HFD mice. (**a**) Body weigh gain, (**b**) measurement of intraperitoneal glucose tolerance test (GTT) in treated mice at the end of dietary intervention after overnight fasting (groups coding: white circle-ND, black circle-HFD, yellow circle-HFD+F), (**c**) area under the curve of coresponding GTT graphs; (**d**) insulinemia after overnight fasting at the end of dietary intervention (n=7-10). Data are presented as mean \pm SEM (n = 6-8 per group); one-way ANOVA, Tukey's multiple comparison test with * p ≤ 0.05, ** p ≤ 0.01, **** p ≤ 0.0001. (groups coding: white column-ND, black line shading-HFD, yellow column-HFD+F)



Supplementary Fig. 2 legend: Bone parameters of investigated mice.

(a-d) Evaluation of bone parameters in tibia: (a) Cortical porosity (Ct.Po), (b) Cortical total volume (Ct.TV); (c) trabecular separation (Tb.Sp) and (d) cortical area fraction (B.Ar/T.Ar) in treated mice (n= 7-10). (e-g) Evaluation of bone parameters in L5 vertebra: (e) Trabecular number (Tb.N), (f) trabecular thickness (Tb.Th), (g) trabecular separation (Tb.Sp). Analysis of circulating (h) bone formation marker P1NP (pg/mL) and (i) bone resorption marker TRAP (U/L). Data are presented as mean \pm SEM (n = 6-8 per group); one-way ANOVA, Tukey's multiple comparison test with * p ≤ 0.05. (groups coding: white column-ND, black line shading-HFD, yellow column-HFD+F)

Supplementary Fig. 3



Supplementary Fig. 3 legend: Metabolomic analysis of plasma, bone marrow (BM) and bone powder samples obtained from investigated mice.

PLS-DA score plots of unique lipid species (top panels) and polar metabolites (bottom panels) for (a) plasma, (b) bone marrow, and (c) bone powder samples.

Plasma

unique lipid species

ND HFD HFD+F



polar metabolites

ND HFD HFD+F





8-iso-Prostaglandin A2 Disaccharide (2) gamma-Butyrobetaine 2-Hydroxybutyric acid Trigonelline Stachydrine (proline betaine) Homocitrulline Hippuric acid Urea N-Acetylphenylalanine N-Acetylleucine 2-Acetamidooctanoic acid Xanthurenic acid Ergothioneine 4-Pyridoxic acid Leucine TMAO Glycine 2-Ethyl-2-hydroxybutyric acid 5-Aminovaleric acid betaine Ophthalmic acid N,N-Dimethylglycine Carnitine 4-Hydroxyphenyllactic acid



Supplementary Fig.4 legend: Metabolomic analysis of plasma samples obtained from investigated mice. Heatmap of top-60 individual lipid species and top-25 polar metablites in plasma (n= 9-10 per group) based on ANOVA with p(FDR) < 0.05,. (gradient color keys used in normalized intensity (A.U.))

BM

unique lipid species



polar metabolites

ND HFD HFD+F





Supplementary Fig.5 legend: Metabolomic analysis of bone marrow (BM) samples obtained from investigated mice. Heatmap of top-60 individual lipid species and top-25 polar metablites in BM (n= 5-6 per group) based on ANOVA with p(FDR) < 0.05. (gradient color keys used in normalized intensity (A.U.))

BP

unique lipid species ND HFD HFD+F PC 36:5 (2) PE 38:5; PE 18:1_20:4 PG 38:5 (1); PG 18:1_20:4 PC 38:4 (2); PC 16:0_22:4 LPC 20:3/0:0 PE 36:4 (2); PE 16:0_20:4 PC 36:4 (2); PC 16:0 20:4 PC 37:4 PC 34:4 (2) LPC 20:4/0:0 (1) PC 38:4 (3); PC 18:0 20:4 PE 36:4 (1); PE 18:2 18:2 PE 38:6 (1); PE 18:2_20:4 PG 38:6 (1); PG 18:2 20:4 PC 38:6 (1); PC 18:2 20:4 LPE 22:4 PE 42:4; PE 18:0_24:4 PS 38:4; PS 18:0_20:4 PE O-38:5 (1); PE O-16:1_22:4 LPC 22:4/0:0 PC 40:4 (2) PC O-37:8(1) PC O-40:4 (2) PE 38:4 (1); PE 16:0_22:4 LPE 20:4 PC O-39:8(1) PE O-40:5 (2); PE O-18:1_22:4 PE O-40:4 (1); PE O-18:0_22:4 PC O-41:11 (2) PE 40:4 (1); PE 18:0_22:4 PE 38:4 (2); PE 18:0_20:4 PC 42:4 PE 44:7;O; PE 22:4 22:3;O PE O-36:6; PE O-16:1 20:5 LPC 20:5/0:0 (1) FA 18:4 TG 57:7; TG 18:1_18:1_21:5 FA 16:4 TG 56:10; TG 18:2_18:3_20:5 TG 54:7 (2); TG 16:0 16:1 22:6 TG 54:8 (2); TG 14:0_18:2_22:6 FA 20:5 (1) PC 44:12; PC 22:6_22:6 TG 50:7; TG 12:0_18:2_20:5 TG 58:9 (1); TG 18:2_18:2_22:5 TG 50:6 (2); TG 12:0_16:0_22:6 TG 52:8; TG 12:0_18:2_22:6 TG 58:8 (1); TG 18:1_18:2_22:5 TG 54:6 (3); TG 16:0_16:0_22:6 TG 52:7; TG 12:0_18:1_22:6 TG 52:6 (2); TG 14:0 16:0 22:6 TG 56:7 (2); TG 16:0_18:1_22:6 DG 40:7; DG 18:1 22:6 TG 60:12 (1); TG 18:1 20:5 22:6 TG 58:10 (1); TG 18:2_18:2_22:6 TG 58:9 (2); TG 18:1_18:2_22:6 TG 56:9; TG 16:1_18:2_22:6 DG 40:8; DG 18:2_22:6 TG 56:8 (2); TG 16:0_18:2_22:6 TG 58:8 (2); TG 18:1_18:1_22:6

polar metabolites

ND HFD HFD+F



A.U. 1 0.5 0 -0.5 -1 5-trans-Prostaglandin D2 N-Acetylalanine Thiamine N-Acetvlhistamine Gly-Gln Guanosine Thymidine 6-trans-12-epi-Leukotriene B4 Prostaglandin F2-beta-Thromboxane B2 12-HETE 12-HHTrE Pantothenic acid 4-Hydroxyphenyllactic acid **Ribose 1-phosphate** N-epsilon-Methyllysine N6,N6,N6-Trimethyllysine TMAO Ergothioneine Stachydrine (proline betaine) N-Acetylphenylalanine Trigonelline 3-Hydroxyisobutyric acid Urea Hippuric acid

Supplementary Fig. 6 legend:

Metabolomic analysis of bone powder (BP) samples obtained from investigated mice. Heatmap of top-60 individual lipid species and top-25 polar metablites in BP (n=6 per group) based on ANOVA with p(FDR) < 0.05. (gradient color keys used in normalized intensity (A.U.)).

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Supplementary Fig. 7
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Supplementary Fig. 7 legend: Cellular characteristics of bone cells obtained from the treated mice. (a) Short-term proliferation assay of primary BMSCs calculated after 1, 3, 6 and 9 days in culture after seeding (n = 3 per group) (groups coding: white circle-ND, black circle-HFD, yellow circle-HFD+F). (b) Gene expression of senescence genes (*p16, p21, Vegfa, Vecam, Tnfa*) measured in mouse HSCs. Data are presented as mean \pm SEM (n = 4 per group); one-way ANOVA, Tukey's multiple comparison test, * p ≤ 0.05 , ** p ≤ 0.01 . (groups coding: white column-ND, black line shading-HFD, yellow column-HFD+F)





Supplementary Fig. 8 legend: The effect of omega-3 PUFA treatment in vitro on osteoclast differentiation

(a) Number of TRAP+ cells after 5 days of differentiation with omega-3 PUFA short term supplementation (n = 4 per group). (b) Gene expression of osteoclastic genes (*Trap, Ctsk*) measured in Ocs after 5 days differentiation. Data are presented as mean \pm SEM (n = 6 per group); one-way ANOVA, Tukey's multiple comparison test, **p \leq 0.01, *** p \leq 0.001, ****p \leq 0.0001. (c) Representative pictures of TRAP+ differentiated OCs after 5 days of differentiation with omega-3 PUFAs (scale bar 250 µm). (groups coding: white column-ND, black line shading-HFD, yellow column-HFD+F)

Supplementary Fig. 9



Supplementary Fig. 9 legend: Uncropped western blot membrane images corresponding to Fig.6b and Fig. 6d

(a) p-S473-AKT, (b) total AKT and corresponding (c) β -actin. (e) p-T308-AKT, (f) total AKT and corresponding (g) β -actin.

Supplementary Fig. 10



Supplementary Fig. 10 legend: Uncropped western blot membrane images corresponding to Fig. 6f. (a) p-NF κ B, (b) total NF κ B and corresponding (c) β -actin.