

Supplementary Materials

Table S1. Diet composition.

	STD ^a -C/-FG	STD ^a -ω3/-FG+ω3	^b HFHS-C/-FG	^b HFHS-ω3/-FG+ω3
Flour (g)	1000 ^a	1000 ^a	1000 ^b	1000 ^b
Porcine gelatin (g)	25	25	25	25
Soybean lecithin (g)	6	6	22	22
Oil (mL)	19 mL soybean	19 mL EPA:DHA 1:1	24 mL soybean	24 mL EPA:DHA 1:1
Protein (% weight)	16.4	16.4	21.7	21.7
Fat (% weight)	6.2	6.2	24.1	24.1
Carbohydrates (% weight)	46.6	46.6	45.0	45.0
Total energy density (kcal/g)	3.1	3.1	4.8	4.8

^aTeklad Global 14% Protein Rodent Maintenance Diet (Envigo, IN, USA). ^bTD.08811 45% Kcal Fat Diet (Envigo, IN, USA). STD (standard diet), HFHS (high-fat high-sucrose diet), ω3 (supplementation with fish oil); FG (supplementation with D-Fagomine).

Table S2. Fatty acid composition of each diet (chow + supplement). Results are expressed as a percentage of total fatty acids (mg/100mg of Total FA).

FATTY ACIDS	STD-C/-FG	STD- ω 3/-FG+ ω 3	HFHS-C/-FG	HFHS- ω 3/-FG+ ω 3
14:00	0.0	0.1	11.5	11.6
16:00	14.8	14.6	32.2	32.0
16:1 ω 7	0.0	0.1	1.7	1.7
18:00	2.9	2.9	13.7	13.7
18:1 ω 9	20.5	20.3	26.4	26.2
18:1 ω 7	0.0	0.0	0.7	0.7
18:2 ω 6	58.6	57.8	9.7	8.7
20:00	n.d.	0.0	n.d.	0.0
18:3 ω 3	2.9	2.9	1.1	1.1
20:1 ω 9	0.0	0.0	0.0	0.0
18:4 ω 3	0.0	0.0	0.0	0.0
20:2 ω 6	0.0	0.0	0.0	0.0
20:3 ω 6	n.d.	0.0	n.d.	0.0
20:4 ω 6	0.0	0.0	0.0	0.0
22:1 ω 11	0.0	0.0	0.0	0.0
22:1 ω 9	0.0	0.0	0.0	0.0
20:4 ω 3	0.0	0.0	0.0	0.0
20:5 ω 3	0.0	0.4	0.0	0.5
24:1 ω 9	0.0	0.0	0.0	0.0
22:5 ω 3	0.0	0.1	0.0	0.1
22:6 ω 3	0.0	0.4	0.0	0.6
ω 3	3.3	3.2	1.6	1.4
ω 6	58.8	58.9	10.4	10.6
ω 6/ ω 3	17.8	18.4	6.5	7.6
SFAs	17.5	18.3	59.4	60.5
MUFAs	21.1	20.3	29.3	28.4
PUFAs	61.1	61.0	10.8	10.7
EPA/DHA	-	1/1	-	1/1.2

¹ STD (standard diet), HFHS (high-fat high-sucrose diet), ω 3 (supplementation with fish oil); FG (supplementation with D-Fagomine); SFAs (saturated fatty acids); MUFAs (monounsaturated fatty acids); PUFAs (polyunsaturated fatty acids); EPA (eicosapentaenoic fatty acid); DHA (docosahexaenoic fatty acid).

Table S3. Carbonylated proteins identified by nanoLC-MS/MS in kidney. Carbonylation index calculated for each protein in each diet are also showed. Protein Spot No. refers to the numbered spots in 2D gels shown in Figure S2.

Spot Nº	Protein ID	Gene Name	Avg. Mass	UniProtKB code	STD-C	STD-FG	STD- ω 3	STD- FG+ ω 3	HFHS-C	HFHS- FG	HFHS- ω 3	HFHS- FG+ ω 3	
1	Phosphatidylethanolamine-binding protein 1 OS=Rattus norvegicus OX=10116 GN=Pebp1 PE=1 SV=3	Pebp1	20801	P31044 PEBP1_RAT		1.02 ^c (0.01)	1.14 ^c (0.14)	0.97 ^c (0.17)	1.07 ^c (0.25)	1.51 ^{ab} (0.01)	1.58 ^a (0.13)	1.11 ^c (0.02)	1.17 ^b (0.06)
2	Superoxide dismutase [Mn] mitochondrial OS=Rattus norvegicus OX=10116 GN=Sod2 PE=1 SV=2	Sod2	24674	P07895 SODM_RAT	1.88(0.50)	1.86(0.22)	1.94(0.58)	1.85(0.17)	2.03(0.44)	1.97(0.15)	2.12(0.22)	2.03(0.12)	
3	Glutathione S-transferase alpha-3 OS=Rattus norvegicus OX=10116 GN=Gsta3 PE=1 SV=3	Gsta3	25319	P04904 GSTA3_RAT	0.36(0.10)	0.38(0.21)	0.37(0.17)	0.34(0.23)	0.38(0.09)	0.35(0.13)	0.38(0.10)	0.37(0.12)	
	Glutathione S-transferase P OS=Rattus norvegicus OX=10116 GN=Gstp1 PE=1 SV=2	Gstp1	23439	P04906 GSTP1_RAT									
	Glutathione S-transferase alpha-4 OS=Rattus norvegicus OX=10116 GN=Gsta4 PE=1 SV=2	Gsta4	25510	P14942 GSTA4_RAT									
4	Peroxisomal trans-2-enoyl-CoA reductase OS=Rattus norvegicus OX=10116 GN=Pecr PE=2 SV=1	Pecr	32433	Q9WVK3 PECR_RAT	0.71(0.22)	0.73(0.25)	0.73(0.31)	0.72(0.34)	0.71(0.18)	0.59(0.15)	0.72(0.15)	0.72(0.20)	
5	Triosephosphate isomerase OS=Rattus norvegicus OX=10116 GN=Tpi1 PE=1 SV=2	Tpi1	26849	P48500 TPIS_RAT	0.54 ^{abc} (0.13)	0.29 ^c (0.07)	0.37 ^{abc} (0.06)	0.29 ^c (0.08)	0.62 ^a (0.16)	0.35 ^{bc} (0.00)	0.61 ^{ab} (0.02)	0.33 ^c (0.11)	
	Electron transfer flavoprotein subunit beta OS=Rattus norvegicus OX=10116 GN=Etfb PE=1 SV=3	Etfb	27687	Q68FU3 ETFB_RAT									
6	Enoyl-CoA delta isomerase 1 mitochondrial OS=Rattus norvegicus OX=10116 GN=Eci1 PE=1 SV=1	Eci1	32254	P23965 ECI1_RAT	0.50 ^{ab} (0.01)	0.36 ^{bc} (0.02)	0.47 ^{abc} (0.05)	0.28 ^c (0.08)	0.57 ^a (0.08)	0.36 ^{bc} (0.03)	0.56 ^a (0.13)	0.44 ^{abc} (0.07)	
7	Carbonic anhydrase 2 OS=Rattus norvegicus OX=10116 GN=Ca2 PE=1 SV=2	Ca2	29114	P27139 CAH2_RAT	0.95 ^b (0.06)	0.87 ^b (0.06)	0.90 ^b (0.06)	0.92 ^b (0.18)	1.87 ^a (0.29)	1.70 ^a (0.29)	1.50 ^a (0.19)	1.49 ^a (0.14)	
8	Omega-amidase NIT2 OS=Rattus norvegicus OX=10116 GN=Nit2 PE=1 SV=1	Nit2	30701	Q497B0 NIT2_RAT	0.62(0.24)	0.57(0.31)	0.61(0.22)	0.56(0.42)	0.66(0.35)	0.55(0.29)	0.67(0.16)	0.66(0.04)	
9	3-hydroxyisobutyrate dehydrogenase mitochondrial OS=Rattus norvegicus OX=10116 GN=Hibadh PE=1 SV=3	Hibadh	35303	P29266 3HIDH_RAT	0.62(0.24)	0.60(0.34)	0.61(0.28)	0.60(0.34)	0.60(0.30)	0.53(0.25)	0.63(0.17)	0.62(0.10)	
10	Malate dehydrogenase cytoplasmic OS=Rattus norvegicus OX=10116 GN=Mdh1 PE=1 SV=3	Mdh1	36483	O88989 MDHC_RAT	0.27(0.08)	0.31(0.19)	0.43(0.03)	0.32(0.37)	0.27(0.06)	0.23(0.05)	0.28(0.12)	0.12(0.04)	
11	Actin cytoplasmic 1 OS=Rattus norvegicus OX=10116 GN=Actb PE=1 SV=1	Actb	41737	P60711 ACTB_RAT	0.19 ^b (0.04)	0.22 ^{ab} (0.09)	0.21 ^{ab} (0.14)	0.20 ^b (0.08)	0.58 ^a (0.15)	0.56 ^{ab} (0.20)	0.37 ^{ab} (0.22)	0.26 ^{ab} (0.04)	
12	Heat shock cognate 71 kDa protein OS=Rattus	Hspa8	70871	P63018 HSP7C_RAT	0.64 ^{ab} (0.26)	0.66 ^{ab} (0.29)	0.63 ^{ab} (0.17)	0.56 ^b (0.20)	1.39 ^a (0.37)	1.16 ^{ab} (0.32)	0.90 ^{ab} (0.35)	0.86 ^{ab} (0.057)	

	SV=2												
28	Glutamate dehydrogenase 1 mitochondrial OS=Rattus norvegicus OX=10116 GN=Glud1 PE=1 SV=2	Glud1	61416	P10860 DHE3_RAT	0.43 ^{abc} (0.03)	0.27 ^{cd} (0.09)	0.33 ^{bcd} (0.12)	0.23 ^d (0.12)	0.62 ^a (0.04)	0.49 ^{ab} (0.01)	0.46 ^{abc} (0.04)	0.41 ^{bcd} (0.01)	
29	Alanine--glyoxylate aminotransferase 2 mitochondrial OS=Rattus norvegicus OX=10116 GN=Agxt2 PE=1 SV=2	Agxt2	57201	Q64565 AG_RAT	0.38(0.08)	0.37(0.07)	0.41(0.19)	0.40(0.17)	0.40(0.08)	0.38(0.02)	0.41(0.11)	0.40(0.02)	
30	Retinal dehydrogenase 1 OS=Rattus norvegicus OX=10116 GN=Aldh1a1 PE=1 SV=3	Aldh1a1	54459	P51647 AL1A1_RAT	0.75 ^a (0.09)	0.55 ^a (0.39)	0.62 ^a (0.14)	0.63 ^a (0.36)	1.17 ^a (0.21)	1.06 ^a (0.03)	0.81 ^a (0.08)	0.78 ^a (0.22)	
31	Succinyl-CoA:3-ketoacid coenzyme A transferase 1 mitochondrial OS=Rattus norvegicus OX=10116 GN=Oxct1 PE=1 SV=1	Oxct1	56204	B2GV06 SCOT1_RAT	0.60 ^a (0.12)	0.38 ^a (0.06)	0.41 ^a (0.06)	0.34 ^a (0.04)	0.71 ^a (0.25)	0.40 ^a (0.04)	0.69 ^a (0.20)	0.69 ^a (0.15)	
	Dihydrolipoyl dehydrogenase mitochondrial OS=Rattus norvegicus OX=10116 GN=Dld PE=1 SV=1	Dld	54038	Q6P6R2 LDLH_RAT									
	Methylmalonate-semialdehyde dehydrogenase [acylating] mitochondrial OS=Rattus norvegicus OX=10116 GN=Aldh6a1 PE=1 SV=1	Aldh6a1	57808	Q02253 MMSA_RAT									
32	Catalase OS=Rattus norvegicus OX=10116 GN=Cat PE=1 SV=3	Cat	59757	P04762 CATA_RAT	0.46 ^b (0.16)	0.31 ^b (0.16)	0.26 ^b (0.09)	0.27 ^b (0.08)	0.89 ^a (0.16)	0.60 ^{ab} (0.09)	0.51 ^b (0.11)	0.51 ^b (0.10)	
	Triokinase/FMN cyclase OS=Rattus norvegicus OX=10116 GN=Tkfc PE=1 SV=1	Tkfc	59444	Q4KLZ6 TKFC_RAT									
33	Acyl-coenzyme A synthetase ACSM2 mitochondrial OS=Rattus norvegicus OX=10116 GN=Acsm2 PE=2 SV=2	Acsm2	64145	O70490 ACSM2_RAT	0.71 ^b (0.25)	0.66 ^b (0.26)	0.56 ^b (0.49)	0.65 ^b (0.40)	2.06 ^a (0.21)	1.40 ^{ab} (0.25)	1.04 ^b (0.17)	0.75 ^b (0.24)	
	Transketolase OS=Rattus norvegicus OX=10116 GN=Tkt PE=1 SV=1	Tkt	67644	P50137 TKT_RAT									
34	Aconitate hydratase mitochondrial OS=Rattus norvegicus OX=10116 GN=Aco2 PE=1 SV=2	Aco2	85433	Q9ER34 ACON_RAT	0.49(0.00)	0.50(0.24)	0.51(0.16)	0.53(0.41)	0.54(0.13)	0.63(0.08)	0.53(0.27)	0.54(0.07)	
35	Serotransferrin OS=Rattus norvegicus OX=10116 GN=Tf PE=1 SV=3	Tf	76395	P12346 TRFE_RAT	0.22 ^{ab} (0.14)	0.19 ^{ab} (0.04)	0.21 ^{ab} (0.10)	0.19 ^b (0.04)	0.35 ^{ab} (0.08)	0.45 ^a (0.11)	0.35 ^{ab} (0.09)	0.34 ^{ab} (0.08)	
36	Cytoplasmic aconitase hydratase OS=Rattus norvegicus OX=10116 GN=Aco1 PE=1 SV=1	Aco1	98127	Q63270 ACOC_RAT	0.14(0.01)	0.16(0.08)	0.18(0.14)	0.16(0.12)	0.16(0.03)	0.30(0.07)	0.15(0.06)	0.16(0.17)	

¹ Two-way ANOVA analyses were conducted, followed by Tukey HSD's post hoc test. * p<0.05 significant differences given by the factor "diet" (STD and HFHS); ^b p<0.05 significant differences given by the factor "supplement" (CONTROL, FG, ω-3, FG+ ω-3). Superscript ^a indicates significant interaction (p<0.05) between the factors diet and supplement. Means with different superscript indicate significant differences (p<0.05) (analyzed by post hoc Tukey HSD).

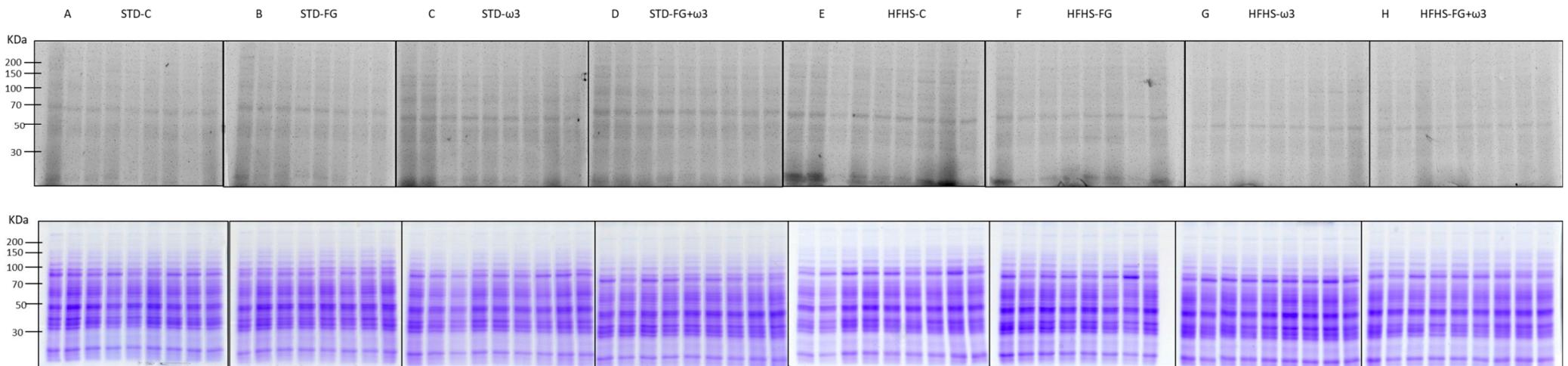


Figure S1. Total proteins carbonylation in rat kidneys. Upper panels show representative images of FTSC-label carbonylated proteins resolved in 1D gels from (A) STD-CONTROL, (B) STD-FG, (C) STD- ω 3, (D) STD-FG+ ω 3, (E) HFHS-CONTROL, (F) HFHS-FG, (G) HFHS- ω 3, (H) HFHS-FG+ ω 3. Lower panels show their corresponding Coomassie stained 1D gels. Images are representatives of three independent labelling experiments performed in triplicates. STD (standard diet), HFHS (high-fat high-sucrose diet), ω 3 (supplementation with fish oil); FG (supplementation with D-Fagomine).

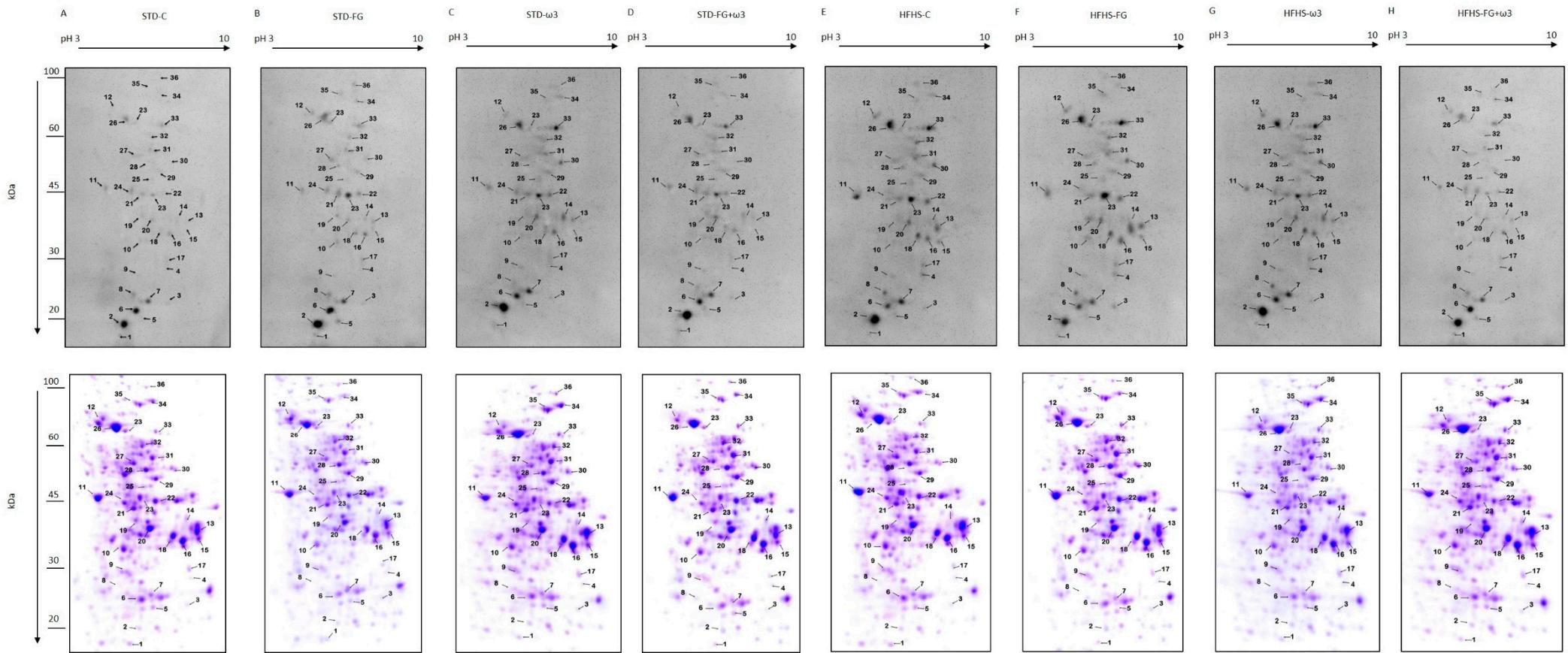


Figure S2. Carbonylated proteins identified in rat kidneys. Upper panels show representative images of FTSC-label carbonylated proteins resolved in 2D gels from (A) STD-CONTROL, (B) STD-FG, (C) STD- ω 3, (D) STD-FG+ ω 3, (E) HFHS-CONTROL, (F) HFHS-FG, (G) HFHS- ω 3, (H) HFHS-FG+ ω 3. Lower panels show their corresponding Coomassie stained 2D gels. Numbered protein spots represent carbonylated proteins confidently identified, and they are listed in Table 5 and Supplementary Table S3. Images are representatives of three independent labelling experiments performed in triplicates. STD (standard diet), HFHS (high-fat high-sucrose diet), ω 3 (supplementation with fish oil); FG (supplementation with D-Fagomine).