

Table S1: Diet composition.

	STD-C/- ω 3 ^{a*}	HFHS-C/- ω 3 ^{b*}
Protein (%)	14.3	17.3
Fat (%)	4.0	23.2
Anhydrous Milkfat (%)		21
Carbohydrate (available) (%)	48.0	47.6
Sucrose (%)	-	34
Fiber (%)	22.1	5
Minerals (%)	4.7 ^c	4.3 ^d
Vitamins (%)	1.2 ^e	1.9 ^f
Total energy density (kcal/g)	2.9	4.7
Calories from Protein (%)	20	14.7
Calories from Fat (%)	13	44.6
Calories from Carbohydrates (%)	67	40.7

^aTeklad Global 14% Protein Rodent Maintenance Diet (Envigo, IN, USA).

^bTD.08811 45% Kcal Fat Diet (Envigo, IN, USA).

^cCalcium (0.7%), phosphorus (0.6%), non-phytate phosphorus (0.3%), sodium (0.1%), potassium (0.6%), chloride (0.3%), magnesium (0.2%), zinc (70 mg/kg), manganese (100 mg/kg), copper (15 mg/kg), iodine (6 mg/kg), iron (175 mg/kg), selenium (0.23 mg/kg).

^d43 g/kg chow of Mineral Mix, AIN-93G-MX (94046): Calcium Carbonate (35.7%), monopotassium phosphate (19.6%), potassium citrate monohydrate (7.1%), sodium chloride (7.4%), potassium sulfate (4.7%), magnesium oxide (2.4%), ferric citrate (0.6%), zinc carbonate (0.2%), manganese carbonate (0.06%), copper carbonate (0.03%), potassium iodate (0.001%), sodium selenate anhydrous (0.001%), ammonium molybdate.4H₂O (0.0008%), sodium metasilicate.9H₂O (0.15%), chromium potassium sulfate.12H₂O (0.3%), lithium chloride (0.002%), boric acid (0.008%), sodium fluoride (0.006%), nickel carbonate (0.003%), ammonium vanadate (0.0007%), powdered sugar (22.1%).

^eVitamin A (17 IU/g), vitamin D3 (1.2 IU/g), vitamin E (150 IU/kg), vitamin K3 (menadione; 58 mg/kg), vitamin B1 (thiamin; 64 mg/kg), vitamin B2 (riboflavin; 16 mg/kg), niacin (nicotinic acid; 84 mg/kg), vitamin B6 (pyridoxine; 17 mg/kg), pantothenic acid (76 mg/kg), vitamin B12 (cyanocobalamin, 0.09 mg/kg), biotin (0.57 mg/kg), folate (5 mg/kg), choline (1030 mg/kg).

^f3 g/kg choline bitartrate + 19 g/kg chow of Vitamin Mix, AIN-93-VX (94047): nicotinic acid (0.3%), D-calcium pantothenate (0.16%), pyridoxine HCl (0.07%), thiamine HCl (0.06%), riboflavin (0.06%), folic acid (0.02%), D-biotin (0.002%), vitamin B12 (0.01% triturated in mannitol) (0.25%), α -tocopherol powder (250 U/g; 3%), vitamin A palmitate (250,000 U/g; 0.16%), vitamin D3 (400,000 U/g; 0.025%), phyloquinone (0.0075%), powdered sucrose (95.97%).

*STD-C and HFHS-C groups received a weekly oral dose of 0.8 ml/kg body weight of soybean oil; STD- ω 3 and HFHS- ω 3 groups received a weekly oral dose of 0.8 ml/kg body weight of fish oil.

Table S2: Fatty acid composition of each diet.

Fatty acid*	STD-C ^a	STD- ω 3 ^b	HFHS-C ^a	HFHS- ω 3 ^b
14:0	0.0	0.1	11.5	11.6
16:0	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
20:0	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
Total SFAs	17.5	18.3	59.4	60.5
Total MUFAs	21.1	20.3	29.3	28.4
Total PUFAs	61.1	61.0	10.8	10.7
Total ω3	3.3	3.2	1.6	1.4
Total ω6	58.8	58.9	10.4	10.6

*Results are expressed as a percentage of total fatty acids (mg/100mg of Total FA).

^aSTD-C and HFHS-C groups received a weekly oral dose of 0.8 ml/kg body weight of soybean oil.

^bSTD- ω 3 and HFHS- ω 3 groups received a weekly oral dose of 0.8 ml/kg body weight of fish oil.

Table S3. Biometrical and biochemical data from Sprague-Dawley rats fed either a standard diet (STD) or a high-fat and high-sucrose diet (HFHS). Control groups (STD-C and HFHS-C) were supplemented with soybean oil. ω 3 groups (STD- ω 3 and HFHS- ω 3) were supplemented with fish oil.^{1,2}

	STD-C	STD- ω 3	HFHS-C	HFHS- ω 3
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
% of initial body Weight ^{z*}	114.07 ^a (2.46)	115.47 ^{ab} (3.27)	118.43 ^b (3.69)	115.90 ^{ab} (3.69)
Adiposity Index (%) [*]	1.67 ^a (0.44)	1.65 ^a (0.38)	2.37 ^b (0.75)	2.32 ^b (0.69)
% Fat in liver ^{y*}	7.01 ^a (0.51)	6.36 ^b (0.28)	7.34 ^a (0.45)	6.55 ^b (0.38)
% Fat in kidney ^{y*}	4.31 ^a (0.23)	4.11 ^{ab} (0.24)	5.65 ^a (0.56)	4.39 ^b (0.39)
% Fat in adipose tissue ^{y*}	96.32 ^a (2.05)	92.73 ^b (3.16)	99.51 ^a (2.03)	97.01 ^b (1.20)
Plasma Insulin ng/mL [*]	0.56 ^a (0.32)	0.65 ^a (0.19)	1.81 ^b (0.82)	1.46 ^b (0.72)
Plasma Glucose mg/mL [*]	63.00 ^a (4.84)	63.44 ^a (4.1)	70.78 ^a (4.99)	71.33 ^a (5.32)
GPx activity (U/g Hb)	85.41 ^a (14.75)	80.87 ^{ab} (19.18)	83.45 ^a (13.06)	71.34 ^b (6.82)
GSSG/GSH in erythrocyte ^{x*§}	0.60 ^a (0.25)	1.07 ^b (0.34)	1.48 ^b (1.09)	1.56 ^b (0.87)
Lipid peroxidation (mmol hydroperoxides/mL plasma) ^{x*}	0.13 ^a (0.03)	0.13 ^a (0.02)	0.21 ^a (0.06)	0.14 ^a (0.02)
Plasma ORAC (μ mol Trolox equivalents/mL) [§]	18.33 ^a (3.76)	19.12 ^a (2.64)	17.41 ^a (4.71)	21.18 ^a (6.12)
Plasma albumin carbonylation index ^{x§}	0.41 ^{ab} (0.04)	0.33 ^{ab} (0.05)	0.43 ^a (0.11)	0.32 ^b (0.04)
Liver carbonylation index ^{x*§}	0.27 ^a (0.02)	0.32 ^b (0.03)	0.45 ^c (0.03)	0.28 ^a (0.03)
Kidney carbonylation index ^{x*§}	0.24 ^a (0.01)	0.30 ^b (0.02)	0.43 ^c (0.04)	0.30 ^a (0.03)

¹Two-way ANOVA analyses were conducted. * p<0.05 significant differences given by the factor “diet” (STD and HFHS); § p<0.05 significant differences given by the factor “supplement” (CONTROL, ω -3). Superscript # 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).

^zA percentage of the initial body weight during the course of the experiment. Body weight percentage was calculated as (final body weight/initial body weight) \times 100.

^yAdiposity index: (total abdominal fat \times 100)/body weight.

ORAC: oxygen radical absorbance capacity. GPx: Glutathione peroxidase. GSSG/GSH: oxidized/reduced glutathione ratio.

² Parameters in the table are already published in:

Dasilva, G; Lois, S; Méndez, L; Miralles-Pérez, B; Romeu, M; Ramos-Romero, S; Torres, J.L.; Medina, I. Fish Oil Improves Pathway-Oriented Profiling of Lipid Mediators for Maintaining Metabolic Homeostasis in Adipose Tissue of Prediabetic Rats, *Front. Immunol.* 12 (2021) 8848161. <https://doi.org/10.3389/fimmu.2021.608875>.

Méndez, L; Muñoz, S; Barros, L; Miralles-Pérez, B; Romeu, M; Ramos-Romero, S; Torres, J.L.; Medina, I. Combined Intake of Fish Oil and D-Fagomine Prevents High-Fat High-Sucrose Diet-Induced Prediabetes by Modulating Lipotoxicity and Protein Carbonylation in the Kidney, *Antioxidants*. 12 (2023) 751. <https://doi.org/10.3390/antiox12030751>.

Hereu, M; Ramos-Romero, S; Busquets, C; Atienza, L; Amézqueta, S; Miralles-Pérez, B; Nogués, M.R; Méndez, L; Medina, I; Torres, J.L. Effects of combined d-fagomine and omega-3 PUFAs on gut microbiota subpopulations and diabetes risk factors in rats fed a high-fat diet. *Sci. Rep.* 2019, 9, 16628. <https://doi.org/10.1038/s41598-019-52678-5>

Hereu, M; Ramos-Romero, S; García-González, N; Amézqueta, S; Torres, J.L. Eubiotic effect of buckwheat d-fagomine in healthy rats. *J. Funct. Foods* 2018, 50, 120–126. <https://doi.org/10.1016/j.jff.2018.09.018>

Hereu, M; Ramos-Romero, S; Marín-Valls, R; Amézqueta, S; Miralles-Pérez, B; Romeu, M; Méndez, L; Medina, I; Torres, J.L. Combined Buckwheat d-Fagomine and Fish Omega-3 PUFAs Stabilize the Populations of Gut Prevotella and Bacteroides While Reducing Weight Gain in Rats. *Nutrients* 2019, 11, 2606. <https://doi.org/10.3390/nu11112606>

Miralles-Pérez, B; Nogués, M.R.; Sánchez-Martos, V.; Taltavull, N.; Méndez, L.; Medina, I.; Ramos-Romero, S.; Torres, J.L.; Romeu, M. The Effects of the Combination of Buckwheat D-Fagomine and Fish Omega-3 Fatty Acids on Oxidative Stress and Related Risk Factors in Pre-Obese Rats. *Foods* 2021, 10, 332. <https://doi.org/10.3390/foods10020332>

Moreno, F.; Méndez, L.; Raner, A.; Miralles-Pérez, B.; Romeu, M.; Ramos-Romero, S.; Torres, J.L.; Medina, I. Fish Oil Supplementation Counteracts the Effect of High-Fat and High-Sucrose Diets on the Carbonylated Proteome in the Rat Cerebral Cortex. *Biomed. Pharmacother.* 2023, 168, 115708. <https://doi.org/10.1016/j.biopha.2023.115708>