

Electronic Supplementary Information

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

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Table S1. Diet composition.

| | STD | HF | FG | ω-3 | FG&ω-3 |
|---|--------------------|--------------------|--------------------|--------------------|--------------------|
| Diet composition | | | | | |
| Flour (g) | 1,000 ^a | 1,000 ^b | 1,000 ^b | 1,000 ^b | 1,000 ^b |
| Fagomine (g) | - | - | 0.96 | - | 0.96 |
| Oil | 0.8 mL | 0.8 mL | 0.8 mL | 0.8 mL | 0.8 mL |
| | SBO/kg b.w. | SBO/kg b.w. | SBO/kg b.w. | FO/kg b.w. | FO/kg b.w. |
| Macronutrients (% weight) | | | | | |
| Protein | 14.3 | 17.0 | 17.0 | 17.0 | 17.0 |
| Fat | 4.0 | 23.0 | 23.0 | 23.0 | 23.0 |
| Carbohydrates | 48.0 | 47.6 | 47.6 | 47.6 | 47.6 |
| Macronutrients (% caloric value) | | | | | |
| Protein | 20.0 | 14.7 | 14.7 | 14.7 | 14.7 |
| Fat | 13.0 | 40.6 | 40.6 | 40.6 | 40.6 |
| Carbohydrates | 67.0 | 40.7 ^c | 40.7 ^c | 40.7 ^c | 40.7 ^c |
| Total energy density (kcal/g) ^d | 2.9 | 4.7 | 4.7 | 4.7 | 4.7 |

Abbreviations: STD, Standard Group; HF, High-Fat Group; FG, Fagomine Group; ω-3, ω-3 PUFA Group; FG&ω-3, Fagomine and ω-3 PUFA Group; SBO, Soybean Oil; FO, Fish Oil; b.w., Body Weight. ^aTeklad Global 14% Protein Rodent Maintenance Diet (Envigo, IN, USA), ^bTD.08811 45% kcal Fat Diet (Envigo, IN, USA), ^cCarbohydrate (available) is calculated by subtracting neutral detergent fiber from total carbohydrates, ^dEnergy density was calculated as estimates of metabolizable energy based on the Atwater conversion factors, assigning 4 kcal/g of protein, 9 kcal/g of fat, and 4 kcal/g of available carbohydrate.

Table S2. Fatty acid composition (mol %) of soybean and fish oils.

| | SBO | FO |
|-----------|--------------|--------------|
| 14:00 | 0.96 ± 0.02 | 4.37 ± 0.05 |
| 15:00 | 0.15 ± 0.01 | 0.29 ± 0.02 |
| 16:00 | 17.78 ± 0.10 | 10.15 ± 0.16 |
| 16:1 ω-7 | 0.90 ± 0.03 | 4.99 ± 0.04 |
| 17:00 | 0.21 ± 0.01 | 0.45 ± 0.004 |
| 18:00 | 2.07 ± 0.01 | 2.94 ± 0.03 |
| 18:1 ω-9 | 18.75 ± 0.03 | 6.41 ± 0.06 |
| 18:1 ω-7 | 1.52 ± 0.02 | 1.91 ± 0.03 |
| 18:2 ω-6 | 47.55 ± 0.01 | 0.65 ± 0.01 |
| 20:00 | n.d | 0.32 ± 0.01 |
| 18:3 ω-3 | 4.00 ± 0.04 | 0.36 ± 0.01 |
| 20:1 ω-9 | 1.43 ± 0.09 | 0.98 ± 0.03 |
| 18:4 ω-3 | 0.15 ± 0.004 | 1.51 ± 0.02 |
| 20:2 ω-6 | 0.20 ± 0.05 | 0.21 ± 0.003 |
| 20:3 ω-6 | n.d | 0.22 ± 0.01 |
| 20:4 ω-6 | 0.40 ± 0.02 | 1.68 ± 0.04 |
| 22:1 ω-11 | 1.08 ± 0.01 | 1.14 ± 0.01 |
| 22:1 ω-9 | 0.25 ± 0.02 | 0.28 ± 0.03 |
| 20:4 ω-3 | 0.20 ± 0.03 | 1.02 ± 0.02 |
| 20:5 ω-3 | 0.70 ± 0.02 | 25.09 ± 0.10 |
| 24:1 ω-9 | 0.28 ± 0.05 | 0.38 ± 0.003 |
| 22:5 ω-3 | 0.26 ± 0.01 | 4.30 ± 0.05 |
| 22:6 ω-3 | 1.15 ± 0.03 | 25.70 ± 0.21 |
| ω-3 | 6.47 ± 0.14 | 58.84 ± 0.16 |
| SFA | 21.17 ± 0.10 | 18.52 ± 0.22 |
| MUFA | 24.21 ± 0.11 | 17.22 ± 0.12 |
| PUFA | 54.62 ± 0.03 | 64.26 ± 0.33 |
| EPA+DHA | 1.85 ± 0.06 | 50.79 ± 0.31 |

Abbreviations: SBO, Soybean Oil; FO, Fish Oil; SFA; n.d., non-detected; SFA, Saturated Fatty acids; MUFA, Monounsaturated Fatty Acids; PUFA, Polyunsaturated Fatty Acids; EPA, Eicosapentaenoic; DHA, Docosahexaenoic.

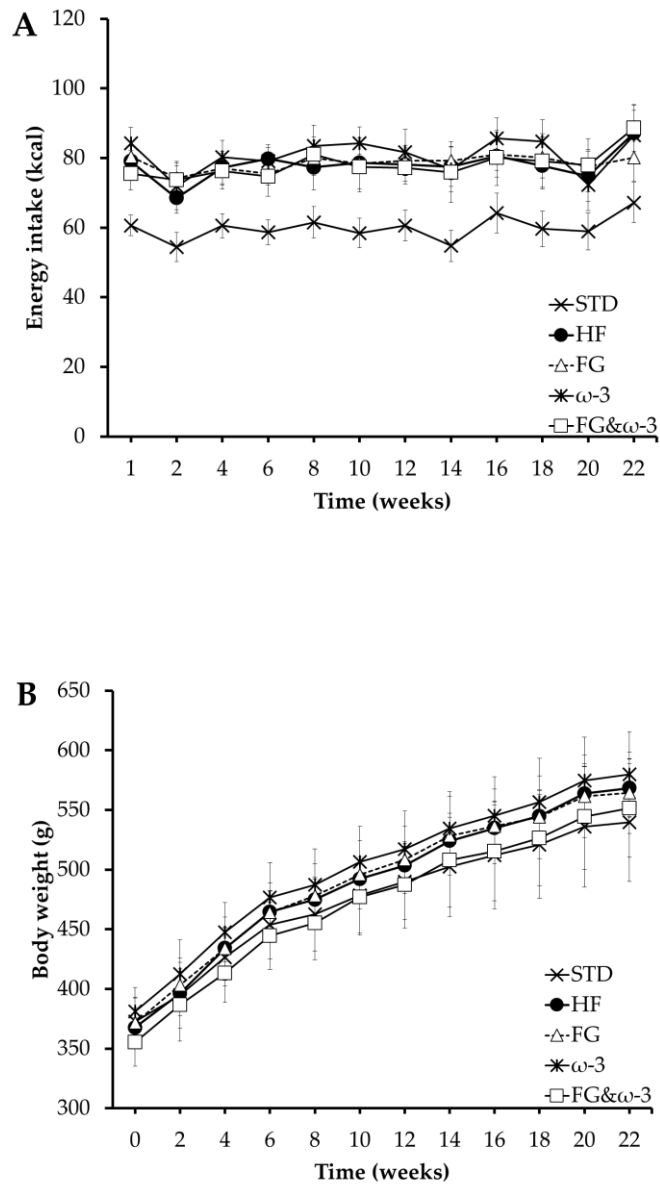


Figure S1. (A) Energy intake and (B) body weight throughout the study. Values are expressed as mean \pm standard deviation, nine rats per group. Abbreviations: STD, Standard group; HF, High-Fat group; FG, Fagomine group; ω -3, ω -3 PUFA group; FG& ω -3, Fagomine and ω -3 PUFA group. Groups fed a HF diet either with supplement or without supplement showed significantly higher energy intake than the STD group (p-value < 0.05). Nevertheless, no significant differences were observed in body weight among the groups.

Table S3. Feed intake, biometric data and insulin resistance biomarkers after 21 weeks of dietary intervention*

| | STD | HF | FG | ω-3 | FG&ω-3 |
|--|--------------|--------------------------|-------------------------|-------------------------|-------------------------|
| Intake data | | | | | |
| Feed intake (g/day) | 20.3 ± 1.6 | 16.3 ± 0.9 ^a | 16.4 ± 1.2 ^a | 16.8 ± 1.0 ^a | 15.9 ± 1.2 ^a |
| Energetic intake (kcal/day) | 58.9 ± 4.6 | 76.5 ± 4.3 ^a | 77.2 ± 5.7 ^a | 79.0 ± 4.7 ^a | 74.8 ± 5.7 ^a |
| Biometric data | | | | | |
| Initial body weight (g) | 373 ± 20 | 368 ± 17 | 370 ± 22 | 381 ± 20 | 355 ± 20 |
| Final body weight (g) | 540 ± 49 | 568 ± 24 | 564 ± 34 | 580 ± 36 | 552 ± 41 |
| Body weight gain (g) | 167 ± 36 | 201 ± 29 | 194 ± 21 | 199 ± 30 | 196 ± 24 |
| Perigonadal adipose tissue (g) | 8.1 ± 1.7 | 13.1 ± 3.9 ^a | 10.8 ± 1.3 | 13.3 ± 4.4 ^a | 9.9 ± 2.1 |
| Adiposity index (%) | 1.5 ± 0.3 | 2.3 ± 0.7 ^a | 1.9 ± 0.3 | 2.3 ± 0.6 ^a | 1.8 ± 0.3 |
| Liver (g) | 14.0 ± 1.7 | 14.0 ± 1.1 | 14.6 ± 1.0 | 14.1 ± 1.0 | 14.6 ± 2.3 |
| Hepatosomatic index (%) | 2.6 ± 0.1 | 2.4 ± 0.2 | 2.6 ± 0.1 | 2.5 ± 0.1 | 2.6 ± 0.3 |
| Insulin resistance biomarkers | | | | | |
| OGTT [#] (AUC, mg/mL per 120 min) | 10,350 ± 739 | 10,335 ± 282 | 10,623 ± 475 | 10,864 ± 625 | 10,338 ± 604 |
| Glucose (mmol/L) | 3.5 ± 0.4 | 3.9 ± 0.3 ^a | 3.9 ± 0.3 ^a | 3.9 ± 0.3 ^a | 3.8 ± 0.2 |
| Insulin (mU/L) | 16.2 ± 9.4 | 52.1 ± 23.9 ^a | 39.3 ± 19.5 | 42.1 ± 20.9 | 40.9 ± 16.5 |

Values are expressed as mean ± standard deviation, nine rats per group. Abbreviations: STD, Standard group; HF, High-Fat group; FG, Fagomine group; ω-3, ω-3 PUFA group; FG&ω-3, Fagomine and ω-3 PUFA group; OGTT, Oral Glucose Tolerance Test; AUC, Area Under the Curve. *These parameters have partially been published in a previous report [1]. [#]OGTT was performed during week 18.

References

1. 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*, 1–12, doi:10.1038/s41598-019-52678-5.