

Supplementary materials

A *Lupinus angustifolius* protein hydrolysate exerts hypocholesterolemic effect in western diet-fed-ApoE^{-/-} mice through the modulation of LDLR and PCSK9 pathways

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Reagents

Dulbecco's modified Eagle's medium (DMEM), L-glutamine, fetal bovine serum (FBS), phosphate buffered saline (PBS), penicillin/streptomycin, chemiluminescent reagent, and 24 or 96-well plates were purchased from Euroclone (Milan, Italy). Bovine serum albumin (BSA), RIPA buffer, pravastatin, the antibody against β -actin, and HMGCoAR assay kit were bought from Sigma-Aldrich (St. Louis, MO, USA). The antibody against HMGCoAR was bought from Abcam (Cambridge, UK). The antibody against phospho-HMGCoAR (Ser872) was purchased from Bioss Antibodies (Woburn, MA, USA). Phenylmethanesulfonyl fluoride (PMSF), Na-orthovanadate inhibitors, and the antibodies against rabbit Ig-horseradish peroxidase (HRP), mouse Ig-HRP, and SREBP-2 were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). The antibodies against the LDLR and the phospho-5' AMP-activated protein kinase (AMPK) (Thr172) were bought from Pierce (Rockford, IL, USA). The antibodies against hepatocyte nuclear factor 1-alpha (HNF1- α) and PCSK9 were bought from GeneTex (Irvine, CA, USA). The inhibitor cocktail Complete Midi from Roche (Basel, Swiss). Mini protean TGX pre-cast gel 7.5%, and Mini nitrocellulose Transfer Packs were purchased from BioRad (Hercules, CA, USA).

Lipid profile quantification

TC, HDL, LDL and TG levels were quantified in plasma through chemiluminescence immunoassay techniques using the COBAS E 601[®] modular analyzer (Roche Diagnostic, Basel, Switzerland). Liver was homogenized in chloroform:isopropanol:Nonidet P-40 (Sigma Aldrich) (7:11:0.1 v/v/v). Samples were centrifuged at 10,000 \times g for 10 min at 4 °C and the supernatant was collected and dried in a Jouan RC1022 evaporator (Thermo Fisher Scientific, Vantaa, Finland). The pellet obtained was dissolved in saline 1% Nonidet P-40. Hepatic total cholesterol (hTC) and hepatic triglycerides (hTG) levels were measured through a chemiluminescence immunoassay using the COBAS E 601[®] modular analyser (Roche Diagnostics).

Table S1. Composition of standard diet

Ingredients	%
Fat ¹	4.0
Palmitic (C16:0)	0.5
Stearic (C18:0)	0.1
Oleic (C18:1 ω 9)	0.7
Linoleic (C18:2 ω 6)	2.0
Linolenic (C18:3 ω 3)	0.1
Total saturated FFAA	0.6
Total monounsaturated FFAA	0.7
Total polyunsaturated FFAA	2.1
Carbohydrate	48.0
Protein	14.3
Cholesterol (mg/kg)	--
Total energy (2.90 kcal/g)	
Fat (ether extract)	13
Carbohydrates	67
Protein	20

Macronutrients percentage of the standard diet and energy provided from the different macromolecules. The complete product description is available in ¹. FFAA, fatty acids.

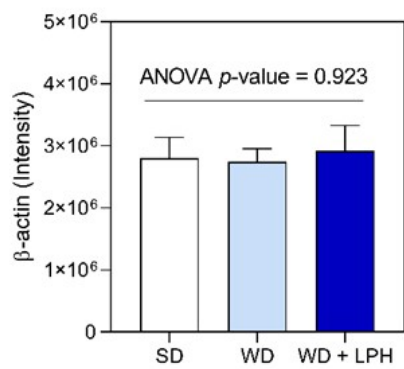
¹The fat of this diet proceeds from soybean oil.

Table S2. Composition of experimental Western diet

Ingredients	%
Fat (2.12 kcal/g)	
Lard	20.68
Soybean oil	2.91
Carbohydrate (1.65 kcal/g)	
Sucrose	20.09
Maltodextrin	11.65
Dextrin	8.48
Powdered cellulose	5.82
Protein (0.83 kcal/g)	
Casein - vitamin tested	23.30
Vitamin Mix	1.16
Mineral Mix	1.16
Food additives	
Potassium citrate. Tribasic monohydrate	1.92
Calcium phosphate	1.51
Calcium carbonate	0.64
L-Cystine	0.35
Choline bitartrate	0.23
Food colouring (FD&C Red No.40)	0.05
Total energy (4.60 kcal/g)	
Fat (ether extract)	46.1
Carbohydrates	35.8
Protein	18.1

Ingredients percentage of the high-fat Western diet and energy provided from the different macromolecules.

Figure S1. β -actin levels concentration among the groups



Notes and references

1. ENVIGO, Teklad Global 14% Protein Rodent Maintenance Diet, <https://insights.envigo.com/hubfs/resources/data-sheets/2014s-datashet-0915.pdf>, (accessed Feb 2022).