Supplemental Material

Serum and Tissue Measurements of Hormones, Metabolites and Reactive Oxidative Species (ROS/RNS)

Removed blood from rats was centrifuged (2000g, 15 min) to obtain serum, which was frozen in liquid nitrogen and stored at -80°C until use. Serum hormone and metabolite levels were measured following manufacturer's protocol: glucose (Accutrend Glucose Analyser, Roche, Switzerland); insulin (Rat Insulin Elisa, Mercodia, Uppsala, Sweden); leptin (Rat Elisa Kit, BioVendor, Brno, Czech Republic); triglycerides (Triglycerides kit, QCA, Barcelona, Spain), cholesterol (Cholesterol Kit, BioSystems, Barcelona, Spain); high-density lipoprotein-cholesterol (HDL-Cholesterol Kit, BioSystems, Barcelona, Spain); non-esterified fatty acids (NEFA-HR Kit, Wako Chemicals, Neuss, Germany).

For reactive oxygen species and reactive nitrogen species (ROS/RNS) quantification, 15 mg of liver was grinded in 1 mL of 50mM phosphate buffer, pH 7.4, using a Dounce homogenizer. Homogenized samples were centrifuged at 10,000g, 4°C for 5 min and supernatants were collected and analysed fluorometrically according to the kit instructions (STA-347, Cell Biolabs, San Diego, CA, USA).

Western blot analysis and immunoblotting

Primary polyclonal antibodies were: anti-PPARβ/δ (1:1000, ab23676), anti-p-S496-AMPK (1:1000, ab39400), anti-AMPK (1:1000, ab39644), anti-p-S9-GSK-3β (1:500, ab30619), anti-GSK-3β (1:500, ab2602), anti-β-actin (1:1000, ab8226) from Abcam, Cambridge, UK; anti-LXRα (1:1000, NB400-157) from Novus Biologicals, Centennial, CO, USA); anti-SREBP-1c (1:200, sc-8984) from Santa Cruz, Dallas, TX, USA.

The secondary antibodies were goat anti-rabbit conjugated with horseradish peroxidase (1:4000, 172-1019) and goat anti-mouse conjugated with horseradish peroxidase (1:4000, 170-6516) from Bio-Rad, Spain. The immune-protein complexes formed were visualized using the ECL Western-blotting detection kit (Amersham Biosciences, Inc., Piscataway, NJ) and the images were subjected to densitometric analysis.

Samples from experimental groups were compared in the same blots and routinely checked with loading control (β-actin). The densitometric values of p-AMPK and p-S9-GSK-3β were normalized with the densitometric values of the corresponding amount of total protein mass of AMPK and S9-GSK-3β, respectively, in the same sample. The densitometric values of LXRα were normalized with the densitometric values of the red ponceau assay.

Supplemental Table 1. Probes used for RT-PCR.

Gene	ABI Assay ID
Pparα	Rn00566193_m1
Pparβ/δ	Rn00565707_m1
Pparγ	Rn00440945_m1
Pdk4	Rn00585577_m1
Fas	Rn00685720_m1
Scd-1	Rn00594894_g1
Cpt-1a	Rn00580702_m1
Fat/Cd36	Rn00580728_m1
Ldlr	Rn005998442_m1
Pgc-1α	Rn00580241_m1
Acox-1	Rn00569216_m1
Fgf21	RN00590706_m1
18Sr RNA	4319413E

Supplemental Table 2. Primer sequences used for RT-PCR.

Gene	Forward Sequence (5' – 3')	Reverse Sequence (5' – 3')
Chrebp-α	AGGCTCAAGCATTCGAAGAG	TGCATCGATCACAGGTCATT
Chrebp-β	CTTGTCCCGGCATAGCAAC	TCTGCAGATCGCGCGGAG
18Sr RNA	CGGCTACCACATCCAAGGAA	GCTGGAATTACCGCGGCT

Supplemental Table 3. Flavanol determination by degree of polymerization (HPLC-FLD-Q-ToF)

	Concentration (mg/g GSE)	Molar %	Representativeness respect weigh extract (%)
Monomers	149.8 ± 0.2	55.5 ± 0.2	15.0 ± 0.0
Galloylated monomers	16.0 ± 0.4	3.9 ± 0.1	1.6 ± 0.0
Dimers	142.0 ± 0.1	26.4 ± 0.1	14.2 ± 0.0
Galloylated dimers	6.5 ± 1.8	0.9 ± 0.3	0.6 ± 0.2
Trimers	58.3 ± 0.3	7.2 ± 0.1	5.8 ± 0.0
Galloylated trimers	1.9 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Tetramers	34.9 ± 0.0	3.2 ± 0.0	3.5 ± 0.0
Galloylated tetramers	12.6 ± 0.0	1.0 ± 0.0	1.3 ± 0.0
Pentamers	14.9 ± 0.2	1.1 ± 0.0	1.5 ± 0.0
Hexamers	5.7 ± 0.1	0.4 ± 0.0	0.6 ± 0.0
Heptamers	1.8 ± 0.0	0.1 ± 0.0	0.2 ± 0.0

Results are expressed as mean ± SD (n=3)

Supplemental Table 4. Flavan-3-ol monomers, dimers and trimers concentration and molar percentage, as well as, the mean degree of polymerization (mDP), galloylation and prodelphynidins percentage, total polyphenolic content, and condensed tannins of GSE determined by spectrophotometric and chromatographic analysis (HPLC-ESI-Q-ToF)

Compounds	Concentration (mg/kg GSE)	Molar proportions (%)
Flavan-3-ols monomers		
(+)-Catechin	83.2 ± 1.5	53.8 ± 0.2
(-)-Epicatechin	59.5 ± 0.9	38.4 ± 0.2
(+)-Gallocatechin	0.7 ± 0.0	0.4 ± 0.0
(-)-Epigallocatechin	0.4 ± 0.0	0.2 ± 0.1
(+)-Catechin gallate	3.9 ± 0.1	1.7 ± 0.0
(-)-Epicatechin gallate	12.5 ± 0.2	5.3 ± 0.0
(+)-Gallocatechin gallate	0.1 ± 0.0	0.0 ± 0.0
(-)-Epigallocatechin gallate	0.2 ± 0.0	0.1 ± 0.0
Total flavan-3-ols[1]	154.7 ± 2.6	
Flavan-3-ols dimers		
Procyanidin B1	54.4 ± 1.1	25.6 ± 0.3
Procyanidin B2	39.5 ± 0.8	18.7 ± 0.1
Procyanidin B3	10.8 ± 0.8	5.1 ± 0.2
Procyanidin B4	14.3 ± 1.3	6.7 ± 0.5
Total galloylated dimers	49.3 ± 0.8	18.3 ± 0.2
Total unknown dimers	54.6 ± 0.7	25.7 ± 0.2
Total flavan-3-ols dimers[2]	212.6 ± 2.1	
Flavan-3-ols trimers		
Procyanidin C1	12.3 ± 0.8	83.9 ± 0.9
Total unknown trimers	2.4 ± 0.2	16.1 ± 0.9
Total flavan-3-ols trimers[3]	14.7 ± 0.9	
Proanthocyanidins		
Total proanthocyanidins[4]	234.5 ± 3.1	
Mean degree of polymerization (mDP)	4.5 ± 0.2	
% Galloylation	7.9 ± 0.2	
% Prodelphynidins	0.5 ± 0.0	
Phenolic acids		
Gallic acid	6.6 ± 0.1	
Spectrophotometric determinations		
Total polyphenol content	761.8 ± 20.6	
Condensed tannins	655.2 ± 72.6	

[1]As (+)-catechin equivalents. [2]As procyanidin B1 equivalents. [3]As procyanidin C1 equivalents. [4]As (+)-catechin equivalents. Results are expressed as mean ± SD (n=3)

Phenolic characterization of the chow diet

Diet formulation described by the fabricant indicate the presence of wheat middlings, ground wheat, ground corn, corn gluten meal and soybean oil as main ingredients. Thus, we proceed to analyse the phenolic composition of the diet following the method of extraction described by Bueno-Herrera & Pérez-Magariño, 2020 employed in wheat phenolic characterization.

Briefly, soluble free polyphenols were extracted three times by solid-liquid extraction from 1 g of sample, the first two with 8 mL of methanol/ 1 M HCl (85:15, v/v) and the third with 8 mL of aqueous acetone (70%), all shacked at room temperature for 30 min at 250 rpm using an orbital shaker (Thermo Scientific MaxQ400, SHKE4000-8CE, Dubuque, IA, USA). The supernatants obtained from the three fractions were pooled, centrifuged at 4000 g, 4°C for 15 min (Beckman Coulter Avanti J-26 XP, Brea, CA, USA) and filtered through 0.20 µm cellulose filters. In order to precipitate and eliminate proteins, the extracts were kept in the freezer at -20ºC for approximately 48 h, after that the extracts were centrifuged and filtered in the same conditions previous described. To determine the phenolic composition by HPLC, 3 mL of extract was concentrated to 0.5 mL using a rotary evaporator at 35ºC to eliminate the organic solvent and 0.5 of solvent A ($CH_3CN/H_2O/HCOOH$, 3:88.5:8.5, v/v/v) was added. Then, the extracts were filtered in 0.20 µm regenerated cellulose filters previous injection in HPLC.

After soluble free fraction preparation, the residue of the chow diet was submitted to alkaline hydrolysis to extract the insoluble bound phenolic compounds. Thus, the residue was digested with 10 mL of 2 M sodium hydroxide at room temperature for 16 h by shaking in orbital shaker at 250 rpm. After that, the solution was acidified with 2 M hydrochloric acid to pH 2.0 and extracted three times with 15 mL of ethyl acetate/ diethyl ether (1:1, v/v) by manual shaking. After each extraction, the mixture was centrifuged at 4000 g, 4°C for 15 min to separate the phases. The three organic fractions were pooled, and 20 mL of the organic phase was evaporated to dryness under vacuum at 35°C using a rotary evaporator. The residue was dissolved in 1.5 mL of solvent A ($CH_3CN/H_2O/HCOOH$, 3:88.5:8.5, v/v/v) and filtered through 0.20 µm cellulose filters previous analysis by HPLC.

Chromatographic identification and quantification of polyphenols presented in both fractions were analysed with an Agilent 1100 Series system (Agilent, Waldbronn, Germany) equipped with a DAD (G1315B) and LC/MSD Trap VL (G2445C VL) ESI-MS/MS system following the chromatographic conditions reported by Pérez-Navarro et al., 2019. The chromatographic separation was carried out on a reversed-phased column ZORBAX Eclipse XDB-C18 (2.1 x 150 mm, 3.5 µm particle, Agilent USA, Santa Clara, CA, USA), thermostated at 40°C with a flow rate of 0.19 mL/min. The composition of the mobile phases was: $CH_3CN/H_2O/HCOOH$ (3:88.5:8.5, v/v/v, solvent A), $CH_3CN/H_2O/HCOOH$ (50:41.5:8.5, v/v/v, solvent B) and $CH_3OH/H_2O/HCOOH$ (90:1.5:8.5, v/v/v, solvent C). The gradient was (time, % solvents B and C): zero min, 4% B; 8 min, 4% B; 37 min, 17% B and 13% C; 51 min, 30% B and 20% C; 51.5 min, 40% B and 30% C; 56 min, 50% B and 50% C; 57 min, 50% B and 50% C; and 64 min, 4% B. Mass spectrometry data were acquired in negative ionization mode with the following parameters: scan range, 100-1000 m/z; dry gas, N_2, 8 L/min; nebulizer, 40 psi; capillary,

3500 V; drying temperature, 350ºC ; skimmer 1, -20 V; skimmer 2, -60 V. The injection volume was 20 µL for the soluble free fraction and 5µL for the insoluble bound phenolic extracts. Polyphenol identification was according to UV-Vis and MS/MS spectra obtained from commercial standards of caffeic acid, *p*-coumaric acid and apigenin from Sigma-Aldrich (Tres Cantos, Madrid, Spain), ferulic acid from Fluka (Sigma-Aldrich, Milan, Italy) and daidzin and genistin from MedChemExpress (Monmouth Junction, NJ, USA) whereas the rest of polyphenols were tentatively identified with data reported in the literature. Quantification was performed using DAD-chromatograms extracted at 320 nm for hydroxycinnamic acids and flavone derivatives and 280 nm for isoflavones, using calibration curves of different commercial standards depending on the compound (see **supplemental Table 5**).

The results of the spectrophotometric determination of the total phenolic content showed a concentration of 2385 ± 220 µg gallic acid equivalents/g of diet of soluble free phenolics and 3159 ± 504 µg gallic acid equivalents/g of diet of insoluble bound phenolic.

The chromatographic characterization of the diet reveals the presence of hydroxycinnamic acids, isoflavones and apigenin derivatives, as shows the **supplementary Table 5**. Particularly, ferulic acid and dehydrodiferulic acids presented in the insoluble bound fraction were the most relevant polyphenols found in diet, being the ferulic acid the principal polyphenol quantified in chow diet (1691 ± 301 µg/g). Other hydroxycinnamic acids were quantified in the soluble free fraction, such as caffeic acid and *p*-coumaric acid, as well as concentrations of isoflavones and apigenin derivatives. The presence of these polyphenols may explain the presence of phenolic metabolites in control rat serum samples used in this study, however the contribution of chow diet polyphenols to the pool of polyphenols provided to the animals did not avoid the differentiation between groups of treatment.

References:

Bueno-Herrera, M., & Pérez-Magariño, S. (2020). Validation of an extraction method for the quantification of soluble free and insoluble bound phenolic compounds in wheat by HPLC-DAD. *Journal of Cereal Science*, *93*(December 2019). https://doi.org/10.1016/j.jcs.2020.102984

Pérez-Navarro, J., Izquierdo-Cañas, P. M., Mena-Morales, A., Martínez-Gascueña, J., Chacón-Vozmediano, J. L., García-Romero, E., … Gómez-Alonso, S. (2019). Phenolic compounds profile of different berry parts from novel Vitis vinifera L. red grape genotypes and Tempranillo using HPLC-DAD-ESI-MS/MS: A varietal differentiation tool. *Food Chemistry*, *295*(January), 350–360. https://doi.org/10.1016/j.foodchem.2019.05.137

Supplemental Table 5. Phenolic composition of chow rat diet determined by spectrophotometric and chromatographic analysis (HPLC-MS-DAD)

Phenolic compounds	Soluble free fraction concentration (µg/g diet)	Insoluble bound fraction concentration (µg/g diet)	Quantification wavelength (nm)	Calibration compound
Caffeic acid	1.3 ± 0.1	N.D.	320	Caffeic acid
p-coumaric acid	N.D.	73.0 ± 7.5	320	*p*-coumaric acid
Ferulic acid	N.D.	1691 ± 301	320	Ferulic acid
Daidzin	10.5 ± 1.0	N.D.	280	Daidzin
Api-6-C-ara-8-C-hex	12.1 ± 1.0	N.D.	320	Apigenin
Api-6-C-ara-8-C-hex	17.4 ± 0.7	N.D.	320	Apigenin
DFA	ND	12.7 ± 2.1	320	Ferulic acid
Api-6-C-ara-8-C-hex	4.0 ± 0.2	N.D.	320	Apigenin
Api-6-C-ara-8-C-hex	79.8 ± 6.1	N.D.	320	Apigenin
Genistin	15.6 ± 0.9	N.D.	280	Genistin
DFA	ND	50.4 ± 4.2	320	Ferulic acid
Api-6-C-β-gal-8-C-β-gluc	12.2 ± 0.9	N.D.	320	Apigenin
Api-6-C-β-gal-8-C-β-gluc	46.7 ± 10.0	N.D.	320	Apigenin
Daidzein	8.7 ± 0.4	N.D.	280	Daidzine
Dicoumaroyl spermidine	17.3 ± 1.4	N.D.	320	*p*-coumaric acid
Genistein	9.3 ± 0.5	N.D.	280	Genistin
DFA	N.D.	68.2 ± 4.8	320	Ferulic acid
DFA	N.D.	76.6 ± 6.6	320	Ferulic acid
DFA	N.D.	45.3 ± 7.6	320	Ferulic acid
Σ Api-6-C-ara-8-C-hex	112.0 ± 6.0	N.D.		
Σ Api-6-C-β-gal-8-C-β-gluc	58.9 ± 12.9	N.D.		
Σ DFA	N.D.	251.6 ± 17.3		
Σ Isoflavones	44.1 ± 1.1	N.D.		
Total phenols	211.6 ± 9.7	2016 ± 303		

Results are expressed as mean ± standard deviation (n=3). DFA: dehydrodiferulic acid; Api-6-C-ara-8-C-hex: Apigenin-6-C-arabinose-8-C-hexoside; Api-6-C-β-gal-8-C-β-gluc: Apigenin-6-C-β-galactosyl-8-C-glucosyl-O-glucopyranoside.