Supplementary Methods

1. Chemical and Reagents for the chromatographic analysis

Standards of *p*-hydroxybenzoic acid, protocatechuic acid, *p*-coumaric acid, syringic acid, chlorogenic acid, (+)-catechin, (-)-epicatechin were purchased from Sigma-Aldrich (St Louis, MO, USA), and vanillic acid from Fluka (Buchs, Switzerland). Dimer B_2, apigenin-7-*O*-glucoside, luteolin-7-*O*-glucoside, naringenin, eriodictyol, quercetin, quercetin-3-O-glucoside, and kaempfeorl-3-O-rutinoside were purchased from Extrasynthese (Genay, France). The standards were prepared in methanol and a stock standard solutions was dissolved in acetone/Milli-Q water/acetic acid (70/29.5/0.5, v/v/v).

Acetone and glacial acetic acid were HPLC grade and were provided by Scharlau Chemie (Barcelona, Spain). Acetonitrile (HPLC grade) was from Romyl (Teknokroma Barcelona, Spain). Water was of Milli-Q quality (Millipore Corp., Bedford, MA, USA).

2. Liquid chromatography (UPLC-ESI-MS/MS) conditions

The phenolic compounds were analyzed by AcQuity Ultra-Performance™ liquid chromatography (UPLC) coupled to a triple quadrupole detector (TQD) mass spectrometer (Waters, Milford). The analytical column was an AcQuity BEH C_{18} column (100 mm × 2.1 mm i.d., 1.7 µm,) equipped with a VanGuard™ Pre-Column AcQuity BEH C_{18} (2.1 × 5 mm, 1.7 µm), also from Waters. During the analysis, the column was kept at 30 °C and the flow rate was 0.3 mL min−1. The mobile phase was 0.2% acetic acid (eluent A), and acetonitrile (eluent B). The elution gradient was 0–5 min, 5–10% B; 5–10 min, 10–12.4% B; 10–18 min, 12.4–28% B; 18–23 min, 28–100% B; 23–25.5 min, 100% B isocratic; 25.5–27 min, 100–5% B; and 27–30 min, 5% B isocratic.

Tandem MS analyses were carried out on a triple quadrupole detector (TQD) mass spectrometer (Waters, Milford, MA, USA) equipped with a Z-spray electrospray interface (ESI). Ionization was achieved using the electrospray interface operating in the negative mode $[M-H]^-$ and the data

were acquired through selected reaction monitoring (SRM). The ionization source parameters were capillary voltage 3 kV, source temperature 150 ◦C and desolvation gas temperature 400 ◦C, with a flow-rate of 800 l/h. Nitrogen (99.99% purity, N2 LCMS nitrogen generator, Claind, Como, Italy) and argon (≥99.99% purity, Aphagaz, Madrid, Spain) were used as cone and collision gases, respectively. The SRM transitions and the individual cone voltage and collision energy for each phenolic compound were evaluated by infusing 10 mg/l of each compound in order to obtain the best instrumental conditions. Two SRM transitions were selected, the most sensitive transition was used for quantification, and a second one for confirmation purposes. The SRM transition for quantification, as well as the individual cone voltage and collision energy for each phenolic compound are shown in Supplementary Table 1. The dwell time established for each transition was 30 ms. Data acquisition was carried out with the MassLynx 4.1 software.

The phenolic compounds were quantified by using own calibration curve. An exception of (epi)catechin glucoside, which was tentatively quantified by using the calibration curve of (epi)catechin; trimer and tetramer by using the calibration curve of dimer B_2; protocatechuic acid glucoside by using the calibratoun curve of protocatechuic acid; apigenin-*C*-glucoside, apigenin-*C*-glucoside-(*C*-arabinoside), and apigenin-di*C*-glucoside by using the calibratoun curve of apigenin-7-*O*-glucoside; naringenin glucoside by using the calibration curve of naringenin; eriodictyol glucoside by using the calibration curve of eriodictyol; quercetin arabinoside by using the calibration curve of quercetin-3-*O*-glucoside; dihydroquercetin by using the calibration curve of quercetin; and clovamide by using the calibration curve of epicatehin.

Supplementary Tables

Supplementary Table 1. Optimized RM conditions for the quantification of the studied phenolic compounds by UPLC-MS/MS.

Compound	SRM quantification	Cone voltage (V)	Collision energy (eV)
p-Hydroxybenzoic acid	137 > 93	40	15
Protocatechuic acid	153 > 109	40	15
Protocatechuic acid glucoside	315 > 153	40	15
p-Coumaric acid	163 > 119	40	10
Vanillic acid	167 > 152	40	15
Syringic acid	197 > 182	30	15
Chlorogenic acid	353 > 191	40	15
Catechin	289 > 245	40	15
Epicatechin	289 > 245	40	15
Catechin glucoside	451 > 289	40	20
Epicatechin glucoside	451 > 289	40	20
Epigallocatechin	305 > 125	40	15
Dimer	577 > 289	40	20
Trimer	865 > 287	60	20
Tetramer	1153 > 865	40	20
Apigenin-7-*O*-glucoside	431 > 268	40	15
Apigenin-*C*-glucoside	431 > 311	40	15
Apigenin-*C*-glucoside-*C*-arabinoside	563 > 353	40	30
Apigenin-di*C*-glucoside	593 > 353	40	30
Luteolin-7-*O*-glucoside	447 > 285	40	15
Naringenin	271 > 151	40	15
Naringenin glucoside	433 > 271	40	10
Eriodictyol	287 > 151	40	15
Eriodictyol glucoside	449 > 287	40	15
Quercetin	301 > 151	40	15
Dihydroquercetin	303 > 285	40	10
Quercetin arabinoside	433 > 300	40	20
Quercetin glucoside	463 > 300	40	25
Kaempferol rutinoside	593 > 285	40	25
Clovamide	358 > 178	40	15

Supplementary Table 2. Specific gene expression probes used for the quantitative real time PCR analyses.

Gene	Description	TaqMan™ Gene Expression Assay
Acox1	Acyl-Coenzyme A oxidase 1, palmitoyl	Rn01460628_m1
Slc27a4	Solute carrier family 27 member 4	Rn01438951_m1
Adipoq	Adiponectin, C1Q and collagen domain containing	Rn00595250_m1
Cebpa	CCAAT/enhancer binding protein (C/EBP), alpha	Rn00560963_s1
Fasn	Fatty acid synthase	Rn00569117_m1
Lep	Leptin	Rn00565158_m1
Pparg	Peroxisome proliferator activated receptor gamma	Rn00440945_m1
Srebf1	Sterol regulatory element binding transcription factor 1	Rn01495769_m1
Tbp	TATA box binding protein. Housekeeping gene control	Rn01455646_m1

Supplementary table 3. RQ-PCR analysis data obtained in retroperitoneal fat samples from HFS, HFS-CCL and HFS-CCH groups. Results are expressed as the fold-difference gene expression levels of each gene in the HFS-CCL and HFS-CCH groups compared with the HFS, calculated with the $2^{-\Delta\Delta Ct}$ method.

	Acox1	*Slc27a4*	*Adipoq*	*Cebpa*	*Fasn*	*Lep*	*Pparg*	*Srebf1*
Anova	ns	ns	0.016	<0.001	0.017	ns	ns	ns
HFS (n=12)	1.00 ± 0.16	1.00 ± 0.64	1.00 ± 0.06 [a]	1.00 ± 0.11 [a]	1.00 ± 0.14 [a]	1.00 ± 0.11	1.00 ± 0.19	1.00 ± 0.18
HFS-CCL (n=11)	0.79 ± 0.09	0.91 ± 0.26	0.77 ± 0.04 [b]	0.43 ± 0.04 [b]	0.59 ± 0.06 [b]	0.89 ± 0.09	0.99 ± 0.13	0.98 ± 0.09
HFS-CCH (n=12)	0.84 ± 0.07	0.92 ± 0.19	0.70 ± 0.02 [b]	0.48 ± 0.04 [b]	0.70 ± 0.09 [ab]	0.92 ± 0.11	1.03 ± 0.22	0.96 ± 0.08

[a] All the results are expressed as the mean ± SD.

ΔΔCt values are calculated as the difference between dCt HFS – dCt HFS-CCL or CCH, for each gene.

dCt values are obtained from the difference of each gene problem with the housekeeping gene (*Tbp*, *TATA box binding protein*).

Statistical analyses were performed using the ANOVA test followed by Student–Newman–Keuls (SNK) test to detect differences between groups. Superscript letters a and b designate groups in which statistically significant differences were observed.

HFS, high-fat-sucrose; CCL, cocoa low dose; CCH, cocoa high dose

Acox1, Acyl-Coenzyme A oxidase 1, palmitoyl ; Slc27a4, Solute carrier family 27 member 4; *Adipoq*, Adiponectin; *Cebpa*, CCAAT/enhancer binding protein, alpha; *Fasn*, Fatty acid synthase; *Lep*, Leptin; *Pparg*, Peroxisome proliferator activated receptor gamma; *Srebf1*, Sterol regulatory element binding transcription factor 1

Supplementary Table 4. Absolute and relative mean organ weights of rats after a dosing period of 60 days.

	Control	CC Low dose (125 mg/kg)	CC Medium dose (250 mg/kg)	CC High dose (500 mg/kg)
Spleen				
Absolute weight (g)	0,618 ± 0,064	0,605 ± 0,050	0,631 ± 0,040	0,608 ± 0,076
Relative to body weight (%)	0,173 ± 0,016	0,160 ± 0,011	0,176 ± 0,009	0,158 ± 0,018
Heart				
Absolute weight (g)	1,050 ± 0,076	1,038 ± 0,110	1,061 ± 0,099	1,122 ± 0,092
Relative to body weight (%)	0,294 ± 0,029	0,274 ± 0,016	0,294 ± 0,014	0,292 ± 0,013
Liver				
Absolute weight (g)	9,958 ± 0,785	10,709 ± 0,928	9,590 ± 0,952	9,987 ± 1,127
Relative to body weight (%)	2,782 ± 0,160	2,832 ± 0,111	2,658 ± 0,116	2,594 ± 0,202
Thymus				
Absolute weight (g)	0,382 ± 0,041	0,383 ± 0,051	0,434 ± 0,077	0,440 ± 0,063
Relative to body weight (%)	0,107 ± 0,011	0,101 ± 0,011	0,120 ± 0,016	0,115 ± 0,017
Kidney				
Absolute weight (g)	1,017 ± 0,237	0,998 ± 0,057	1,008 ± 0,094	0,966 ± 0,134
Relative to body weight (%)	0,267 ± 0,024	0,264 ± 0,012	0,279 ± 0,013	0,251 ± 0,032
Adrenal				
Absolute weight (g)	0,055 ± 0,010	0,066 ± 0,020	0,047 ± 0,014	0,060 ± 0,024
Relative to body weight (%)	0,015 ± 0,002	0,017 ± 0,005	0,013 ± 0,003	0,015 ± 0,006
Testicle				
Absolute weight (g)	1,817 ± 0,115	1,817 ± 0,068	1,813 ± 0,188	1,844 ± 0,067
Relative to body weight (%)	0,509 ± 0,042	0,482 ± 0,022	0,503 ± 0,029	0,481 ± 0,019

Data are mean ± SD.

Supplementary Table 5. Clinical biochemistry of rats at day 60 after the starting dosing period.

Day 60	Control	CC Low dose (125 mg/kg)	CC Medium dose (250 mg/kg)	CC High dose (500 mg/kg)
Glucose (mg/dL)	150 ± 40	162 ± 33	142 ± 24	143 ± 43
Cholesterol (mg/dL)	82 ± 9	80 ± 10	91 ± 20	77 ± 6
Albumin (g/dL)	4,1 ± 0,2	4,1 ± 0,1	4,2 ± 0,1	4,0 ± 0,1
Urea (mg/dL)	51 ± 7	48 ± 9	47 ± 8	52 ± 5
Aspartate Aminotransferase (U/L)	76 ± 8	89 ± 10	105 ± 43	94 ± 32
Alanine Aminotransferase (U/L)	48 ± 9	43 ± 6	54 ± 13	58 ± 23
Total Bilirubin (mg/dL)	0,12 ± 0,04	0,11 ± 0,02	0,11 ± 0,01	0,15 ± 0,05
Calcium (mg/dL)	10,07 ± 0,17	10,35 ± 0,12 (*)	10,25 ± 0,16	9,84 ± 0,11
Creatinine (mg/dL)	0,48 ± 0,06	0,46 ± 0,06	0,46 ± 0,08	0,48 ± 0,09
Total Protein (g/dL)	6,2 ± 0,3	6,3 ± 0,2	6,3 ± 0,1	6,2 ± 0,1
Sodium (mmol/L)	144 ± 5	139 ± 1 (**)	144 ± 4	144 ± 5
Potasium (mmol/L)	4,07 ± 0,24	4,28 ± 0,17	4,12 ± 0,30	4,36 ± 0,20
Clorine (mmol/L)	95 ± 1	95 ± 1	95 ± 1	95 ± 1

Data are mean ± SD. In bold, values that were significantly different (Mann-Whitney U Test) from the control group (* $p < 0.05$, ** $p < 0.01$).

Supplementary table 6. Haematological profile of rats at day 60 after the starting dosing period.

	Control	CC Low dose (125 mg/kg)	CC Medium dose (250 mg/kg)	CC High dose (500 mg/kg)
RBC (x10^6 cel/mL)	9,87 ± 0,36	9,08 ± 1,23	9,79 ± 0,49	9,82 ± 0,09
WBC (x10^3 cel/mL)	8,78 ± 1,83	8,52 ± 2,03	8,32 ± 1,01	8,47 ± 1,90
Haemoglobin (g/dL)	16,8 ± 1,1	15,9 ± 2,3	17,4 ± 0,7	17,0 ± 0,8
Haematocrit (%)	48,5 ± 2,1	45,1 ±6,1	48,9 ± 1,8	47,2 ± 2,0
MCV (fl)	49,2 ± 0,5	49,6 ± 1,1	50,0 ± 3,2	48,1 ± 1,9
MCH (pg)	17,1 ± 1,3	17,5 ± 0,4	17,8 ± 1,0	17,3 ± 0,7
MCHC (g/dL)	34,7 ± 2,6	35,3 ± 0,5	35,6 ± 0,5	36,1 ± 0,2
Platelets (x10^3 cel/mL)	743 ± 296	695 ± 273	787 ± 158	722 ± 176
Eosinophils (%)	2,2 ± 1,5	1,7 ± 0,7	1,1 ± 0,2 (*)	1,8 ± 0,8
Neutrophils (%)	16,6 ± 1,3	16,0 ± 2,7	18,9 ± 3,8	16,2 ± 2,3
Lymphocytes (%)	78,2 ± 3,3	78,5 ± 1,5	76,7 ± 3,9	78,7 ± 2,2
Monocytes (%)	3,0 ± 1,4	3,7 ± 0,8	3,3 ± 0,8	3,3 ± 0,6

Data are mean ± SD.
RBC, red blood cells; WBC, white blood cells; MCV, mean corpuscular volume; MCH, mean cell haemoglobin; MCHC, mean corpuscular haemoglobin concentration.
In bold, values that were significantly different (Mann-Whitney U Test) from the control group (* $p < 0.05$, ** $p < 0.01$).