# Seasonal consumption of polyphenol-rich fruits affects the hypothalamic leptin signaling system in a photoperiod-dependent mode

Maria Ibars, Gerard Aragonès, Andrea Ardid-Ruiz, Albert Gibert-Ramos, Anna Arola-Arnal, Manuel Suárez & Cinta Bladé

**Supplementary Table S1.**
Nutrients present in raw Sweet Cherry (*Prunus avium* L.)

| Nutrient | Unit | Value per 100 g |
|---|:---:|:---:|
| **Proximates** | | |
| Water | g | 82.25 |
| Energy | kcal | 63 |
| Energy | kJ | 263 |
| Protein | g | 1.06 |
| Total lipid (fat) | g | 0.2 |
| Ash | g | 0.48 |
| Carbohydrate, by difference | g | 16.01 |
| Fiber, total dietary | g | 2.1 |
| Sugars, total | g | 12.82 |
| Sucrose | g | 0.15 |
| Glucose (dextrose) | g | 6.59 |
| Fructose | g | 5.37 |
| Maltose | g | 0.12 |
| Galactose | g | 0.59 |
| **Minerals** | | |
| Calcium, Ca | mg | 13 |
| Iron, Fe | mg | 0.36 |
| Magnesium, Mg | mg | 11 |
| Phosphorus, P | mg | 21 |
| Potassium, K | mg | 222 |
| Zinc, Zn | mg | 0.07 |
| Copper, Cu | mg | 0.06 |
| Manganese, Mn | mg | 0.07 |
| Fluoride, F | µg | 2 |
| **Vitamins** | | |
| Vitamin C, total ascorbic acid | mg | 7 |
| Thiamin | mg | 0.027 |
| Riboflavin | mg | 0.033 |
| Niacin | mg | 0.154 |
| Pantothenic acid | mg | 0.199 |
| Vitamin B-6 | mg | 0.049 |
| Folate, total | µg | 4 |

| | | |
|---|---|---|
| Folate, food | µg | 4 |
| Folate, DFE | µg | 4 |
| Choline, total | mg | 6.1 |
| Vitamin A | µg | 3 |
| Carotene, beta | µg | 38 |
| Lutein + zeaxanthin | µg | 85 |
| Vitamin E | mg | 0.07 |
| Tocopherol, beta | mg | 0.01 |
| Tocopherol, gamma | mg | 0.04 |
| Vitamin K (phylloquinone) | µg | 2.1 |

**Lipids**

| | | |
|---|---|---|
| Fatty acids, total saturated | g | 0.038 |
| 14:00 | g | 0.001 |
| 16:00 | g | 0.027 |
| 18:00 | g | 0.009 |
| Fatty acids, total monounsaturated | g | 0.047 |
| 16:1 undifferentiated | g | 0.001 |
| 18:1 undifferentiated | g | 0.047 |
| Fatty acids, total polyunsaturated | g | 0.052 |
| 18:2 undifferentiated | g | 0.027 |
| 18:3 undifferentiated | g | 0.026 |
| Phytosterols | mg | 12 |

**Amino Acids**

| | | |
|---|---|---|
| Tryptophan | g | 0.009 |
| Threonine | g | 0.022 |
| Isoleucine | g | 0.02 |
| Leucine | g | 0.03 |
| Lysine | g | 0.032 |
| Methionine | g | 0.01 |
| Cystine | g | 0.01 |
| Phenylalanine | g | 0.024 |
| Tyrosine | g | 0.014 |
| Valine | g | 0.024 |
| Arginine | g | 0.018 |
| Histidine | g | 0.015 |
| Alanine | g | 0.026 |
| Aspartic acid | g | 0.569 |
| Glutamic acid | g | 0.083 |
| Glycine | g | 0.023 |
| Proline | g | 0.039 |
| Serine | g | 0.03 |

**Supplementary Table S2.**
Polyphenols present in raw Sweet Cherry (*Prunus avium* L.)

| Polyphenol Type | Unit | Value per 100 g |
|---|---|---|
| Total polyphenols | mg | 174.90 |
| **Flavonoids** | | |
| **Anthocyanins** | | |
| Cyanidin 3-O-glucoside | mg | 18.73 |
| Cyanidin 3-O-rutinoside | mg | 143.27 |
| Pelargonidin 3-O-rutinoside | mg | 1.24 |
| Peonidin 3-O-glucoside | mg | 0.76 |
| Peonidin 3-O-rutinoside | mg | 7.42 |
| **Flavanols** | | |
| (+)-Catechin | mg | 1.5 |
| (-)-Epicatechin | mg | 7.78 |
| (-)-Epicatechin 3-O-gallate | mg | 0.09 |
| (-)-Epigallocatechin | mg | 0.05 |
| Procyanidin dimer B1 | mg | 0.23 |
| Procyanidin dimer B2 | mg | 2.1 |
| Procyanidin dimer B3 | mg | 0.08 |
| Procyanidin dimer B4 | mg | 0.18 |
| Procyanidin dimer B5 | mg | 0.2 |
| Procyanidin dimer B7 | mg | 1.01 |
| Procyanidin trimer C1 | mg | 1.85 |
| **Phenolic acids** | | |
| **Hydroxycinnamic acids** | | |
| 3-Caffeoylquinic acid | mg | 44.71 |
| 3-Feruloylquinic acid | mg | 0.43 |
| 3-p-Coumaroylquinic acid | mg | 38.43 |
| 4-Caffeoylquinic acid | mg | 0.77 |
| 4-p-Coumaroylquinic acid | mg | 1.27 |
| 5-Caffeoylquinic acid | mg | 2.2 |

*Phenol-Explorer, database on polyphenol content in foods, software version 3.6, 2015.*

**Supplementary Table S3.**

Nutrients present in raw (black) Grape (*Vitis vinifera* L.)

| Nutrient | Unit | Value per 100 g |
|---|---|---|
| **Proximates** | | |
| Water | g | 80.54 |
| Energy | kcal | 69 |
| Protein | g | 0.72 |
| Total lipid (fat) | g | 0.16 |
| Carbohydrate, by difference | g | 18.1 |
| Fiber, total dietary | g | 0.9 |
| Sugars, total | g | 15.48 |
| **Minerals** | | |
| Calcium, Ca | mg | 10 |
| Iron, Fe | mg | 0.36 |
| Magnesium, Mg | mg | 7 |
| Phosphorus, P | mg | 20 |
| Potassium, K | mg | 191 |
| Sodium, Na | mg | 2 |
| Zinc, Zn | mg | 0.07 |
| **Vitamins** | | |
| Vitamin C, total ascorbic acid | mg | 3.2 |
| Thiamin | mg | 0.069 |
| Riboflavin | mg | 0.07 |
| Niacin | mg | 0.188 |
| Vitamin B-6 | mg | 0.086 |
| Folate, DFE | µg | 2 |
| Vitamin A, | µg | 3 |
| Vitamin E (alpha-tocopherol) | mg | 0.19 |
| Vitamin K (phylloquinone) | µg | 14.6 |
| **Lipids** | | |
| Fatty acids, total saturated | g | 0.054 |
| Fatty acids, total monounsaturated | g | 0.007 |
| Fatty acids, total polyunsaturated | g | 0.048 |

*USDA National Nutrient Database for Standard Reference, software version 3.9.2 2018-04-30*

**Supplementary Table S4.**
Polyphenols present in raw (black) Grape (*Vitis vinifera* L.)

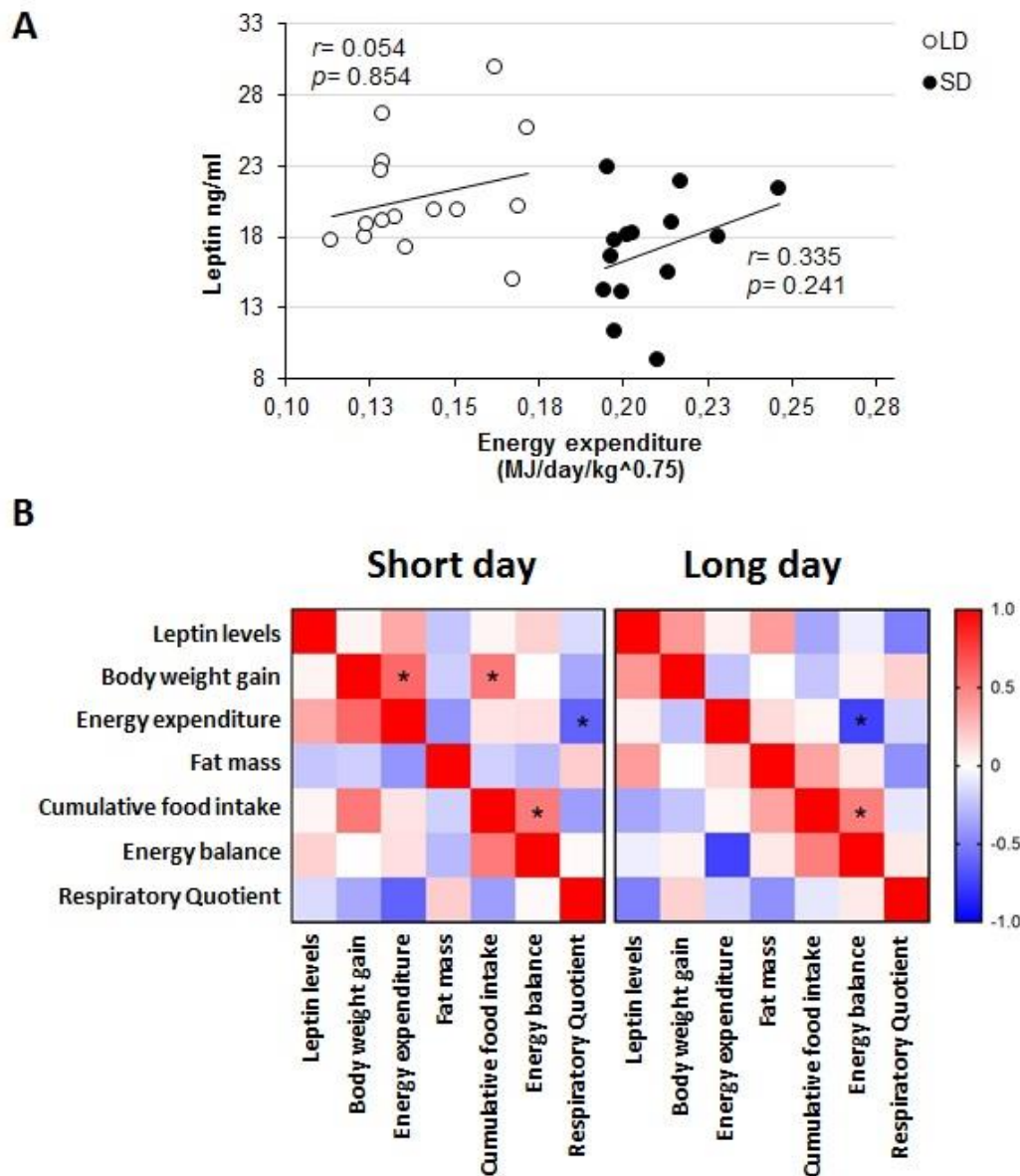| Polyphenols | Unit | Value per 100 g |
|---|---|---|
| Total polyphenols | mg | 174.90 |
| **Flavonoids** | | |
| **Anthocyanins** | | |
| Cyanidin 3-O-(6"-p-coumaroyl-glucoside) | mg | 0.1 |
| Cyanidin 3-O-glucoside | mg | 1.08 |
| Delphinidin 3-O-(6"-acetyl-glucoside) | mg | 0.54 |
| Delphinidin 3-O-glucoside | mg | 2.63 |
| Malvidin 3-O-(6"-acetyl-glucoside) | mg | 9.66 |
| Malvidin 3-O-(6"-p-coumaroyl-glucoside) | mg | 9.91 |
| Malvidin 3-O-glucoside | mg | 39.23 |
| Peonidin 3-O-(6"-p-coumaroyl-glucoside) | mg | 0.34 |
| Peonidin 3-O-glucoside | mg | 5.8 |
| Petunidin 3-O-(6"-p-coumaroyl-glucoside) | mg | 0.05 |
| Petunidin 3-O-glucoside | mg | 2.76 |
| **Flavanols** | | |
| (+)-Catechin | mg | 5.46 |
| (-)-Epicatechin | mg | 5.24 |
| (-)-Epicatechin 3-O-gallate | mg | 1.68 |
| (-)-Epigallocatechin | mg | 0.03 |
| Procyanidin dimer B1 | mg | 0.43 |
| Procyanidin dimer B2 | mg | 0.36 |
| Procyanidin dimer B3 | mg | 0.12 |
| Procyanidin dimer B4 | mg | 0.33 |
| Procyanidin trimer C1 | mg | 0.38 |
| **Flavonols** | | |
| Quercetin 3-O-galactoside | mg | 0.93 |
| Quercetin 3-O-glucuronide | mg | 2.15 |
| **Phenolic acids** | | |
| **Hydroxycinnamic acids** | | |
| Caffeoyl tartaric acid | mg | 1.13 |
| p-Coumaroyl tartaric acid | mg | 0.56 |
| **Stilbenes** | | |
| **Stilbenes** | | |
| Piceatannol | mg | 5.20E-03 |
| Resveratrol | mg | 0.15 |
| Resveratrol 3-O-glucoside | mg | 0.03 |
| Trans-Resveratrol | mg | 0.15 |
| Trans-Resveratrol 3-O-glucoside | mg | 1.00E-02 |

*Phenol-Explorer, database on polyphenol content in foods, software version 3.6, 2015.*
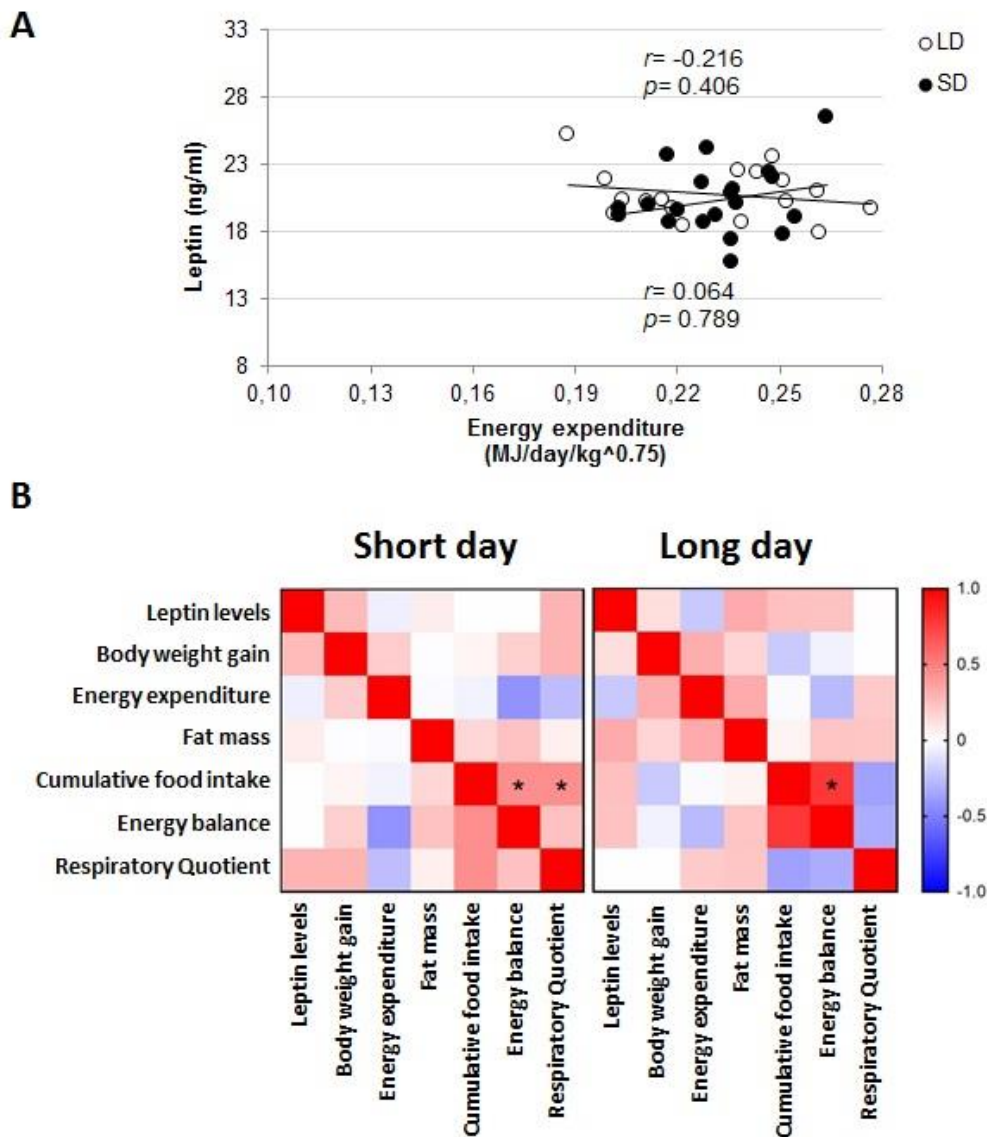
**Supplementary Table S5.**
Primer sequences used in qPCR amplification

| Gene | Sequence | Product size (bp) | GenBank accession no/reference |
|---|---|---|---|
| *Obrb* | Forward 5'-CCAGTACCCAGAGCCAAAGT<br>Reverse 5'-GGATCGGGCTTCACAACAAGC | 122 | NM_012596.1 |
| *Socs3* | Forward 5'-CTGGACCCATTCGGGAGTTC<br>Reverse n'-CTGGGAGCTACCGACCATTG | 148 | NM_053565.1 |
| *Ptp1b* | Forward 5'-CCCTTTTGACCACAGTCGGA<br>Reverse 5'-TTGGTAAAGGGCCCTGGGTG | 119 | NM_012637.2 |
| *Pomc* | Forward 5'-GAGGCGACGGAGGAGAAAAG<br>Reverse 5'-TGAGGCTCTGTCGCGGAAA | 98 | NM_139326.2 |
| *AgRP* | Forward 5'-GAGAACTCTGGGAACAGGGC<br>Reverse 5'-CAAGCAAAGGCCATGCTGAC | 140 | NM_033650.1 |
| *Npy* | Forward 5'-CTATCCCTGCTCGTGTGTTTGG<br>Reverse 5'-TGGTGATGAGATTGATGTAGTGTCG | 136 | Sun B et al. 2014 |
| *Mc4r* | Forward 5'-CAACTCCTTTGCAAGCTCCG<br>Reverse 5'-TCCAACCTCCTAGGTCAGGG | 129 | NM_013099.3 |
| *Npy1r* | Forward 5'-TCTTCTCTGCCCTTCGTGATC<br>Reverse 5'-TGAACGCCGCAAGTGATACA | 73 | NM_001113357.1 |
| *Ppia* | Forward 5'-CTTCGAGCTGTTTGCAGACAA<br>Reverse 5'-AAGTCACCACCCTGGCACATG | 138 | NM_017101.1 |
| *Rplp0* | Forward 5'-GGACCTCACCGAGATTAGGG<br>Reverse 5'-CCCACCTTGTCTCCAGTCTT | 225 | NM_022402.2 |

Obrb, leptin receptor isoform b; Socs3, suppressor of cytokine signaling 3; Ptp1b, protein tyrosine phosphate 1B; Pomc, proopiomelanocortin; Agrp, agouti-related protein; Npy, neuropeptide Y; Mc4r, melanocortin 4 receptor; Npy1r, neuropeptide Y receptor Y1; Ppia, peptidylprolyl isomerase A; Rplp0, ribosomal protein lateral stalk subunit P0.

**Supplementary Figure S1. Correlation analyses of metabolic variables in <u>non-obese animals</u>.** **(A)** Global correlation plot between 24 h energy expenditure and circulating leptin levels of all non-obese animals in relation to either long-day (LD) or short-day (SD). **(B)** Heatmaps showing the Pearson correlation (r) coefficients between serum leptin (ng/ml), body weight gain (g), 24 h energy expenditure (MJ/day/kg^0.75), fat mass %, cumulative food intake (MJ), 24 h energy balance (MJ) estimated by the difference between energy intake and energy expenditure and finally the 24 h respiratory quotient in relation to either long-day (LD) or short-day (SD). Strengh and direction of the association between two variables is represented by *r* values ranging from -1 to 0 for negatively associated and 0 to 1 for positively associated parameters, coloured in blue to red respectively. Values are presented as the mean ± SEM of six animals per group. *adjusted P-value ≤0.05 taking the variables on the Y axis as reference.

**Supplementary Figure S2. Correlation analyses of metabolic variables in <u>obese animals</u>. (A)** Global correlation plot between 24 h energy expenditure and circulating leptin levels of all obese animals in relation to either long-day (LD) or short-day (SD). **(B)** Heatmaps showing the Pearson correlation (r) coefficients between serum leptin (ng/ml), body weight gain (g), 24 h energy expenditure (MJ/day/kg^0.75), fat mass %, cumulative food intake (MJ), 24 h energy balance (MJ) estimated by the difference between energy intake and energy expenditure and finally the 24 h respiratory quotient in relation to either long-day (LD) or short-day (SD). Strengh and direction of the association between two variables is represented by *r* values ranging from -1 to 0 for negatively associated and 0 to 1 for positively associated parameters, coloured in blue to red respectively. Values are presented as the mean ± SEM of six animals per group. *adjusted P-value ≤0.05 taking the variables on the Y axis as reference.