**Effect of dark sweet cherry powder consumption on the gut microbiota, short-chain fatty acids, and biomarkers of gut health in obese db/db mice (SUPPLEMENTARY INFORMATION)**

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**Supplementary Figures**





**Supplementary Figure S1.** Cherry intake improved colon barrier measured through area of outer colon wall (continue line box) relative to total area colon wall (continue and dashed line boxes). (A) Representative photomicrographs taken from colon sections stained with H&E. Bar = 300 µm, 20X. Mice fed control diet (lean and obese) or cherry supplemented diet (10%). Photomicrographs were taken with Aperio CS2 digital pathology scanner (Leica Biosystems Inc. Buffalo Grove, IL). (B) Quantitative results of outer colon layer relative to total colon wall area. Photomicrographs were blinded analyzed with ImageJ software (http://rsb.info.nih.gov/ij/). Areas were measured along the colon tissue (10 or more measurements each picture) from different animals (n ≥ 5). Box plots represent median (line inside the box) and whiskers (min to max). Data was analyzed with Kruskal-Wallis test followed by Dunn’s multiple comparison test (p = 0.08) using GraphPad Prism 5.01 Software Inc.

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**Supplementary Figure S2.** Spearman’s correlation matrix of fecal bacteria versus end point biomarkers of intestinal health (mRNA levels of ATF4 and VCAM-1 in colonic mucosal cells). The direction of ellipses represents positive or negative correlations and the width of ellipses represents the strength of correlation (narrow ellipse = stronger correlation).

**Supplementary Tables**

**Supplementary Table S1.** Nutritional contents of cherry powder.

|  |  |  |
| --- | --- | --- |
| **Cherry powder** | | **Content (g/100 g powder)** |
| Protein | | 4.2% |
| Ash | | 4.8% |
| Moisture | | 3.6% |
| Total dietary fiber | | 5.1% |
| Maltodextrin | | 20% |
| Silicon dioxide | | 2% |
| Sugars | Fructose | 20.4% |
| Glucose | 35% |
| Lactose | < 0.1% |
| Maltose | 1.3% |
| Sucrose | < 0.1% |
| Total extractable phenolics (mg GAE/100 g) | | 629 ± 39 |
| Total bound non-extractable phenolics (mg GAE/100 g) | | 130 ± 3.9 |

Dark sweet cherries (Bing variety) were processed and freeze dried by Powder Pure (The Dalles, OR) to obtain cherry powder used for mice diet.

**Supplementary Table S2.** Targets, primers and references for all bacterial groups use in qPCR.

|  |  |  |
| --- | --- | --- |
| Target | Primer sequence (5’-3’) | Reference |
| Firmicutes | TGAAACTYAAAGGAATTGACG  ACCATGCACCACCTGTC | Bacchetti De Gregoris et al. (2011) |
| *Lactobacillus* spp. | AGCAGTAGGGAATCTTCCA  CACCGCTACACATGGAG | Walter et al. (2001), Heilig et al. (2002) |
| *Lactobacillus plantarum* | CTCTGGTATTGATTGGTGCTTGCAT  GTTCGCCACTCACTCAAATGTAAA | Matsuda et al. (2009) |
| *Lactobacillus acidophilus* | GCAGATCGCATGATCAGCTTATA  TCAGTCTCTCAACTCGGCTATG | Firmesse et al. (2008) |
| Ruminococcaceae | ACTGAGAGGTTGAACGGCCA  CCTTTACACCCAGTAAWTCCGGA | Garcia-Mazcorro et al. (2012) |
| *Faecalibacterium* | GAAGGCGGCCTACTGGGCAC  GTGCAGGCGAGTTGCAGCCT | Garcia-Mazcorro et al. (2012) |
| *Clostridium butyricum* | GTGCCGCCGCTAACGCATTAAGTAT  ACCATGCACCACCTGTCTTCCTGCC | Bartosch et al. (2004) |
| *Clostridium* cluster IV (*C. leptum* group) | GCACAAGCAGTGGAGT  CTTCCTCCGTTTTGTCAA | Matsuki et al. (2004) |
| *Eubacterium halii* | GCGTAGGTGGCAGTGCAA  GCACCGRAGCCTATACGG | Ramirez-Farias et al. (2009) |
| *Enterococcus* | CCCTTATTGTTAGTTGCCATCATT  ACTCGTTGTACTTCCCATTGT | Rinttilä et al. (2004) |
| *Turicibacter* | CAGACGGGGACAACGATTGGA  TACGCATCGTCGCCTTGGTA | Suchodolski et al. (2012) |
| CFB (Cytophaga-Flavobacterium-Bacteroides phylum) | CCGGAWTYATTGGGTTTAAAGGG  GGTAAGGTTCCTCGCGTA | Mühling et al. (2008) |
| Bacteroidetes | GGARCATGTGGTTTAATTCGATGAT  AGCTGACGACAACCATGCAG | Guo et al. (2008) |
| *Bacteroides/Prevotella* | GAGAGGAAGGTCCCCCAC  CGCTACTTGGCTGGTTCAG | Layton et al. (2006) |
| *Bacteroides* spp. | CGATGGATAGGGGTTCTGAGAGGA  GCTGGCACGGAGTTAGCCGA | Bergström et al. (2012) |
| *Bacteroides fragilis* | CTGAACCAGCCAAGTAGCG  CCGCAAACTTTCACAACTGACTTA | Liu et al. (2003) |
| *Bacteroides vulgatus* | GCATCATGAGTCCGCATGTTC  TCCATACCCGACTTTATTCCTT | Wang et al. (1996) |
| *Bacteroides thetaiotaomicron* | GGCAGCATTTCAGTTTGCTTG  GGTACATACAAAATTCCACACGT | Wang et al. (1996) |
| *Bacteroides eggerthi* | GTCATATTAACGGTGGCG  GGGTTBCCCCATTCGG | Liu et al. (2003) |
| *Parabacteroides distasonnis* | TGATCCCTTGTGCTGCT  ATCCCCCTCATTCGGA | Liu et al. (2003) |
| Betaproteobacteria | AACGCGAAAAACCTTACCTACC  TGCCCTTTCGTAGCAACTAGTG | Yang et al. (2015) |
| *Bifidobacterium* | GCGTGCTTAACACATGCAAGTC  CACCCGTTTCCAGGAGCTATT | Penders et al. (2005) |
| *Bifidobacterium adolescentes* | CTCCAGTTGGATGCATGTC  CGAAGGCTTGCTCCCAGT | Matsuki et al. (1998) |
| *Bifidobacterium breve* | AATGCCGGATGCTCCATCACAC  GCCTTGCTCCCTAACAAAAGAGG | Rinne et al. (2005) |
| Enterobacteriaceae | CATTGACGTTACCCGCAGAAGAAGC  CTCTACGAGACTCAAGCTTGC | Bartosch et al. (2004) |
| *E. coli* | CATGCCGCGTGTATGAAGAA  CGGGTAACGTCAATGAGCAAA | Huijsdens et al. (2002) |
| *Desulfovibrio* | CCGTAGATATCTGGAGGAACATCAG  CCGTAGATATCTGGAGGAACATCAG | Fite et al. (2004) |
| *Akkermansia muciniphila* | CAGCACGTGAAGGTGGGGAC  CCTTGCGGTTGGCTTCAGAT | Collado et al. (2007) |
| Deferribacteres | CTATTTCCAGTTGCTAACGG  GAGHTGCTTCCCTCTGATTATG | Yang et al. (2015) |
| Tenericutes | ATGTGTAGCGGTAAAATGCGTAA  CMTACTTGCGTACGTACTACT | Yang et al. (2015) |

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**Supplementary Table S3.** Primers used for mRNA analysis.

|  |  |  |
| --- | --- | --- |
| Target | Forward primer (5’ to 3’) | Reverse primer (5’ to 3’) |
| IL-1β | TCGCTCAGGGTCACAAGAAA | CATCAGAGGCAAGGAGGAAAAC |
| TNF-α | AAATGGGCTCCCTCTCATCAGTTC | TCTGCTTGGTGGTTTGCTACGAC |
| NF-kB | GGA TGG TGA GGT CAC TCT | TCC TGA ACT CCA GCA CTC TCT TC |
| ATF4\* | GAGCTTCCTGAACAGCGAAGTG | TGGCCACCTCCAGATAGTCATC |
| CHOP | CCTAGCTTGGCTGACAGAGG | CTGCTCCTTCTCCTTCATGC |
| PG | ATGAAGACCATTTACTTTG | CGGTTCCTCTTGGTGTTCATCAAC |
| ZO-1 | ACCCGAAACTGATGCTGTGGATAG | AAATGGCCGGGCAGAACTTGTGTA |
| Occ | ATGTCCGGCCGATGCTCTC | TTTGGCTGCTCTTGGGTCTGTAT |
| F4/80 | TGACAACCAGACGGCTTGTG | CAGGCGAGGAAAAGATAGTGT |
| MCP-1 | CAAGCAGAAGTGGGTTCAGGAT | TCTTCGGAGTTTGGGTTTGC |
| VCAM-1\* | GTCACGGTCAAGTGTTTGGC | AGATCCGGGGGAGATGTCAA |
| RPL19 | GAAGGTCAAAGGGAATGTGTTCA | CCTTGTCTGCCTTCAGCTTGT |

IL-1 β; interleukin-1β, TNF-α; tumor necrosis factor alpha, NF-*k*B; nuclear factor kappa B, ATF4; activating transcription factor 4, CHOP; CCAAT/enhancer binding protein homologous protein, PG; proglucagon, ZO-1; zonula occludens-1, Occ; occludin, F4/80; macrophage F4/80 receptor, MCP-1; monocyte chemoattractant protein-1, VCAM-1, vascular cell adhesion molecule 1, RPL19; ribosomal protein L19.

**Supplementary Table S4.** Parameters of host physiology and serum biomarkers. Medians and interquartile ranges are provided. Those parameters or biomarkers that showed statistical significant difference are boldface for better recognition.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter/biomarker | Obese controls | Obese cherry-supplemented | Lean controls | P value |
| **Body weight (g)** | **35.9a**  **(32.7-44.3) (n=10)** | **41.1a,b**  **(35.7-47.6) (n=12)** | **30.9c**  **(29.2-33.6) (n=10)** | **0.002** |
| **BMIs** | **4.4a**  **(4.1-4.8)**  **(n=10)** | **4.5a,b**  **(4.0-4.9)**  **(n=12)** | **3.3c**  **(3.1-3.5) (n=10)** | **<0.001** |
| **Weight cecum contents (mg)** | **191a**  **(104-234)** | **314a,b**  **(198-439)** | **128a,c**  **(93-152)** | **0.003** |
| Relative thickness of outer colon wall | 0.64 (0.6-0.7) | 0.73 (0.7-0.8) | 0.72 (0.6-0.7) | 0.08 |

Different letters state statistical significance difference.

**Supplementary Table S5.** mRNA levels of biomarkers involved in inflammation, cellular stress, and gut barrier function in colonic mucosal cells.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Genes | mRNA levels/RPL19 mRNA | Obese controls | Lean controls | Obese cherry-supplemented |
| Inflammation/ Cellular stress | IL-1β | 3.42  (1.0; 39.2) | 19.35  (5.6; 55.9 | 8.61  (2.5; 23.1) |
| TNF-α | 3.34  (1.2; 22.3) | 6.86 (3.1; 9.1) | 6.59  (1.1; 12.7) |
| NF-kB | 7.13  (1.6; 18.9) | 10.66  (6.4; 17.1) | 7.06  (4.1; 31.2) |
| ATF4\* | 4.10  (1.1; 8.2) | 5.85  (2.7; 10.2) | 3.33  (1.7; 6.2) |
| CHOP | 6.58  (1.0; 9.9) | 7.73  (2.5; 11.2) | 6.79  (2.2; 9.2) |
| Intestinal permeability and gut barrier function | PG | 8.07  (1.8; 28.4) | 5.2  (1.1; 14.6) | 7.45  (2.6; 21.2) |
| ZO-1 | 3.92  (3.1; 5.6) | 3.69  (1.7; 6.8) | 3.70  (1.1; 5.5) |
| Occ | 4.38  (1.1; 6.5) | 3.24  (2.6; 5.3) | 3.40  (2.1; 3.8) |
| Monocyte infiltration/Cell adhesion/Inflammation | F4/80 | 4.61  (1.4; 11.3) | 9.49  (6.4; 28.6) | 5.04  (2.6; 26.5) |
| MCP-1 | 6.84  (1.2; 28.5) | 7.8  (4.0; 16.4) | 8.7  (1.2; 27.1) |
| VCAM-1 \* | 6.42  (1.0; 32.0) | 8.51  (3.8; 12.0) | 3.98  (2.6; 7.0) |

ATF4: activating transcription factor 4, CHOP: CCAAT/enhancer binding protein homologous protein, IL-1β: interleukin 1β, Occ: occluding, PG: proglucagon, TNF- α: tumor necrosis factor- α; ZO-1, zonula occludens-1, F4/80: macrophage F4/80 receptor. Values of fold expression are median, range. \*Outlier detection: http://www.miniwebtool.com/outlier-calculator. Data are median (min, max). Data was analyzed with Kruskal-Wallis test, \*, p <0.05.

**Supplementary Table S6.** mRNA levels of biomarkers involved in inflammation, cellular stress, and gut barrier function in colon tissues.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Genes | mRNA levels/RPL19 mRNA | Obese controls | Lean controls | Obese cherry-supplemented |
| Inflammation/ Cellular stress | IL-1 | 65.81  (1.8; 295.3) | 86.89  (1.9; 154.6) | 141.90  (1.1; 309.7) |
| TNF-α | 150.10 (1.2; 384.2) | 253.70  (2.7; 326.9) | 318.90  (15.0; 382.1) |
| NF-κB | 46.86  (1.1; 192.0) | 57.27  (1.6; 88.6) | 93.31  (1.1; 142.2) |
| ATF4 | 9.82  (1.1; 33.3) | 12.57  (5.4; 17.5) | 17.12  (2.0; 29.5) |
| CHOP | 18.03  (2.2; 110.3) | 41.85  (1.2; 124.6) | 60.95  (1.6; 97.0) |
| IL-8 | 85.41  (1.6; 240.6) | 107.90  (3.9; 177.3) | 178.50  (70.3; 256.2) |
| Intestinal permeability and gut barrier function | OCC | 45.18  (1.4; 280.7) | 63.11  (1.6; 124.4) | 98.61  (5.3; 173.6) |
| Monocyte infiltration/Cell adhesion/Inflammation | F4/80 | 31.01  (1.1; 79.0) | 42.40  (2.9; 68.1) | 65.06  (1.2; 98.1) |
| VCAM1 | 12.70  (3.0; 46.5) | 21.86  (1.5; 29.9) | 31.38  (2.0; 69.7) |

IL: interleukin; TNF- α: tumor necrosis factor- α; NF-κB: nuclear factor kappa B transcription factor; ATF4: activating transcription factor 4; CHOP: CCAAT/enhancer binding protein homologous protein; OCC: occluding; VCAM1: vascular cell adhesion molecule 1. Data are median (min; max). Outlier detection: GraphPad Prism 6.