**SUPPLEMENTARY MATERIAL**

**METHODS**

### Plasma endocannabinoids analysis

The AEA and 2-AG were quantitated by UPLC-MS/MS after protein precipitation and solid-phase extraction using the Ostro matrix (Waters Corp, Milford, MA). Briefly, 200 µL plasma was placed in wells of an Ostro 96-well plate and three volumes of acidified acetonitrile (1% formic acid) with internal standards, D4-AEA and D5-2-AG was added. Samples were triturated and incubated at least two minutes at room temperature before being pushed through the plate using a Waters positive pressure manifold (Waters Corp, Milford, MA). Samples were dried under a stream of nitrogen at 42 °C and then re-suspended in 100 µL acetonitrile with 0.2% acetic acid and 0.1 mM ammonium acetate.

Samples were then analyzed by triplicate injection (7.5 µL) onto a 2.1 x 100 mM Kinetex XB C18 column packed with 1.7 µm particles and equipped with a guard column (Phenomenex, Torrance, CA) using a Waters Acquity UPLC system (Waters Corp, Milford, MA). Analytes were eluted from the column using a gradient method with the A solvent being water with 0.2% acetic acid and solvent B being acetonitrile with 0.2% acetic acid. From time 0 to 3.5 minutes the gradient increased from 70% to 75% B. At 3.6 minutes the gradient increased to 95% B and was held there until 4.8 minutes. The gradient was then returned to 70% B at 5 minutes and held there for one minute. The column eluate was introduced into an AB/Sciex Q-Trap 5500 triple quadrupole mass spectrometer (SCIEX, Framingham, MA). Analytes and standards were monitored using a two-period MRM, positive ion mode analysis in which the first two minutes of the gradient were diverted to waste. From time 0 to 3 minutes the AEA compounds were monitored using three transitions each (analyte parent 348:products 287, 245 and 62; ISTD parent 352:products 287, 245, 66). During this analysis the declustering potential was 46 V, the ion spray voltage was 3500 V, and the temperature was 500 °C. From time 3 to 6 minutes the 2-AG compounds were monitored with three transitions (analyte parent 379:products 287, 269, 203; ISTD parent 384:products 287, 269, 203). During this time the declustering potential was 75 V, the ion spray voltage was 4000 and the temperature was 650 °C. Data from the runs were processed using the MultiQuant analysis software (SCIEX, Framingham, MA). A calibration curve of standards diluted in water (0.3 to 60 nM) in the Ostro plate and processed in parallel with samples was used to determine analyte concentrations in subject plasma. Area under the curve of analytes relative to internal standards was used to construct the calibration curve using a quadratic model with 1/x weighting. Points showing greater than 15% variation in accuracy were eliminated from the calibration curve. As an additional control for assay performance high (30 nM 2-AG; 8 nM AEA), mid (8 nM 2-AG; 1 nM AEA) and low-concentration (1 nM 2-AG; 0.3 nM AEA) quality control samples diluted in water and processed in parallel to samples were included in each analysis run. Failure (QC > 15% different from expected) of more than one in four QC samples was cause for re-analysis.

### PBMC isolation and mRNA analysis

PBMC were isolated from sodium heparin blood by gradient centrifugation using Histopaque®-1077, following the manufacturer’s instruction with minor modifications. Briefly, 20 mL blood was diluted with 15 mL isotonic, sterile, pyrogen-free PBS and then carefully loaded onto 15 mL Histopaque®-1077 in a clear, 50 mL centrifuge tube. After centrifugation at 400 × g for 30 min at room temperature, the interface layer containing mononuclear cells was collected and washed twice with PBS. Cell number was then determined on a TC10™ automated cell counter (Bio-Rad, Hercules, CA) and around 1 × 106 cells were transferred to a new centrifuge tube and pelleted at 250 × g for 10 min at room temperature. The pellet was suspended in 1 mL RNAlater, mixed well by vortexing, and stored at 4 °C overnight. The next day, the cells were pelleted at 8000 × g for 5 min at 4 °C and stored at -80 °C until RNA extraction.

 RNA was extracted by TRIzol reagent following the manufacturer’s recommended protocol. Briefly, cells were homogenized by pipetting up and down in TRIzol reagent. Chloroform was added for phase separation, and aqueous phase was removed and mixed with isopropanol to precipitate RNA. RNA was pelleted by centrifugation, washed with 75% ethanol, and dissolved in RNase-free water. The extracted RNA was further purified using an RNeasy mini kit (Qiagen, Valencia, CA). Briefly, the RNA solution was mixed with the supplied lysis buffer and ethanol, and then loaded onto a column, digested with an RNase-free DNase and RNA was eluted with RNase-free water. RNA concentration was then determined by fluorometric quantitation on the Qubit (Life Technologies, Carlsbad, CA). Then, cDNA was synthesized from 1 μg RNA using an iScript™ cDNA synthesis kit (Bio-Rad, Hercules, CA) on a Veriti® thermal cycler (Life Technologies, Carlsbad, CA), resulting in a 20 μL reaction volume. Then, 5 μL of the newly synthesized cDNA product was pooled for preparation of a standard curve.

 Reverse transcription quantitative PCR (RT-qPCR) was performed using the iTaq™ Universal SYBR® Green Supermix (Bio-Rad, Hercules, CA), on a Bio-Rad CFX96 system (Bio-Rad, Hercules, CA), following the manufacture’s protocol. To prevent amplification of genomic DNA, specific primers were designed by the NCBI primer design tool to span an intron-exon boundary of genes (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome>) ([Altschul, Gish et al. 1990](#_ENREF_1)). Primers were manufactured by Integrated DNA Technologies (Coralville, IA) at 25 nmol scale. The genes and primer sequences determined by RT-qPCR are listed in **Supplementary Table S1**. The controls consisted of *RNA18S5* and *RPRL0* genes that are stably expressed in PBMC ([Bas, Forsberg et al. 2004](#_ENREF_3), [Dheda, Huggett et al. 2004](#_ENREF_7)). The RT-qPCR reaction volume was 10 μL, consisting of 4 μL of 20-times diluted cDNA and 6 μL of master mix (5 μL of SYBR® Green Supermix; 0.5 μL of each 2.5 μM primer). Each RT-qPCR run included no cDNA and no reverse transcriptase as negative controls. A 6-point standard curve was prepared using the 4-fold serial dilution of the samples cDNA pool and the unknown samples were quantified by the relative standard curve method using Bio-Rad CFX manager 2.1 software (Bio-Rad, Hercules, CA) ([Grala, Roche et al. 2014](#_ENREF_11)).

## Supplementary Table S1 Primer sequences of target and reference PBMC genes.

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **GenBank accession number** | **Forward (primer sequence 5′–3′)** | **Reverse (primer sequence 5′–3′)** |
| *RNA18S5* | NR\_003286.2  | CTG AGA AAC GGC TAC CAC ATC | GCC TCG AAA GAG TCC TGT ATT G |
| *RPLP0* | NM\_001002.3 | CTC GTG GAA GTG ACA TCG TCT | GCT TGG AGC CCA CAT TGT CT |
| *TLR4* | NM\_003266.3 | GTC CCT GAA CCC TAT GAA CTT T | AAC CAG CCA GAC CTT GAA TAC |
| *RELA* | NM\_001145138.1 | CCA GAC CAA CAA CAA CCC CT | GGG GGC ACG ATT GTC AAA GA |
| *NFKBIA* | NM\_020529.2 | GAG CTC CGA GAC TTT CGA GG | TGT AGA CAC GTG TGG CCA TT |
| *PTGS2* | NM\_000963.3 | AAC TGC TCA ACA CCG GAA T | CCC TTG AAG TGG GTA AGT ATG TAG |
| *NCF1* | NM\_000265.5 | CTG AGC CCA ACT ATG CAG GT | TGA CGT CGT CTT TCC TGA TG |
| *TNF* | NM\_000594.3 | CCT CTC TCT AAT CAG CCC TCT | AGG GTT TGC TAC AAC ATG GG |
| *IFNG* | NM\_000619.2 | GGC TTT TCA GCT CTG CAT CG | TCT GTC ACT CTC CTC TTT CCA |
| *TGFB1* | NM\_000660.5 | CGA CTC GCC AGA GTG GTT AT | CGG TAG TGA ACC CGT TGA TGT |

*RNA18S5*, encoding 18s rRNA; *RPLP0*, encoding ribosomal protein large P0; *TLR4*, encoding Toll-like receptor 4; *RELA*, encoding p65 subunit of nuclear factor kappa B; *NFKBIA*, encoding nuclear factor kappa B inhibitor alpha; *PTGS2*, encoding cyclooxygenase-2, or COX-2; *NCF1*, encoding the p47 subunit of nicotinamide adenine dinucleotide phosphate-oxidase, or NADPH oxidase; *TNF*, encoding tumor necrosis factors, or TNF; IFNG, encoding interferon-γ; *TGFB1*, encoding transforming growth factor beta 1, or TGFβ1.

## Supplementary Table S2 Pearson correlation coefficients between paired biomarkers of all participants at baseline1.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|   | BMI (kg/m2) | IL-6 (pg/mL) | hsCRP (mg/L) | TNF-α (pg/mL) | sTNF-RII (pg/mL) | LPS (EU/mL) | LBP (μg/mL) | sCD14 (ng/mL) | IgM EndoCAb (MMU/mL) | AEA (nM) |
| IL-6 (pg/mL) | 0.51\*\* |  |  |  |  |  |  |  |  |  |
| hsCRP (mg/L) | 0.54\*\* | 0.39\*\* |  |  |  |  |  |  |  |  |
| TNF-α (pg/mL) | 0.28\*\* | 0.29\*\* | 0.20 |  |  |  |  |  |  |  |
| sTNF-RII (pg/mL) | 0.44\*\* | 0.31\*\* | 0.30\*\* | 0.39\*\* |  |  |  |  |  |  |
| LPS (EU/mL) | 0.05 | -0.02 | 0.12 | -0.01 | 0.06 |  |  |  |  |  |
| LBP (μg/mL) | 0.41\*\* | 0.36\*\* | 0.54\*\* | 0.07 | 0.24\*\* | 0.29\*\* |  |  |  |  |
| sCD14 (ng/mL) | 0.05 | 0.16 | 0.18 | -0.09 | 0.29\*\* | 0.01 | 0.10 |  |  |  |
| IgM EndoCAb (MMU/mL) | -0.32\*\* | -0.14 | -0.13 | -0.07 | -0.25\*\* | -0.22\* | -0.34\*\* | -0.09\*\* |  |  |
| AEA (nM) | 0.60\*\* | 0.29\*\* | 0.46\*\* | 0.12 | 0.22\* | -0.03 | 0.31\*\* | 0.10 | -0.20\* |  |
| 2-AG (nM) | 0.25\*\* | 0.07 | 0.39\*\* | 0.21\* | 0.13 | 0.12 | 0.17 | -0.14 | -0.16 | 0.28\*\* |

 1\* *P* < 0.05; \*\* *P* < 0.01. IL-6, interleukin-6; LPS, lipopolysaccharide; LBP, LPS-binding protein; sCD14, soluble CD14; hsCRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor alpha; sTNF-RII, soluble tumor necrosis factor receptor II; IgM EndoCAb, immunoglobulin M endotoxin-core antibody; AEA, anandamide; 2-AG, 2-arachidonoylglycerol.

2Pearson correlation coefficient.

**Supplementary Table S3** Self-reported dietary intakes at baseline and near the end of intervention, determined by 3-day dietary record (mean ± SEM)1.

|  |  |
| --- | --- |
| **Intakes** (unit) | **Group** |
| CN (n = 29)2 | CO (n = 30) | YN (n = 30) | YO (n = 30) |
| Baseline | End | Baseline | End | Baseline | End | Baseline | End |
| Energy (kcal)\* | 1657 ± 54 | 1733 ± 48 | 1869 ± 61 | 1900 ± 67 | 1666 ± 60 | 1727 ± 60 | 1893 ± 69 | 1962 ± 63 |
| Total Fat (g)\* | 67.4 ± 3.1 | 58.8 ± 2.7 ↓ | 75.2 ± 3.6 | 67.6 ± 3.7 | 62.8 ± 3.7 | 59.7 ± 3.5 | 77.5 ± 3.8 | 71.8 ± 3.8 |
| Carbohydrate (g)\* | 197.1 ± 7.8 | 234.2 ± 7.5 ↑ | 226.4 ± 8.4 | 251.6 ± 9.2 | 205.7 ± 6.4 | 226.7 ± 6.7 | 223.7 ± 9.6 | 255.1 ± 8.1 ↑ |
| Total Protein (g)\* | 71 ± 2.8 | 70.2 ± 2.5 | 78 ± 3.5 | 72.9 ± 3.0 | 72.8 ± 3.4 | 70.9 ± 3.3 | 78.6 ± 3.1 | 76.4 ± 3.0 |
| Total Fat (% 3) | 35.7 ± 1.1 | 29.3 ± 0.9 ↓ | 34.8 ± 1.0 | 30.4 ± 0.9 ↓ | 31.8 ± 0.9 | 28.8 ± 0.9 ↓ | 35.4 ± 1.0 | 31.3 ± 0.9 ↓ |
| Carbohydrate (% 3) | 46.0 ± 1.2 | 52.2 ± 1.0 ↑ | 48.0 ± 1.1 | 51.7 ± 1.1 ↑ | 49.8 ± 1.1 | 52.3 ± 1.1 | 46.0 ± 1.1 | 50.6 ± 0.9 ↑ |
| Total Protein (% 3) | 17.3 ± 0.7 | 18.1 ± 0.6 | 16.7 ± 0.5 | 17.1 ± 0.6 | 17.7 ± 0.7 | 17.8 ± 0.5 | 17.3 ± 0.6 | 17.1 ± 0.5 |
| Cholesterol (mg) | 268 ± 23 | 223 ± 22 | 249 ± 22 | 240 ± 23 | 258 ± 21 | 256 ± 22 | 332 ± 25 | 255 ± 20 ↓ |
| SFA (g)\* | 22.5 ± 1.2 | 20.5 ± 1.1 | 25.4 ± 1.7 | 23.4 ± 1.7 | 20.9 ± 1.5 | 19.7 ± 1.4 | 27.2 ± 1.4 | 24.0 ± 1.3 |
| Vitamin D (μg) | 5.4 ± 0.6 | 10.9 ± 0.5 ↑ | 4.5 ± 0.5 | 9.3 ± 0.3 ↑ | 4.2 ± 0.5 | 9.9 ± 0.4 ↑ | 4.4 ± 0.4 | 10.1 ± 0.3 ↑ |
| Calcium (mg) | 819 ± 36 | 926 ± 38 ↑# | 811 ± 45 | 887 ± 49$ | 713 ± 35 | 1058 ± 38 ↑# | 835 ± 47 | 1166 ± 38 ↑$ |
| Sodium (mg)\* | 2751 ± 135 | 2492 ± 96 | 3350 ± 185 | 2823 ± 131 | 2989 ± 187 | 2573 ± 140 ↓ | 3116 ± 175 | 2954 ± 175 |
| Potassium (mg) | 2492 ± 101 | 2186 ± 80 ↓ | 2476 ± 140 | 2264 ± 96$ | 2433 ± 85 | 2425 ± 89 | 2473 ± 107 | 2695 ± 101 ↑$ |
| Total Sugars (g)\* | 71.7 ± 3.7 | 111.3 ± 3.3 ↑ | 87.2 ± 4.4 | 116.6 ± 4.4 ↑ | 77.7 ± 3.7 | 104.6 ± 3.5 ↑ | 87.7 ± 5.0 | 115.1 ± 4.1 ↑ |
| Added Sugars (g)\* | 37.2 ± 3.3 | 70.3 ± 2.5 ↑ | 54.5 ± 4.1 | 83.4 ± 4.3 ↑ | 38.8 ± 3.2 | 71.2 ± 3.2 ↑ | 52.8 ± 5.2 | 81.6 ± 3.6 ↑ |

1Abbreviations: CN, control non-obese; CO, control obese; YN, yogurt non-obese; YO, yogurt obese; SFA, total saturated fatty acids.

2One subject was excluded in the CN group due to incomplete dietary record.

3Percentage of calories from total fat, carbohydrate and protein, respectively.

\*At baseline, significant differences between obese and non-obese participants determined by independent T-test (PROC TTEST), *P* < 0.05.

#At the end of intervention, significant difference between CN and YN, determined by ANCOVA with baseline intakes as covariate (PROC GLM), *P* < 0.05.

$At the end of intervention, significant difference between CO and YO, determined by ANCOVA with baseline intakes as covariate (PROC GLM), *P* < 0.05.

↓ Significant decrease from baseline determined by paired T-test (PROC TTEST), *P* < 0.05.

↑ Significant increase from baseline determined by paired T-test (PROC TTEST), *P* < 0.05.



## Supplementary Figure S1 Baseline fasting PBMC relative gene expression, normalized to 18s rRNA and RPLP0. The relative expression of target genes between groups was compared by independent T-test. No significant differences between YO or CO gene expression was detected. *TLR4*, encoding Toll-like receptor 4; *RELA*, encoding p65 subunit of nuclear factor kappa B; *NFKBIA*, encoding nuclear factor kappa B inhibitor alpha; *PTGS2*, encoding cyclooxygenase-2, or COX-2; *NCF1*, encoding the p47 subunit of nicotinamide adenine dinucleotide phosphate-oxidase, or NADPH oxidase; *TNF*, encoding tumor necrosis factors, or TNF; IFNG, encoding interferon-γ; *TGFB1*, encoding transforming growth factor beta 1, or TGFβ1.