**Supplementary Material to:**

# Citrate enrichment in a Western diet reduces weight gain via browning of adipose tissues without resolving diet-induced insulin resistance in mice

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Running title: Citrate induces weight loss but not reverses insulin resistance

**Rational for citrate dose used**

Considering the consumption of 1 liter of carbonated soft drinks (50 g total citrate anion), and 5 meals containing UPF (industrialized ready-to-eat pasta, sausages, powdered and packaged instant soups, noodles, and desserts, an individual take, at least 150 g of citrate anion per day. For an average male of 70 kg, it is a little bit more than 2 g/kg citrate anion. We have previously stablished a dose of 40 mg/g of Chow diet to mimic citrate consumption by humans in a mice protocol (Branco et al., 2021). Considering that mice consume 32.5% less HFHS diet than Chow, we have increased this dose to 67 mg/kg of HFHS to achieve a similar citrate intake compared to the previously reported (Branco et al., 2021).

Branco, J. R., Esteves, A. M., Leandro, J. G. B., Demaria, T. M., Godoi, V., Marette, A., … Sola-Penna, M. (2021). Dietary citrate acutely induces insulin resistance and markers of liver inflammation in mice. *The Journal of Nutritional Biochemistry*, *98*, 108834. https://doi.org/10.1016/j.jnutbio.2021.108834

**Table S1: Chow and High-Fat High-Sucrose (HFHS) diets composition**

| Diet | Chow | | | HFHS | | |
|---|---|---|---|---|---|---|
| **Ingredients** | **g/100g** | **Energy provided (kcal/100g)** | **Energy (%)** | **g/100g** | **Energy provided (kcal/100g)** | **Energy (%)** |
| Casein | 20.00 | 80.0 | 21.5 | 20.00 | 80.0 | 14.7 |
| L-cystine | 0.18 | 0.7 | 0.2 | 0.18 | 0.7 | 0.1 |
| Sucrose | 10.00 | 40.0 | 10.8 | 28.69 | 107.6 | 19.8 |
| Alpha-cellulose | 5.00 | | | 5.00 | | |
| Starch | 40.00 | 160.0 | 43.0 | | | |
| Dextrinized Starch | 13.09 | 53.4 | 14.3 | | | |
| Mineral mix (AIN-93) | 3.50 | | | 4.90 | | |
| Vitamin mix (AIN-93) | 1.00 | | | 1.40 | | |
| Lard | - | | | 19.80 | 178.2 | 32.7 |
| Soy oil | 7.00 | 38.1 | 10.2 | 19.80 | 178.2 | 32.7 |
| Choline bitartrate | 0.20 | | | 0.20 | | |
| Tert-butylhydrytoluene (BHT) | 0.03 | | | 0.03 | | |
| Total | 100.00 | 372.2 | 100 | 100.00 | 544.7 | 100 |

Table S2. Antibodies used

| Antibody | Brand | Catalog # | Dilution |
|---|---|---|---|
| ACC | Cell Signaling* | 3662 | 1:1000 |
| ACLY | Abcam& | 40793 | 1:1000 |
| Akt | Cell Signaling | 9272 | 1:1000 |
| Anti-mouse | Jackson Labs# | 115-035-146 | 1:10000 |
| Anti-rabbit | Jackson Labs | 115-035-144 | 1:20000 |
| mTOR | Cell Signaling | 2972 | 1:1000 |
| Phospho-S6 ribosomal protein (S235/236) | Cell Signaling | 4858 | 1:1000 |
| Phospho-ACLY (S455) | Abcam | 46796 | 1:1000 |
| Phospho-Akt (S473) | Cell Signaling | 9271 | 1:1000 |
| Phospho-Akt (T308) | Cell Signaling | 9275 | 1:1000 |
| Phospho-mTOR (S2448) | Cell Signaling | 2971 | 1:1000 |
| S6 ribosomal protein | Cell Signaling | 2217 | 1:1000 |
| SCD1 | Cell Signaling | 2794 | 1:1000 |
| β-actin | Cell Signaling | 4970 | 1:1000 |

* Cell signaling Technology, Danvers, MA, USA
& Abcam, Cambridge, UK
# Jackson ImmunoResearch Labs, West Grove, PA, USA

Table S3. Oligo pairs.

| Gene | Forward sequence | Reverse sequence | Amplicon length (bp) | Efficiency (%) |
|---|---|---|---|---|
| *Acaca* | CCGAGAAAGCAGGGGATCTG | TACCCGACGCATGGTTTTCA | 91 | 101 |
| *Acly* | TGGATGCCACAGCTGACTAC | GGTTCAGCAAGGTCAGCTTC | 150 | 95 |
| *Actb* | TGAACCCTAAGGCCAACC | CACAATGCCTGTGGTACG | 128 | 98 |
| *Cpt1a* | TGGACCCAAATTGCAGTGGT | CTCCCACCAGTCACTCACATAA | 73 | 97 |
| *Fasn* | CCATGGCAGCTGTTGGTTTG | GTGTCCTCAGAGTTGTGGCA | 88 | 98 |
| *Fpr2* | CCAGTGATTCAAGCACCAGTT | TCACAGACTTCATGGGGCCTT | 71 | 99 |
| *Hprt* | CCCTGGTTAAGCAGTACAGCCCC | AGTCTGGCCTGTATCCAACACTTCG | 90 | 101 |
| *Il12b* | GCAGCAAAGCAAGATGTGTCC | TGGAGACACCAGCAAAACGA | 70 | 96 |
| *Pgc1a* | CTFTGGAACTGCAGGCCTAA | CAAGAGGGCTTCAGCTTTGG | 96 | 99 |
| *Ppara* | GCAACAACCCGCCTTTTGTC | TTGGCCACAAGCGTCTTCTC | 70 | 99 |
| *Pparg* | GCCTATGAGCACTTCACAAGAAAT | GCTGATTCCGAAGTTGGTGG | 76 | 97 |
| *Rpl7* | GAAGCTCATCTATGAGAAGGC | AAGACGAAGGAGCTGCAGAAC | 202 | 105 |
| *Scd1* | CACCTGCCTCTTCGGGATTTT | GGCCCATTCGTACACGTCA | 166 | 98 |
| *Tnfa* | TGAGAAGTTCCCAAATGGCCT | CCACTTGGTGGTTTGTGAGTG | 74 | 101 |
| *Ucp1* | ACTGCCACACCTCCAGTCATT | CTTTGCCTCACTCAGGATTGG | 123 | 108 |

Table S4. Tissues weight (% of body weight)

| **Tissue** | **Chow** | **HFHS** | **HFHS+cit** |
|---|---|---|---|
| Heart | 0.53±0.01 | 0.43±0.01* | 0.53±0.02# |
| Pancreas | 0.51±0.03 | 0.52±0.03 | 0.52±0.01 |
| Spleen | 0.26±0.01 | 0.25±0.01 | 0.29±0.01# |
| BAT | 0.38±0.04 | 0.26±0.03* | 0.23±0.02* |
| Kidneys | 1.09±0.03 | 0.97±0.03* | 1.11±0.02# |
| Adrenal | 0.03±0.01 | 0.03±0.01 | 0.03±0.01 |
| Soleus | 0.06±0.01 | 0.05±0.01 | 0.07±0.01*,# |
| Gastrocnemius | 1.08±0.03 | 0.95±0.03* | 1.20±0.02*,# |
| Quadriceps | 1.16±0.03 | 1.01±0.04* | 1.30±0.04*,# |
| Stomach | 0.59±0.02 | 0.55±0.04 | 0.80±0.03*,# |

* $P < 0.05$ as compared to Chow

\# $P < 0.05$ as compared to HFHS

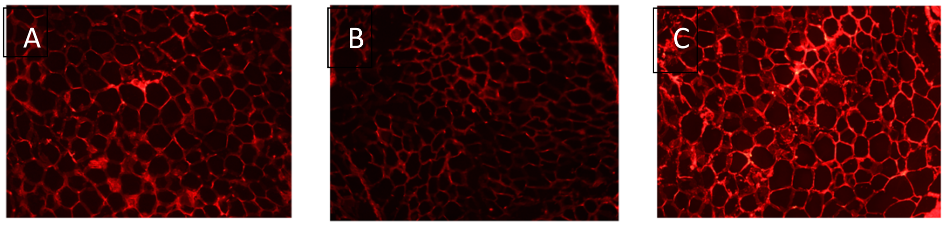


Figure S1. Citrate supplemented Western diet promote mitochondria biogenesis. Mitotracker deep red stained eWAT samples were analyzed for fluorescence emission using a Slight DS-5-M1 digital camera (Nikon, Melville,NY) connected to an epifluorescence Nikon Eclipse 50i light microscope under 20x objective. Panel A: Chow; Panel B: HFHS; Panel C: HFHS+Cit.