

## **SUPPLEMENTARY MATERIAL**

### **Host or the hosted? Effects of non-nutritive sweeteners on intestinal and microbial mechanisms of glycemic control**

Braden D. Rose<sup>1,2,3,4</sup>, Nektaria Pezos<sup>1,2,4</sup>, Jocelyn M. Choo<sup>5,6</sup>, Tongzhi Wu<sup>2,3</sup>, Geraint B. Rogers<sup>5,6</sup>, Kerry L. Ivey<sup>7,8</sup>, Christopher K. Rayner<sup>2,3</sup>, Richard L. Young<sup>1,2,3,4†</sup>

<sup>1</sup>Intestinal Sensing Group, <sup>2</sup>Adelaide Medical School and <sup>3</sup>Centre of Research Excellence in Translating Nutritional Science to Good Health, The University of Adelaide, Adelaide, SA 5005, Australia

<sup>4</sup>Diabetes, Nutrition & Gut Health and <sup>5</sup>Precision Medicine, Lifelong Health, South Australian Health & Medical Research Institute (SAHMRI), Adelaide, SA 5000, Australia

<sup>5</sup>Microbiome and Host Health, Lifelong Health, South Australia Health and Medical Research Institute, Adelaide, South Australia, Australia. <sup>6</sup>Infection and Immunity, College of Medicine & Public Health, Flinders University of South Australia, Bedford Park, SA 5042, Australia.

<sup>7</sup>Department of Medicine, Brigham and Women's Hospital, Boston, MA 02115, USA

<sup>8</sup>Department of Medicine, Harvard Medical School, Boston, MA 02115, USA

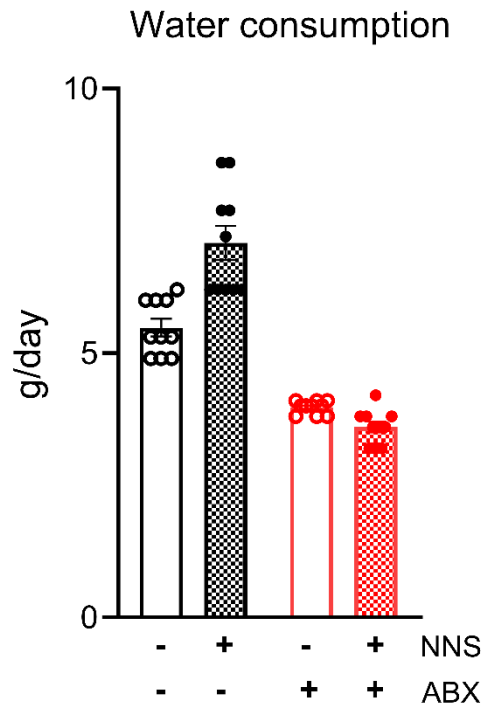
#### **Corresponding Author†**

Richard L Young PhD

Nutrition, Diabetes & Gut Health, Lifelong Health, South Australian Health & Medical Research Institute (SAHMRI)

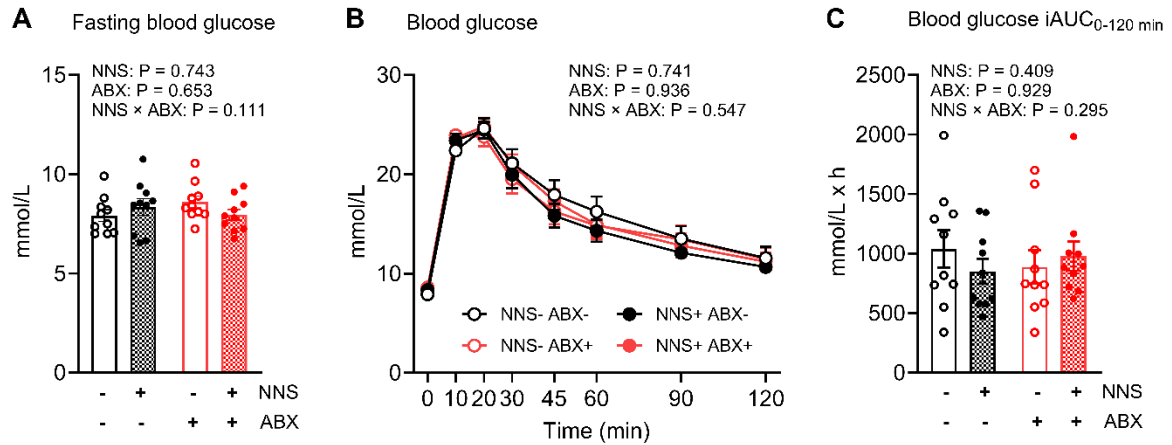
PO Box 11060 Adelaide SA 5001, AUSTRALIA

Tel: +61 8 8128 4845; Email: richard.young@adelaide.edu.au



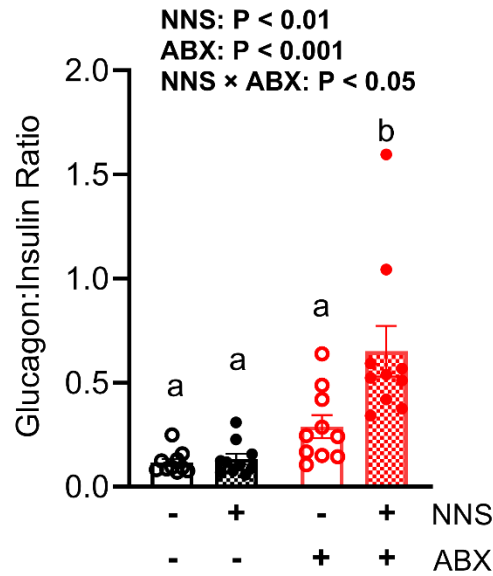
**Supplementary Figure 1: Daily drinking water intake.**

Daily drinking water intake in study cohorts. N = 10. ABX, antibiotic-supplemented; NNS, non-nutritive sweetener-supplemented.



**Supplementary Figure 2: Pre-study fasting blood glucose concentrations and oral glucose tolerance (Day -1).**

(A) Fasting blood glucose concentrations (Day -1), (B) OGTT blood glucose responses, and (C) iAUC<sub>0-120</sub>. P values above for main and interaction effects with bold signifying significance. N=10. Data are mean  $\pm$  SEM. ABX, antibiotic-supplemented; iAUC, incremental area under the curve; NNS, non-nutritive sweetener-supplemented; OGTT, oral glucose tolerance test.



**Supplementary Figure 3: Plasma glucagon-to-insulin ratio in response to intrajejunal glucose infusion.**

Plasma glucagon-to-insulin ratio as an estimate of endogenous glucose production after 30-min intrajejunal glucose infusion. P values above for main and interaction effects with bold signifying significance; a, b denote interaction differences (post-hoc multiple comparison) at  $P < 0.05$ . N = 10. ABX, antibiotic-supplemented; NNS, non-nutritive sweetener-supplemented.

**Supplementary Table 1: Hormone assay sensitivity**

<b>Analyte</b>	<b>Source (Catalogue No.)</b>	<b>Sensitivity (pg/ml)</b>	<b>CV %</b>
<b>GLP-1 (total)</b>	Merck Millipore (MPMMMHE-44K)	22.8	3.6
<b>Glucagon</b>	Merck Millipore (MPMMMHE-44K)	14.4	1.7
<b>GIP</b>	Merck Millipore (MPMMMHE-44K)	1.4	4.4
<b>Insulin</b>	Merck Millipore (MPMMMHE-44K)	62.4	12.3
<b>PYY</b>	Merck Millipore (MPMMMHE-44K)	9.5	1.9
<b>PP</b>	Merck Millipore (MPMMMHE-44K)	6.1	1.8

**Supplementary Table 2: Primers used in qRT-PCR assays**

<b>Gene (Protein)</b>	<b>QuantiTect Primer Assay (Locus, Qiagen Cat No.)</b>	<b>Amplicon length (bp)</b>
<i>Tas1R2</i> (T1R2)	<b>Mm_Tas1r2_1_SG</b> (NM_031873, XM_980714; QT00142639)	137
<i>Slc5a1</i> (SGLT-1)	<b>Mm_Slc5a1_1_SG</b> (NM_019810; QT00112679)	120
<i>Slc2a2</i> (GLUT2)	<b>Mm_Slc2a2_1_SG</b> (NM_031197; QT00103537)	105
<i>Gcg</i> (Proglucagon)	<b>Mm_Gcg_1_SG</b> (NM_008100, XM_006498770; QT00124033)	94
<i>Pcsk1</i> (PC1)	<b>Mm_Pcsk1_1_SG</b> (NM_013628, XM_006517153, XM_006517155, XM_006517154; QT00106190)	100
<i>Pcsk2</i> (PC2)	<b>Mm_Pcsk2_1_SG</b> (NM_008792; QT00167426)	118
<i>Dpp4</i> (DPP-IV)	<b>Mm_Dpp4_1_SG</b> (NM_001159543, NM_010074, XM_006498691, XM_006498690, XM_006498692; QT00095284)	70
<i>B2m</i> ( $\beta$ 2M)	<b>Mm_B2m_2_SG</b> (NM_009735; QT01149547)	143
<i>Hprt</i> (HPRT1)	<b>Mm_Hprt_1_SG</b> (NM_013556; QT00166768)	168