**Supplemental Data**

**SUPPLEMENTAL FIGURE 1**. Assessment of health and well-being in subjects at study baseline and following treatment with placebo or β2-1 fructan based on results from the HRQoL SF-36 questionnaire (*n* = 30, bars correspond to the mean ± standard error). Scores range from 0 (worst possible health status) to 100 (best possible health status). MCS; mental component summary. PCS; physical component summary. Baseline PCS (●) MCS (○); placebo PCS (▲) MCS (Δ); β2-1 fructan PCS (■) MCS (□). No significant differences between baseline, placebo and β2-1 fructan phases were detected in the mental or physical components.



**SUPPLEMENTAL TABLE 1.** Antibodies used in peripheral cell population analysis

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Marker | Description1 | Clone | Conjugate2 | Manufacturer3 |
| Control | Isotype – IgG1 | T1B9 | PE | Invitrogen |
| Control | Isotype – IgG1 | T1B9 | FITC | Invitrogen |
| CD34 | T lymphocytes | SK7 | FITC | BD Biosciences |
| CD44 | TH1 and TH2 T cells, monocytes, macrophages | SK3 | APC | BD Biosciences |
| CD84 | cytotoxic T cells | SK1 | PE | BD Biosciences |
| CD11c | myeloid DC | S-HCL-3 | APC | BD Biosciences |
| CD14 | monocytes | M5E2 | APC | BD Biosciences |
| CD16 | NK cells and granulocytes | 3G8 | AF647 | BD Biosciences |
| CD164 | NK cells and granulocytes | B73.1 | PE | BD Biosciences |
| CD194 | B cells | SJ25C1 | APC | BD Biosciences |
| CD45 | leukocytes | 2D1 (HLe-1) | FITC | BD Biosciences |
| CD454 | leukocytes | 2D1 (HLe-1) | PerCP | BD Biosciences |
| CD564 | NK cells | NCAM 16.2 | PE | BD Biosciences |
| CD86 | monocytes, activated B cells, DC | 2331 (FUN-1) | PE | BD Biosciences |
| CD123 | plasmacytoid DC | 9F5 | PE | BD Biosciences |
| CD282 | TLR-2 | TLR2.1 | PE | BioLegend |
| CD284 | TLR-4 | HTA125 | PE | BioLegend |
| HLA-DR | MHC II, B cells, monocytes, activated T cells | L243 | PerCP | BD Biosciences |
| IL-12 | intracellular IL-12 | C8.6 | FITC | Invitrogen |
| lin1 | T lymphocytes, monocytes, macrophages, neutrophils, eosinophils, neutrophils, NK cells, B lymphocytes | SK7, 3G8, SJ25C1, L27, MφP9, NCAM16.2 | FITC | BD Biosciences |
| TNF-α | intracellular TNF-α | 6401.1111 | PE | BD Biosciences |

1 DC, dendritic cell; Ig, immunoglobulin; IL, interleukin, MHC II, major histocompatibility complex class II molecule; NK, natural killer cells; TNF, tumour necrosis factor.

2 AF647, Alexa Fluor 647; APC, allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin–chlorophyll–protein complex.

3 Location of manufacturers: BD Biosciences, San Jose CA; BioLegend, San Diego CA, USA; Invitrogen, Carlsbad CA, USA.

4 Part of a BD Mulitest™ kit.

**SUPPLEMENTAL TABLE 2.** Individual participant demographic data at study baseline

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ID | Gender | Age | Height (m) | Weight (kg) | BMI (kg/m2) | Sys BP1 | Dia BP |
| 001 | M | 26 | 1.790 | 73.4 | 22.91 | 135 | 75 |
| 002 | M | 28 | 1.846 | 90.3 | 26.50 | 135 | 76 |
| 003 | F | 27 | 1.496 | 50.4 | 22.52 | 107 | 70 |
| 004 | F | 29 | 1.699 | 59.4 | 20.58 | 115 | 79 |
| 005 | F | 25 | 1.670 | 69.5 | 24.92 | 112 | 84 |
| 006 | M | 34 | 1.830 | 79.0 | 23.59 | 131 | 83 |
| 007 | M | 28 | 1.830 | 71.7 | 21.41 | 123 | 82 |
| 008 | F | 27 | 1.635 | 52.0 | 19.45 | 104 | 69 |
| 009 | F | 20 | 1.603 | 52.7 | 20.51 | 112 | 68 |
| 010 | F | 23 | 1.617 | 70.2 | 26.85 | 115 | 68 |
| 011 | F | 25 | 1.634 | 57.9 | 21.69 | 94 | 65 |
| 012 | M | 25 | 1.770 | 73.4 | 23.43 | 135 | 79 |
| 013 | F | 32 | 1.765 | 72.0 | 23.11 | 132 | 89 |
| 015 | M | 27 | 1.780 | 78.4 | 24.74 | 120 | 63 |
| 016 | M | 36 | 1.718 | 73.3 | 24.83 | 139 | 87 |
| 018 | M | 35 | 1.657 | 80.0 | 29.14 | 130 | 65 |
| 022 | F | 26 | 1.595 | 57.5 | 22.60 | 121 | 87 |
| 023 | F | 22 | 1.622 | 67.2 | 25.54 | 116 | 82 |
| 024 | M | 24 | 1.860 | 91.3 | 26.39 | 152 | 94 |
| 025 | M | 25 | 1.770 | 75.5 | 24.10 | 136 | 76 |
| 026 | F | 24 | 1.695 | 66.6 | 23.18 | 116 | 68 |
| 027 | F | 30 | 1.630 | 77.0 | 28.98 | 111 | 82 |
| 028 | M | 29 | 1.680 | 82.8 | 29.34 | 119 | 86 |
| 029 | F | 42 | 1.515 | 58.0 | 25.27 | 122 | 93 |
| 030 | F | 24 | 1.600 | 57.8 | 22.58 | 110 | 78 |
| 031 | M | 38 | 1.840 | 96.7 | 28.56 | 122 | 76 |
| 032 | F | 34 | 1.655 | 62.4 | 22.78 | 108 | 81 |
| 033 | M | 23 | 1.660 | 66.2 | 24.02 | 127 | 72 |
| 034 | F | 25 | 1.652 | 81.0 | 29.68 | 114 | 79 |
| 035 | F | 30 | 1.665 | 50.0 | 18.04 | 120 | 80 |
| Median | | 27 | 1.668 | 71.0 | 23.8 | 120 | 79 |
| Mean | | 28.1 | 1.693 | 69.8 | 24.2 | 121 | 78 |
| 95% CI | | 26.3 – 29.9 | 1.66 – 17.3 | 65.4 – 74.2 | 23.2 – 25.3 | 116.7 – 125.5 | 74.9 – 80.9 |

1 Sys BP, systolic blood pressure; Dia BP, diastolic blood pressure.

**SUPPLEMENTAL TABLE 3.** Estimated daily background dietary β2-1 fructan intakes in subjects during placebo and β2-1 fructan phases (*n* = 30, midpoint values). Collected categorical data from a food frequency questionnaire was converted to numerical values (β2-1 fructan/100 g food item) and ranges of intake of β2-1 fructan as g/day were calculated over each phase. Midpoints of reported ranges were used to estimate total consumption of β2-1 fructan as g/day between phases; no significant differences in dietary β2-1 fructan intake were detected.

|  |  |  |
| --- | --- | --- |
| **ID** | **Placebo phase(g β2-1 fructan)** | **β2-1 fructan phase (g β2-1 fructan)** |
| 001 | 1.29 | 0.52 |
| 002 | 0.83 | 1.14 |
| 003 | 1.86 | 2.40 |
| 004 | 0.58 | 0.94 |
| 005 | 1.45 | 0.92 |
| 006 | 1.10 | 0.50 |
| 007 | 0.69 | 0.77 |
| 008 | 1.31 | 1.39 |
| 009 | 1.17 | 0.35 |
| 010 | 0.80 | 0.68 |
| 011 | 1.06 | 1.13 |
| 012 | 1.82 | 2.13 |
| 013 | 0.49 | 0.42 |
| 015 | 2.36 | 0.74 |
| 016 | 1.18 | 1.48 |
| 018 | 0.22 | 0.52 |
| 022 | 2.04 | 1.53 |
| 023 | 0.47 | 0.30 |
| 024 | 0.74 | 0.65 |
| 025 | 1.40 | 0.98 |
| 026 | 1.28 | 0.92 |
| 027 | 0.32 | 0.22 |
| 028 | 1.13 | 0.70 |
| 029 | 2.29 | 2.05 |
| 030 | 1.74 | 2.12 |
| 031 | 0.13 | 0.42 |
| 032 | 0.95 | 0.95 |
| 033 | 1.24 | 0.69 |
| 034 | 0.17 | 0.13 |
| 035 | 0.46 | 1.03 |
| **Estimated β2-1 fructan intake (g)** | 1.09 | 0.96 |

**SUPPLEMENTAL TABLE 4**. Relative content of Bifidobacteria (% of total community) as determined by qPCR in the faeces of test subjects at the initiation and termination of the placebo and β2-1 fructan phases.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Relative Bifidobacteria content (% of total community)** | | | | | |
|  |  | **Placebo (P)** | | **β2-1 Fructan (F)** | |
| **Subject** | **Treatment Order** | **Day 0** | **Day 28** | **Day 0** | **Day 28** |
| 1 | PF | 2.6 | 2.9 | 2.1 | 3.5 |
| 3 | PF | 0.1 | 1.2 | 0.3 | 2.1 |
| 4 | PF | 0.4 | 0.6 | 0.7 | 4.7 |
| 5 | PF | 0.7 | 2.0 | 1.9 | 1.6 |
| 7 | PF | 0.6 | 0.4 | 0.4 | 6.0 |
| 9 | PF | 1.4 | 0.7 | 1.6 | 1.8 |
| 13 | PF | 3.1 | 1.6 | 2.0 | 3.6 |
| 18 | PF | 4.0 | 6.5 | 13.9 | 10.2 |
| 22 | PF | 0.2 | 1.9 | 0.6 | 6.9 |
| 24 | PF | 4.7 | 4.7 | 5.0 | 5.1 |
| 29 | PF | 10.6 | 8.1 | 16.0 | 27.1 |
| 31 | PF | 0.5 | 0.8 | 0.0 | 2.5 |
| 33 | PF | 2.2 | 1.8 | 6.8 | 4.8 |
| 34 | PF | 1.9 | 3.2 | 2.1 | 3.8 |
| 35 | PF | 0.2 | 0.2 | 1.3 | 3.0 |
|  |  |  |  |  |  |
| 2 | FP | 1.2 | 3.4 | 2.2 | 7.2 |
| 6 | FP | 0.6 | 0.5 | 0.3 | 4.5 |
| 8 | FP | 11.1 | 2.6 | 7.2 | 8.6 |
| 10 | FP | 1.9 | 3.8 | 0.8 | 2.6 |
| 11 | FP | 1.6 | 1.8 | 3.0 | 12.1 |
| 12 | FP | 0.1 | 0.0 | 0.0 | 0.0 |
| 15 | FP | 3.6 | 2.7 | 5.6 | 2.0 |
| 16 | FP | 0.0 | 0.0 | 1.6 | 9.7 |
| 23 | FP | 0.6 | 1.6 | 2.0 | 17.1 |
| 25 | FP | 0.6 | 0.1 | 1.2 | 5.6 |
| 26 | FP | 1.6 | 2.0 | 4.1 | 6.4 |
| 27 | FP | 0.6 | 0.2 | 0.4 | 4.0 |
| 28 | FP | 0.1 | 1.1 | 0.1 | 0.1 |
| 30 | FP | 0.0 | 0.0 | 0.2 | 0.0 |
| 32 | FP | 2.8 | 0.6 | 2.6 | 4.5 |
|  |  |  |  |  |  |
|  | Mean | 2.0a | 1.9a | 2.9a | 5.7b |
|  | SD | 2.7 | 1.9 | 3.8 | 5.5 |

a,b values with different letters are different at the *P* < 0.05 level as determined by Tukey’s HSD.

**SUPPLEMENTAL TABLE 5**. Individual SCFA and BCFA proportions in faeces (% of total) at the termination of the placebo and β2-1 fructan phases. Total faecal fatty acid concentrations (µmol g-1 wet weight, mean ± standard error) were significantly different (*P* < 0.001) between phases.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  | Placebo Phase | | β2-1 Fructan Phase | |
|  | Fatty Acid | n | Mean  (µmol gww-1) | SEM | Mean  (µmol gww-1) | SEM |
|  | Acetic (%) | 30 | 57.7a | 1.1 | 55.5 | 1.1 |
| SCFA | Propionic (%) | 30 | 17.1 | 0.9 | 17.8a | 1.1 |
|  | Butyric (%) | 30 | 17.0 | 0.7 | 20.0a | 1.4 |
|  |  |  |  |  |  |  |
|  | Iso-Butyric (%) | 30 | 2.3a | 0.2 | 1.8 | 0.2 |
|  | Iso-Valeric (%) | 30 | 3.1a | 0.4 | 2.4 | 0.3 |
| BCFA | Valeric (%) | 30 | 2.3a | 0.2 | 2.0 | 0.2 |
|  | Caproic (%) | 30 | 0.6a | 0.1 | 0.4 | 0.1 |
|  | Heptanoic (%) | 30 | 0.1 | 0.0 | 0.0 | 0.0 |
|  |  |  |  |  |  |  |
| **Total** | **(µmol gww-1)** | **30** | **81.6** | **5.8** | **89.5a** | **6.2** |

aMean value was significantly different between phases determined by repeated measures t-test corrected for false discovery rate.

**SUPPLEMENTAL TABLE 6.** Participant routine biochemical and haematological biomarkers1

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Marker2** | **Placebo d-02** | **Placebo d-28** | **Fructan d-0** | **Fructan d-28** |
| BMI | 24.1 ± 0.5 | 24.2 ± 0.5 | 24.1 ± 0.5 | 24.2 ± 0.5 |
| BUN | 5.0 ± 0.2 | 4.7 ± 0.3 | 4.9 ± 0.3 | 4.8 ± 0.3 |
| fast Glu | 4.9 ± 0.1 | 4.9 ± 0.1 | 4.9 ± 0.1 | 4.9 ± 0.1 |
| Creat | 77.1 ± 2.7 | 76.7 ± 0.1 | 75.8 ± 2.4 | 75.4 ± 2.3 |
| eGFR | 91.0 ± 2.5 | 90.8 ± 2.2 | 91.4 ± 2.4 | 92.5 ± 2.2 |
| AST | 24.1 ± 1.6 | 23.4 ± 1.2 | 23.8 ± 0.9 | 24.1 ± 1.0 |
| GGT | 18.9 ± 1.7 | 20.2 ± 1.8 | 19.8 ± 2.2 | 20.4 ± 1.8 |
| Glob | 26.6 ± 0.5 | 27.2 ± 0.5 | 26.5 ± 0.5 | 27.2 ± 0.6 |
| T-Pr | 71.9 ± 0.5 | 72.9 ± 0.5 | 71.9 ± 0.5 | 72.7 ± 0.6 |
| Alb | 45.3 ± 0.5 | 45.7 ± 0.5 | 45.7 ± 0.5 | 45.5 ± 0.4 |
| hs CRP | 2.7 ± 0.8 | 3.4 ± 1.3 | 3.0 ± 1.0 | 2.5 ± 0.9 |
| TC | 4.6 ± 0.1 | 4.5 ± 0.1 | 4.5 ± 0.1 | 4.7 ± 0.1 |
| LDL | 2.6 ± 0.1 | 2.5 ± 0.1 | 2.4 ± 0.1 | 2.6 ± 0.1 |
| HDL | 1.5 ± 0.1 | 1.5 ± 0.1 | 1.5 ± 0.1 | 1.5 ± 0.1 |
| TC/HDL | 3.3 ± 0.2 | 3.1 ± 0.1 | 4.0 ± 0.9 | 3.2 ± 0.2 |
| TG | 1.0 ± 0.1 | 1.3 ± 0.3 | 1.1 ± 0.1 | 1.2 ± 0.1 |
| Hgb | 138.0 ± 2.2 | 137.9 ± 2.4 | 137.1 ± 2.0 | 138.7 ± 0.6 |
| HCT | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 |
| WBC | 6.4 ± 0.4 | 6.4 ± 0.4 | 6.5 ± 0.3 | 6.3 ± 0.3 |
| RBC | 4.7 ± 0.1 | 4.7 ± 0.1 | 4.6 ± 0.1 | 4.7 ± 0.1 |
| MCV | 89.0 ± 0.6 | 89.1 ± 0.7 | 88.9 ± 0.7 | 88.9 ± 0.7 |
| MCH | 29.6 ± 0.3 | 29.6 ± 0.3 | 29.5 ± 0.3 | 29.8 ± 0.3 |
| MCHC | 333.3 ± 1.9 | 332.6 ± 1.7 | 332.5 ± 1.7 | 334.7 ± 1.8 |
| RDW | 13.0 ± 0.1 | 13.0 ± 0.1 | 13.0 ± 0.1 | 13.1 ± 0.1 |
| Pl.Ct. | 257.9 ± 14.3 | 264.0 ± 13.6 | 270.4 ± 13.6 | 264.2 ± 13.5 |

1 Values expressed as mean ± SEM (*n* = 30).

2 Alb, albumin; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; Creat, creatinine; eGFR, calculated glomerular filtration rate; fast GLU, fasting glucose; GGT, gamma glutamyltransferase; Glob, globulin protein; HCT, haematocrit; Hgb, haemoglobin; HDL, high-density lipoprotein; hs CRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; Pl.Ct., platelet count; RBC, red blood cell count; RDW, red cell distribution width; T-Pr, total protein; TC, total cholesterol; TC/HDL, total cholesterol/high-density lipoprotein ration; TG, triglycerides; WBC, white blood cell count.