Supplementary Materials

Title: Consumption of barley flour increases gut fermentation and improves glucose intolerance via the short-chain fatty acid receptor GPR43 in obese male mice

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Supplemental Table 1: The nutrient components of barley flour.

(g/100g dw)	Barley
Fat	3.4
Protein	9.4
Ash	1.0
Available carbohydrate	67.8
Total dietary fiber	18.4
β-(1-3)-(1,4) Glucan	10.1

Fat: Acid hydrolysis method

Protein: Kjeldahl method

Ash: Dry ashing method

Total dietary fiber: AOAC 991.43 method

β-(1-3)-(1,4) Glucan: McCleary method (AOAC 995.16)

Available carbohydrate: (100 − ("Fat" + "Protein" + "Ash" + "Total dietary fiber"))

Supplemental Table 2: Primer sequence for real time PCR.

gene symbol	Forward	Reverse
G6pd	5'-TACTGGCAGAGCAGGT-3'	5'-GATCTGGTCCTCACG-3'
Pepck	5'-CCACAGCTGCTGCAGAACA-3'	5'- GAAGGGTCGCATGGCAAA-3'
Glut2	5'-CGCAATGGTCGCCTCATT-3'	5'-CAGTCCTGATACACTTCGTC-3'
Pk	5'-TGCAATTGGAGCCGTGGA-3'	5'-CCTGGGCAGAACGAGTCACA-3'
Gpr43	5'-GGGATCTGGGTCACATGCTTAT-3	5'-ATGTCAGACAGACGGGTACCAA-3'
Pc1/3	5'-AGACAGCATTACACCATCTCTA-3'	5'-AGAACACTTCTCTGCATACCAAGGT-3'
Pgcg	5'-ATTGCCAAACGTCATGATGA-3'	5'-GGCGACTTCTTCTGGGAAGT-3'
NeuroD	5'-CTTGGCCAAGAACTACATCTGG-3'	5'-CGTGTTTGAAAGAGAAGTTGCC-3'
Reference		
36B4	5'-GGCCCTGCACTCTCGCTTTC-3	5'-TGCCAGGACGCGCTTGT-3'

Supplemental Table 3: Body weight, food intake, and food efficiency ratio in C57BL/6J mice fed the experimental diets.

	HC (C57BL/6J)	HB (C57BL/6J)	P value
Initial weight (g)	20.9 ± 0.49	20.7 ± 0.44	0.84
Final weight (g)	43.7 ± 1.47	42.1 ± 1.15	0.29
Body weight gain (g/d)	0.26 ± 0.01	0.23 ± 0.01	0.26
Food intake (g/d)	2.83 ± 0.06	2.85 ± 0.06	0.84
Food efficiency ratio (%)	8.99 ± 0.32	8.27 ± 0.46	0.21
Liver weight (g)	1.52 ± 0.11	1.29 ± 0.04	0.05
Cecum with contents (g)	0.29 ± 0.01	0.37 ± 0.02	0.0086
Retroperitoneal fat (g)	1.06 ± 0.07	0.91 ± 0.06	0.13
Epididymal fat (g)	2.50 ± 0.10	2.47 ± 0.17	0.91
Mesenteric fat (g)	1.17 ± 0.14	0.91 ± 0.09	0.12

Data are presented as mean ± SE (n=8).

Food efficiency ratio = body weight gain / food intake × 100

Abbreviations: HC, control group; HB, barley group.

Supplemental Table 4: Body weight, food intake, and food efficiency ratio in GPR43-knockout mice fed the experimental diets.

	*gpr*43(-/-) HC	*gpr*43(-/-) HB	P value
Initial weight (g)	22.3 ± 0.36	22.3 ± 0.59	0.95
Final weight (g)	38.4 ± 1.50	36.5 ± 2.09	0.49
Body weight gain (g/d)	0.16 ± 0.01	0.14 ± 0.01	0.42
Food intake (g/d)	2.60 ± 0.14	2.69 ± 0.18	0.71
Food efficiency ratio (%)	6.31 ± 0.55	5.24 ± 0.69	0.24
Liver weight (g)	1.51 ± 0.10	1.44 ± 0.13	0.67
Cecum with contents (g)	0.26 ± 0.02	0.40 ± 0.02	<0.0001
Retroperitoneal fat (g)	0.91 ± 0.07	0.75 ± 0.10	0.20
Epididymal fat (g)	1.90 ± 0.13	1.63 ± 0.18	0.22
Mesenteric fat (g)	0.77 ± 0.10	0.68 ± 0.15	0.63

Data are presented as mean ± SE (n=8~10).

Food efficiency ratio = body weight gain / food intake × 100

Abbreviations: *gpr*43(-/-) HC, Gpr43-knockout mice (control group); *gpr*43(-/-) HB, Gpr43-knockout mice (barley group)
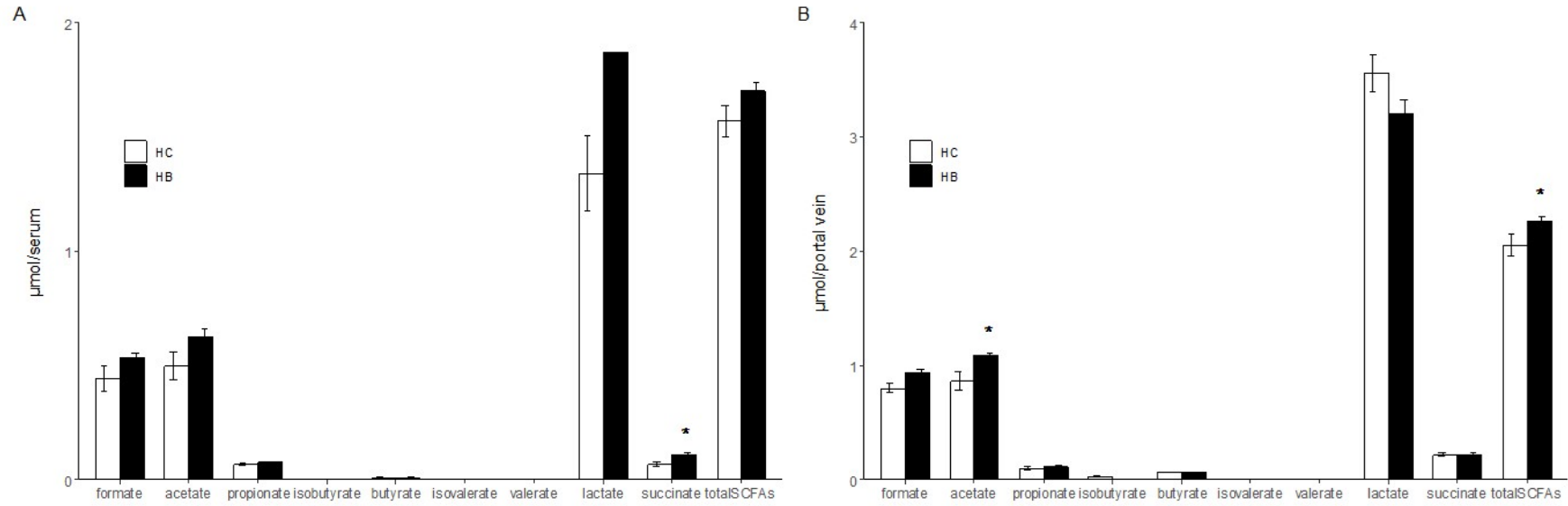
Supplemental Figure 1
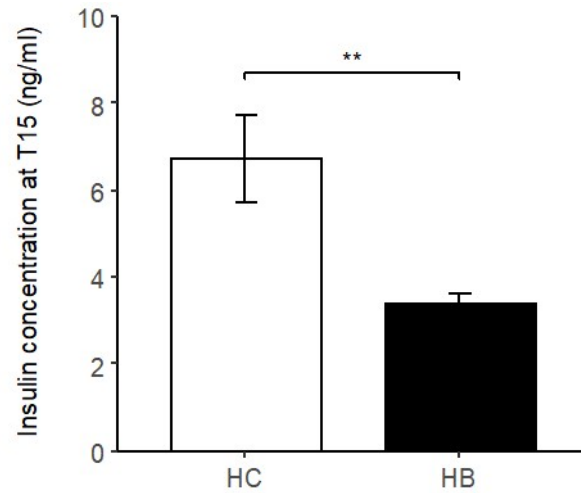
The concentration of insulin by glucose stimulated at T15.

Data are presented as mean ± SE (pooled data, n=4 by repetition).

Superscripts in the figure show significant differences between the experimental groups (* P<0.05, ** P<0.01).

Abbreviations: HC, control group; HB, barley group.

Supplemental Figure 2

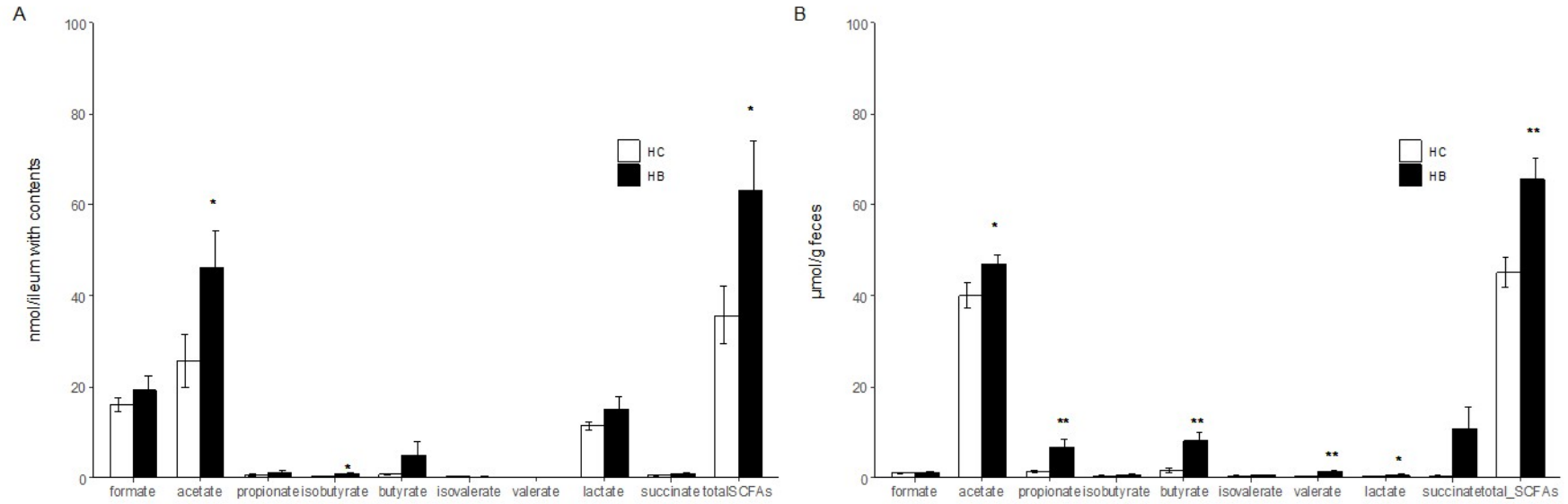The concentration of SCFAs and organic acids in the serum (A) and portal vein (B).

Data are presented as mean ± SE (n=8).

Superscripts in the figure show significant differences between the experimental groups (* P<0.05, ** P<0.01).

Abbreviations: HC, control group; HB, barley group.

Supplemental Figure 3

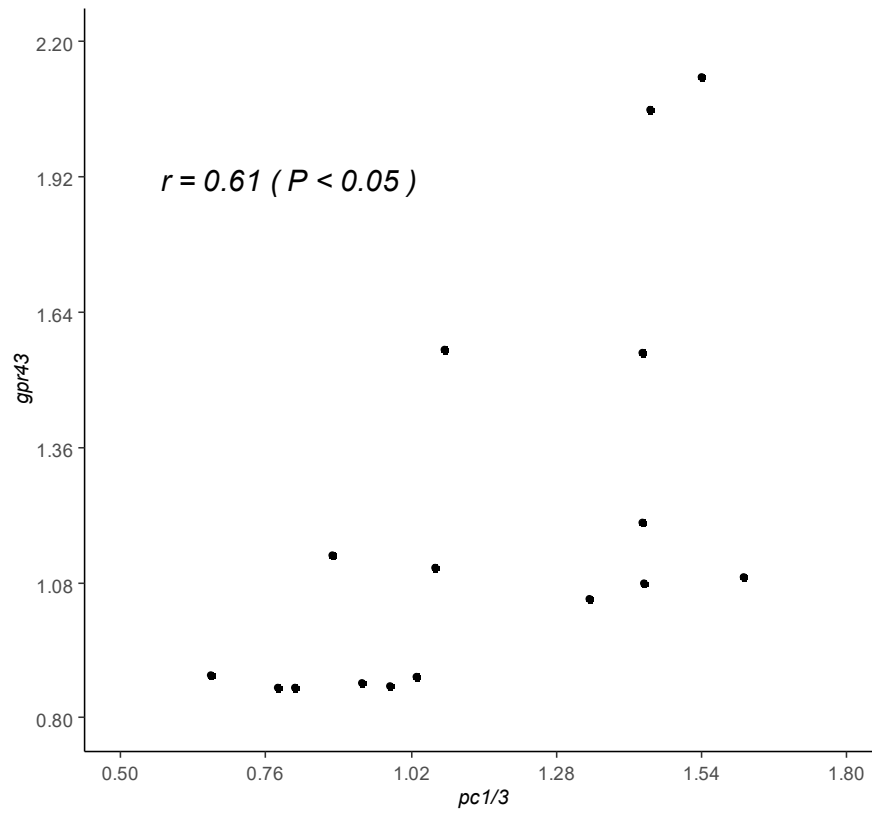The concentration of SCFAs and organic acids in the ileum (A) and feces (B).

Data are presented as mean ± SE (n=8).

Superscripts in the figure show significant differences between the experimental groups (* P<0.05, ** P<0.01).
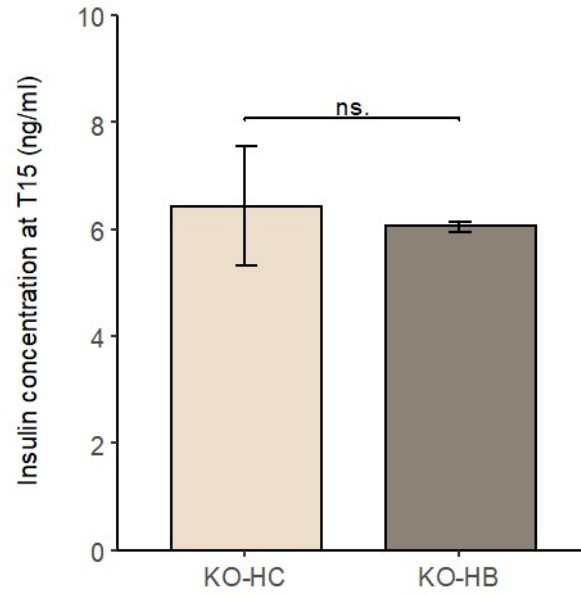
Abbreviations: HC, control group; HB, barley group.

Supplemental Figure 4

Spearman's correlation coefficient matrix between mRNA expression of gpr43 and pc1/3 in ileum of the C57BL/6J mice.

r value indicates Spearman's correlation coefficient.

Supplemental Figure 5

The concentration of insulin by glucose stimulated at T15.

Data are presented as mean ± SE (pooled data, n=4 by repetition).

Superscripts in the figure show significant differences between the experimental groups (* P<0.05, ** P<0.01).

Abbreviations: KO-HC, gpr43-knockout mice (control group); KO-HB, gpr43-knockout mice (barley group).

Supplemental Figure 6
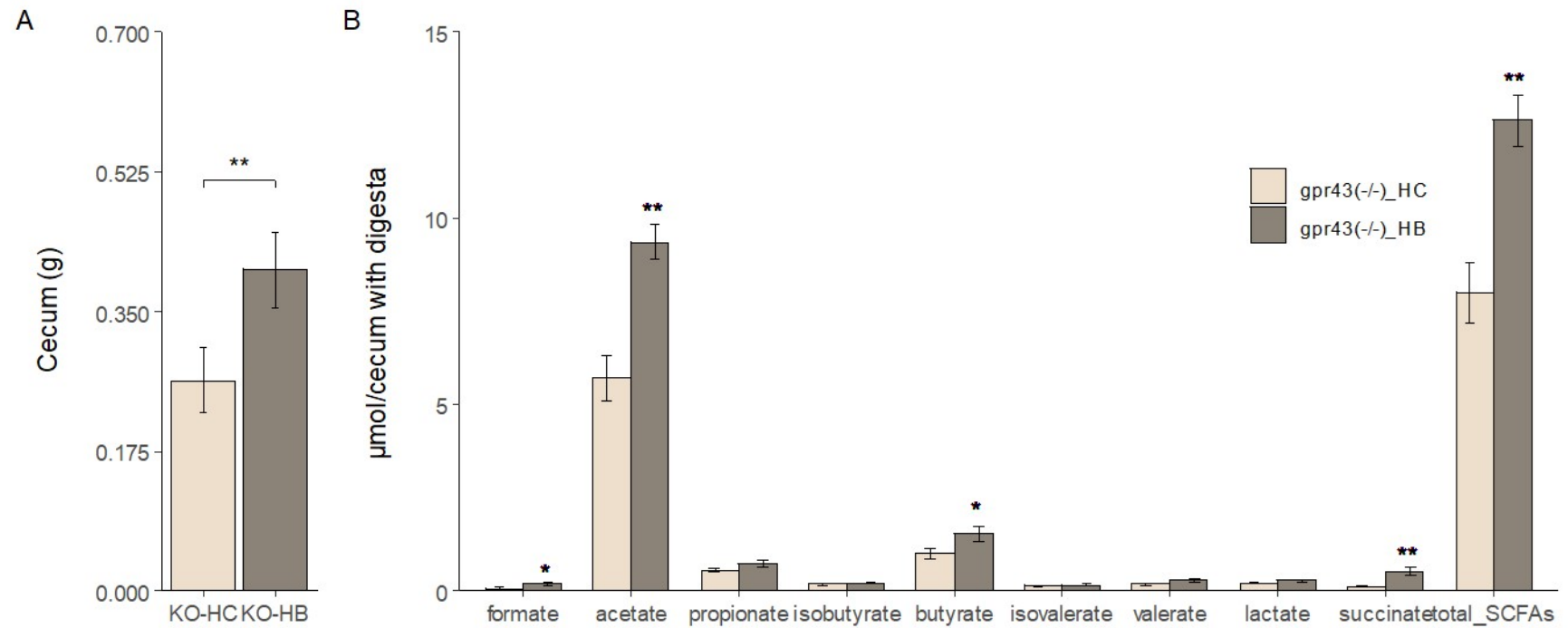
The weight of cecum (A) and the concentration of SCFAs and organic acids in the cecum (B) in GPR43-knockout mice.

Data are presented as mean ± SE (n=8~10).

Superscripts in the figure show significant differences between the experimental groups (* P<0.05, ** P<0.01).

Abbreviations: KO-HC, gpr43-knockout mice (control group); KO-HB, gpr43-knockout mice (barley group).