environmental microbiology reports

Environmental Microbiology Reports (2013) **5**(5), 765–775 doi:10.1111/1758-2229.12079

Strict vegetarian diet improves the risk factors associated with metabolic diseases by modulating gut microbiota and reducing intestinal inflammation

Min-Soo Kim,[1] Seong-Soo Hwang,[2] Eun-Jin Park[3] and Jin-Woo Bae[1]*

[1]*Department of Life and Nanopharmaceutical Sciences and Department of Biology, Kyung Hee University, 1 Hoegi-dong, Dongdaemun-gu, Seoul 130-701, Republic of Korea.*

[2]*Hwangseongsoo Clinic, Jamwon-dong, Seocho-gu, Seoul 137-909, Republic of Korea.*

[3]*Department of Food Bioengineering, Jeju National University, Jeju 690-756, Republic of Korea.*

Summary

Low-grade inflammation of the intestine results in metabolic dysfunction, in which dysbiosis of the gut microbiota is intimately involved. Dietary fibre induces prebiotic effects that may restore imbalances in the gut microbiota; however, no clinical trials have been reported in patients with metabolic diseases. Here, six obese subjects with type 2 diabetes and/or hypertension were assigned to a strict vegetarian diet (SVD) for 1 month, and blood biomarkers of glucose and lipid metabolisms, faecal microbiota using 454-pyrosequencing of 16S ribosomal RNA genes, faecal lipocalin-2 and short-chain fatty acids were monitored. An SVD reduced body weight and the concentrations of triglycerides, total cholesterol, low-density lipoprotein cholesterol and haemoglobin A1c, and improved fasting glucose and postprandial glucose levels. An SVD reduced the *Firmicutes*-to-*Bacteroidetes* ratio in the gut microbiota, but did not alter enterotypes. An SVD led to a decrease in the pathobionts such as the *Enterobacteriaceae* and an increase in commensal microbes such as *Bacteroides fragilis* and *Clostridium* species belonging to clusters XIVa and IV, resulting in reduced intestinal lipocalin-2 and short-chain fatty acids levels. This study underscores the benefits of dietary fibre for improving the risk factors of metabolic diseases and shows that increased fibre intake reduces gut inflammation by changing the gut microbiota.

Received 16 June, 2013; accepted 19 June, 2013. *For correspondence. E-mail baejw@khu.ac.kr; Tel. (+82) 2 961 2312; Fax (+82) 2 961 0244.

Introduction

The human intestine is densely populated by trillions of microbial symbionts. Symbiotic gut microbiota help nutrient absorption through the fermentation of dietary fibre (Flint *et al*., 2008) and provide protection from invading pathogens (Endt *et al*., 2010). They also help to develop and regulate the immune system (Chung *et al*., 2012; Olszak *et al*., 2012). Industrialization is associated with an increase in the incidence of metabolic syndrome and autoimmune diseases (Bach, 2002), as are Western-style dietary pattern and reduced exposure to environmental microorganisms by excessive hygiene (Fung *et al*., 2001; Heidemann *et al*., 2008; Blaser and Falkow, 2009); dysbiosis of the gut microbiota is thought to be responsible (Kau *et al*., 2011).

A global view of the human microbiome suggests that the establishment of the human gut microbiota is dependent on social and cultural factors, rather than on inherited factors (Yatsunenko *et al*., 2012). Indeed, diet is regarded as the main factor contributing to the make-up of the gut microbiota (De Filippo *et al*., 2010), and long-term Western-style dietary patterns are associated with in this make-up (Ley *et al*., 2006; Wu *et al*., 2011). Metabolic syndromes such as obesity, diabetes and cardiovascular disease develop in response to low-grade chronic inflammation (Wellen and Hotamisligil, 2005). Changes in the gut microbiota are considered to be central to this, although the exact underlying mechanisms have not yet been identified (Musso *et al*., 2011). It is thought that an altered gut microbiota harvests excess calories in the form of volatile fatty acids, which increases adiposity (Turnbaugh *et al*., 2006; Cho *et al*., 2012). Endotoxin-induced systemic inflammation, caused by lipopolysaccharides produced by the altered gut microbiota passing through the impaired intestinal barrier (Cani *et al*., 2008), is directly associated with a Western-style diet (Pendyala *et al*., 2012). Interestingly, these metabolic phenotypes are transmissible by transplantation of the gut microbiota, proving the direct involvement of altered gut microbiota in metabolic dysfunction (Turnbaugh *et al*., 2006; Vijay-Kumar *et al*., 2011).

An intake of dietary fibre is thought to reduce the risk for obesity and metabolic diseases by modulating the composition of the gut microbiota (Parnell and Reimer, 2012).

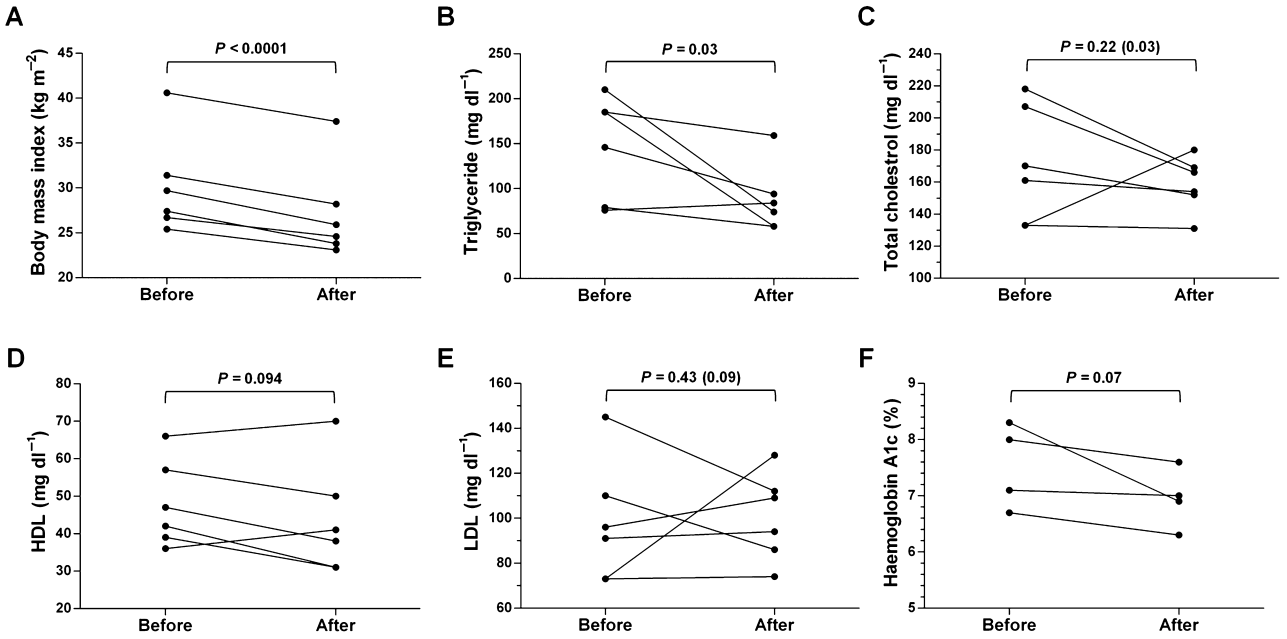

Fig. 1. Comparison of body mass index and the concentrations of biomarkers linked to metabolic syndrome. Comparison of body mass index (A), plasma concentrations of triglycerides (B), total cholesterol (C), high-density lipoprotein (HDL) cholesterol (D), and low-density lipoprotein (LDL) cholesterol (E); and HbA1c (F), both before and after 1 month on an SVD (one-tailed paired *t*-test). The *P*-value is shown in parentheses (except subject HE). HbA1c levels were only measured in subjects diagnosed with type 2 diabetes (subjects HA, HB, HE and HF).

The inverse relationship between dietary fibre consumption and the incidence of metabolic disease supports the health effects of dietary fibre (Slavin, 2008). A recent study demonstrated the structural resilience of the gut microbiota in response to dietary changes (Zhang *et al.*, 2012). Another study showed that direct transplantation of the microbiota from lean donors increased the insulin sensitivity of recipients with metabolic syndrome (Vrieze *et al.*, 2012a). Considering the critical role played by the altered gut microbiota in the development of metabolic diseases (Musso *et al.*, 2011), we questioned whether the prebiotic effect of dietary fibre would reduce the risk factors associated with metabolic diseases by promoting gut microbial homeostasis. However, no clinical trials have been reported.

This study used diet therapy using a strict vegetarian diet (SVD) to evaluate whether prebiotic consumption reduced the risk factors associated with metabolic diseases by modulating the composition of the gut microbiota. Six obese patients diagnosed with type 2 diabetes and/or hypertension were placed on an SVD for 1 month, and changes in the markers related to glucose/lipid metabolism, the gut microbial composition and indicators of gut inflammation were determined using plasma and faecal samples. Taking interindividual variations into account, we monitored plasma glucose levels and blood pressure daily, and assessed the dynamics of the microbial communities by pyrosequencing of polymerase chain reaction-amplified 16S ribosomal RNA (rRNA) genes. This study shows that dietary fibre has a prebiotic effect on the gut microbiota and contributes to metabolic and immunologic improvements in patients with metabolic diseases.

Results and discussion

Diet therapy improves metabolic risk factors

Six obese volunteers [HA, HB, HC, HD, HE, and HF; mean body mass index (in kg m^{-2}): 30.2; range: 25.4–40.6] diagnosed with type 2 diabetes and/or hypertension were recruited (Table S1), and fed an SVD comprising 16% protein, 72% carbohydrate (including dietary fibre 18%, 42 g day^{-1}), and 12% fat as calories (Table S2) for a month. Body weight and the concentrations of plasma metabolic biomarkers were compared before and after an SVD. A significant reduction in body mass index was observed after one month on an SVD ($P < 0.0001$) (Fig. 1A). Plasma concentrations of triglycerides were also significantly decreased ($P < 0.05$) (Fig. 1B); indeed, those of subjects HC and HD were decreased below the normal range (150 mg dl^{-1}) (Grundy *et al.*, 2005). Total cholesterol levels were also significantly decreased below the normal range (< 200 mg dl^{-1}), except in subject HE ($P < 0.05$) (Fig. 1C). In addition, all subjects (except HE) showed normal

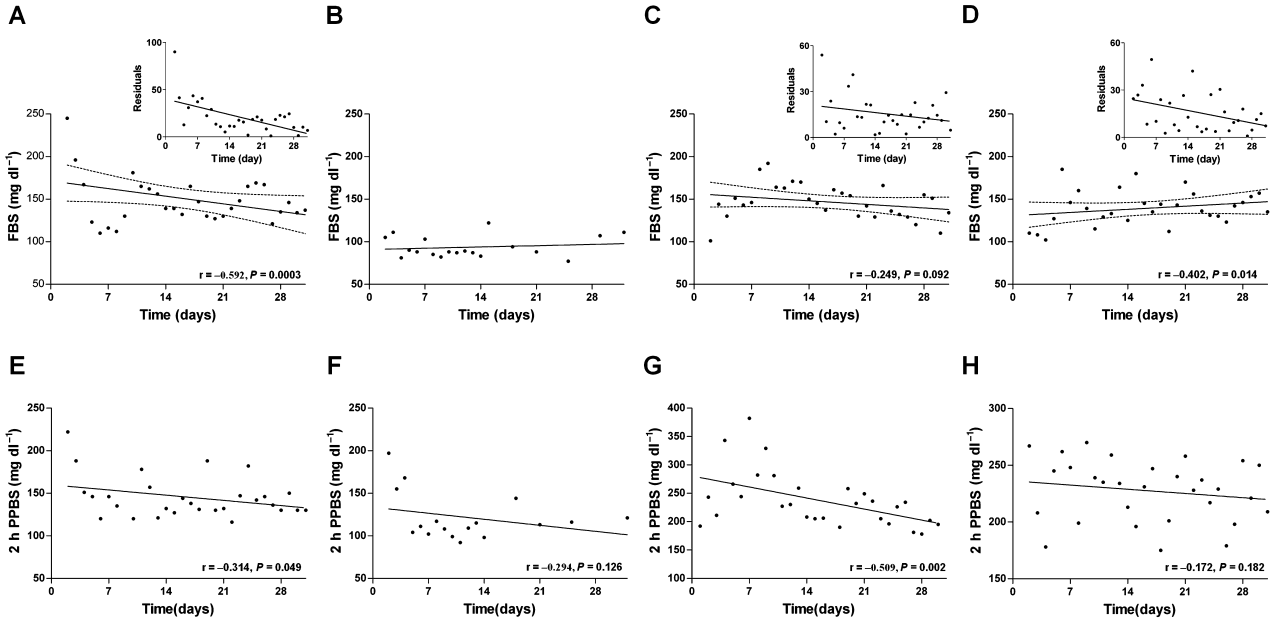

Fig. 2. An SVD improves glucose homeostasis. FBS (A–D) and 2 h PPBS (E–H) levels in subjects diagnosed with type 2 diabetes (subjects HA, HB, HE and HF) were measured on a daily basis. Consumption of an SVD reduced residual FBS and 2 h PPBS levels (Pearson's correlation coefficient).

low-density lipoprotein cholesterol levels (< 130 mg dl^{-1}) after 1 month on the SVD (Fig. 1D). However, there was no improvement in high-density lipoprotein cholesterol levels (Fig. 1E). Together, these results suggest that an SVD is associated with a significant improvement in lipid metabolism.

Plasma glucose levels and blood pressure were monitored daily. Fasting blood sugar (FBS) levels did not fall below normal criteria (110 mg dl^{-1}); however, the FBS levels in subjects HA and HF stabilized significantly over time based on residual analysis ($P < 0.05$) (Fig. 2A and D). A similar trend was observed for subject HC, but the results showed only weak statistical significance (Fig. 2C). Subject HE maintained normal FBS concentrations (< 110 mg dl^{-1}) while on an SVD (Fig. 2B). The 2 h postprandial blood sugar (2 h PPBS) levels were also improved by an SVD. For subjects HA and HE, the levels dropped below the threshold considered diagnostic for diabetes (< 200 mg dl^{-1}) and were negatively correlated with an SVD ($P < 0.05$) (Fig. 2E and G). A similar trend was noted for subjects HB and HF, although the statistical significance was weak (Fig. 2F and H). In addition, the plasma concentrations of haemoglobin A1c fell in all subjects after the SVD (Fig. 1F). Together, these results suggest that an SVD improves impaired glucose tolerance by stabilizing FBS levels and reducing 2 h PPBS levels. However, there was no apparent improvement in blood pressure (Fig. S1); therefore, long-term consumption of an SVD may be required to clarify its effects on hypertension.

Changes in the gut microbial community structure induced by diet therapy

Changes in the gut microbiota were monitored over time by 454-pyrosequencing of V1-V2 region amplicons of 16S rRNA genes from periodically collected faecal samples. After quality filtering, a total of 122 979 high-quality sequences were obtained ($n = 41$ samples; 2570 ± 1159 reads/sample). Operational taxonomic units (OTUs) were determined by clustering the sequences at 97% similarity. UniFrac distances between the communities were calculated to assess any differences at the OTU level. The bacterial community in each subject on day 1 was set as the baseline and then compared with the composition on day 3, 5, 7, 14, 21 and 28. Based on the unweighted and weighted UniFrac distances, the gut microbial community in individual subjects showed marked changes over time, and these changes were positively correlated with the consumption of an SVD ($P < 0.05$ and $P < 0.01$ respectively) (Fig. 3). To confirm whether these changes were induced by the dietary intervention, the communities were compared backwards using day 28 as the baseline, but no significant changes in the gut microbiota were observed (Fig. S2). This result indicates that an SVD had a significant effect on the composition of the gut microbiota at the OTU level. However, there was no correlation between the consumption of an SVD and bacterial diversity (Table S4). This indicates that SVD-induced changes in the composition of the gut microbiota were not associated with changes in bacterial diversity.

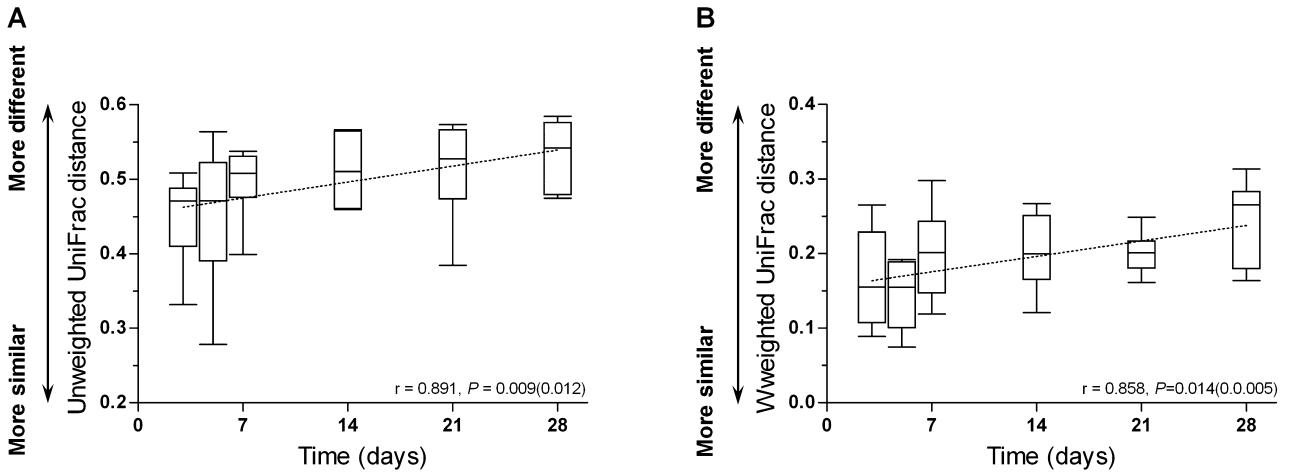

Fig. 3. An SVD induces changes of the gut microbial community over time. Changes in the gut microbial community were monitored by comparing the community of day 1 with those of all other days in order. The data are based on unweighted UniFrac distance (A) and weighted UniFrac distance (B). The *P*-values were calculated using Pearson's correlation. The *P*-values in parenthesis were derived from a linear regression.

Obese individuals appear to harbour a high *Firmicutes*-to-*Bacteroidetes* ratio in their gut microbiota (Turnbaugh *et al*., 2009). Therefore, we examined the effect of an SVD diet on correcting the *Firmicutes*-to-*Bacteroidetes* ratio in these subjects by assessing the relative changes in abundance of these two phyla. As the study subjects lost weight on an SVD (an average of $10.0 \pm 2.4\%$ over the study period), the abundance of *Firmicutes* was decreased over time ($P = 0.052$) (Fig. 4A); however, the abundance of *Bacteroidetes* increased significantly ($P < 0.05$) (Fig. 4B). Taken together, these results suggest that an SVD corrected the *Firmicutes*-to-*Bacteroidetes* ratio in these subjects and that this change might be correlated with the observed weight loss.

Consumption of an SVD for 1 month changed the composition of the gut microbiota but did not affect bacterial diversity. Although somewhat controversial, a high *Firmicutes*-to-*Bacteroidetes* ratio and low bacterial diversity in the gut are thought to be associated with obesity (Turnbaugh *et al*., 2009). An increase in the *Firmicutes*

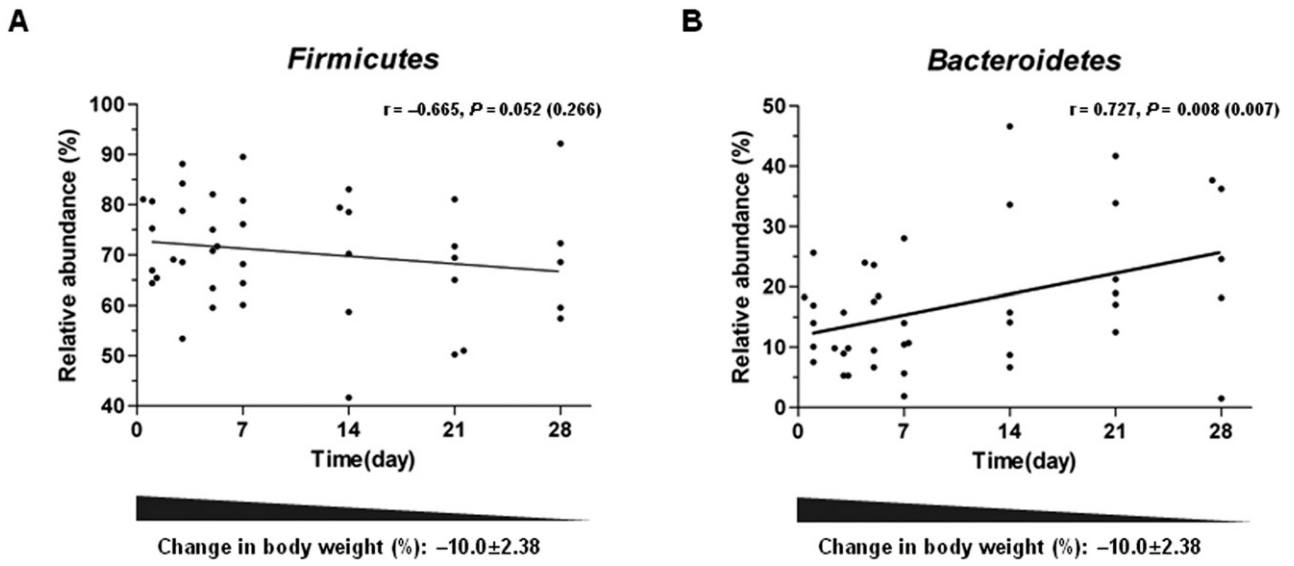

Fig. 4. Changes in the relative abundance of *Firmicutes* and *Bacteroidetes*. Changes in the relative abundance of *Firmicutes* (A) and *Bacteroidetes* (B) correlated with the consumption of an SVD. *P*-values were derived using Pearson's correlation. The *P*-values in parenthesis were derived from linear regression analysis.

population leads to weight gain through excessive energy extraction via increased carbohydrate transport and utilization (Turnbaugh *et al*., 2006); specifically, a 20% increase in the *Firmicutes* population relative to the *Bacteroidetes* population results in a net gain of 150 kcal (Jumpertz *et al*., 2011). Interestingly, restoration of the *Firmicutes*-to-*Bacteroidetes* ratio by a fat- or carbohydrate-restricted low-calorie diet results in weight loss (Ley *et al*., 2006; Zhang *et al*., 2012), suggesting that diet-induced changes may lead to an improvement in metabolic dysfunction in obese patients. However, previous studies do not support the notion that a lack of bacterial diversity is correlated with metabolic dysfunction (Ley *et al*., 2006; Martínez *et al*., 2012).

Effect of diet therapy on enterotypes

It was proposed that the human gut microbiota can be categorized into three enterotypes, driven by *Bacteroides*, *Prevotella* and *Ruminococcus* (Arumugam *et al*., 2011). We examined whether SVD-induced changes in the bacterial community led to a change in enterotypes. Partitioning around medoids clustering generated four distinct clusters (Fig. S3). One cluster comprised the community present in subjects HA, HB and HE, which was identified as *Prevotella*. The three enterotypes clustered separately for each subject, although the *Bacteroides* and *Ruminococcus* enterotypes could not be identified unequivocally because each has a different driver. Significant changes in the composition of the gut microbiota accompanied with physiological changes in the host did not result in a change of enterotypes, indicating that enterotypes are not influenced by an SVD.

Although an SVD induces significant changes in the composition of the gut bacterial community, these changes failed to affect gut enterotypes. Enterotypes are driven primarily by *Prevotella* and *Bacteroides* (Yatsunenko *et al*., 2012). This study shows that the relative abundance of these two genera tended to increase in response to an SVD, suggesting that unstable enterotypes might be indicative of disrupted microbial homeostasis in the gut. Because of interindividual variation, there is a need to develop a simplified model of the human gut microbiota. Thus, the concept of the 'enterotype' was developed (Arumugam *et al*., 2011), which may serve as a prognostic tool to aid the diagnosis of gut microbial properties. However, this study did not find any association between change patterns in gut microbial composition and enterotypes; this suggests that enterotypes might not serve as microbial shared features. A recent report suggests that enterotypes may not be as stable as previously thought (Rajilić-Stojanović *et al*., 2012). Therefore, we need to develop a more advanced concept, along with long-term studies of the composition of the gut microbiome.

Key players in the gut microbiota respond to diet therapy

One of the main objectives of this study was to determine which bacterial groups are associated with the prebiotic effects induced by an SVD. Comparative analyses of previous metagenomic studies reveals a striking feature: alterations in the composition of the gut microbiota or in certain bacterial blooms may be an aggravating factor or a consequence of a disease, rather than playing an early role in the pathogenesis of a disease (Mukhopadhya *et al*., 2012); the loss or gain of certain taxonomic groups may be regarded as an aggravating factor in the development of disease. Changes in the abundance of the different taxonomic groups were monitored at the genus and species levels on an SVD. Despite interindividual variation, a marked reduction in the abundance of *Escherichia* and *Klebsiella* in the *Enterobacteriaceae* family was observed in subjects HA, HC, HE and HF, which was negatively correlated with an SVD in subjects HA, HC and HE ($P < 0.05$) (Fig. 5 and Fig. S4). Moreover, the number of OTUs identifying *Clostridium clostridioforme* (which causes bacteremia) (Finegold *et al*., 2005) declined over time, except in subject HE (Table S5). The abundance of *Lactobacillus ruminis* and *Lactobacillus mucosae* (in subjects HB, HC and HF), *Streptococcus lutetiensis* (in subjects HD and HE) and *Veillonella parvula* (in subjects HC and HE) also declined over time (Table S5); these genera can cause systemic infections such as bacteremia, endocarditis and meningitis (Bhatti and Frank, 2000; van't Wout and Bijlmer, 2005; Joly *et al*., 2010; Neville *et al*., 2012), as well as overrepresented in the altered gut microbiota (Tana *et al*., 2010; Koren *et al*., 2012). The OTUs obtained for subject HD showed that the abundance of *S. lutetiensis*, which belongs to the virulent *Streptococcus bovis* group D (van't Wout and Bijlmer, 2005), declined over time; however, *Streptococcus salivarius* exhibiting anti-inflammatory effects on gut epithelial cells and monocytes (Kaci *et al*., 2011) was more abundant (Table S5). The class *Mollicutes* and the genus *Succinivibrio*, which were decreased in subject HA, are associated with abnormalities in the gut microbiota of populations consuming a Western-style diet or those not fed on lysozyme-deficient breast milk respectively (Turnbaugh *et al*., 2008; Maga *et al*., 2012). The abundance of all the above genera was negatively correlated with an SVD, supporting the hypothesis that an SVD inhibits the growth of pathobionts.

The search for specific bacterial species that trigger low-grade inflammation in the intestine is ongoing, and the *Enterobacteriaceae* family are a strong candidate (Mukhopadhya *et al*., 2012). The members of the *Enterobacteriaceae* family act as strong colitogenic pathobionts causing aberrant immune responses when gut homeostasis is disrupted (Garrett *et al*., 2010; Carvalho

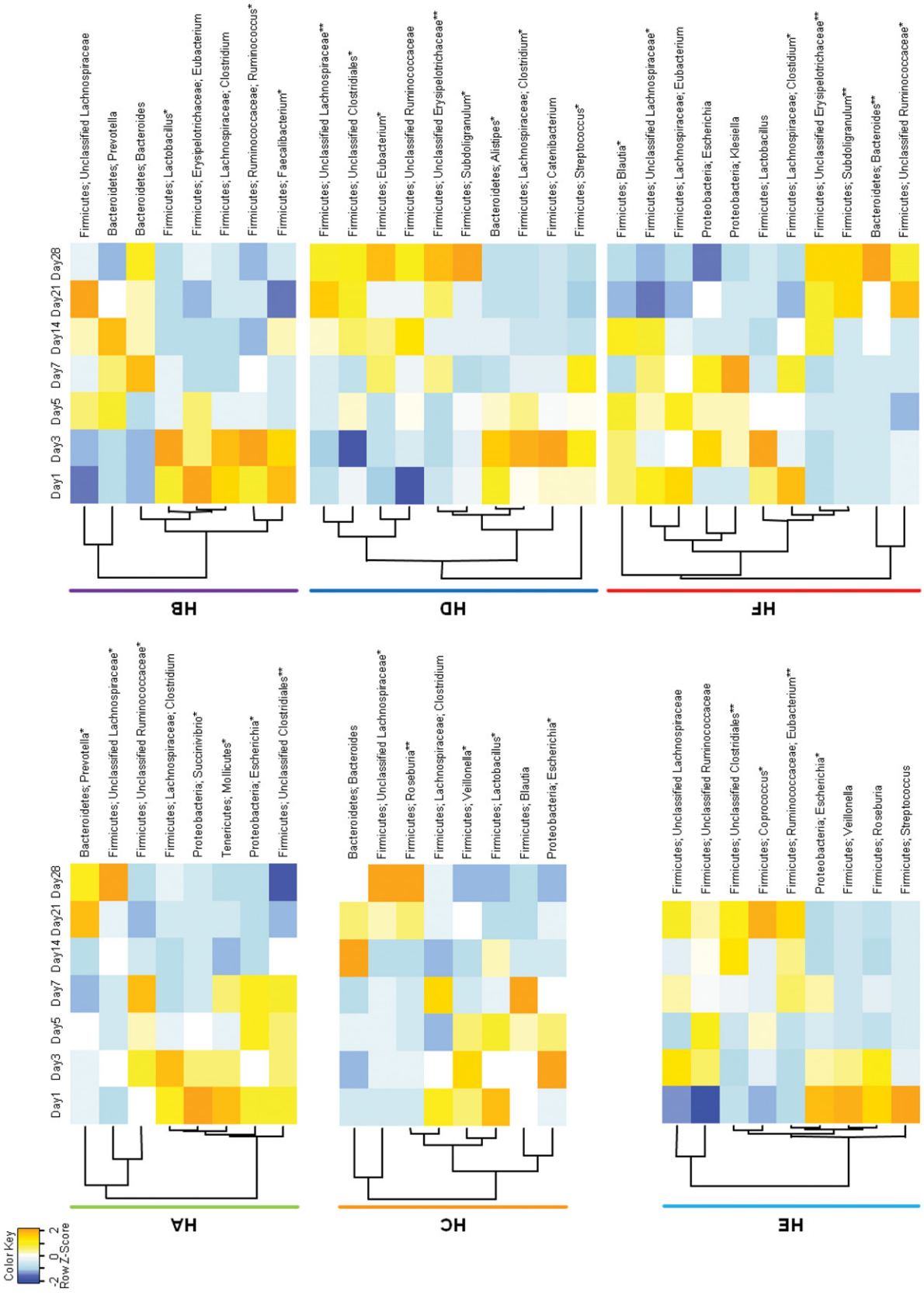

Fig. 5. The main taxonomic groups affected by an SVD. Changes of relative abundance of different taxonomic groups in the gut microbiota were visualized on a heat map (using row Z-score normalization for all data sets). Asterisks indicate a significant correlation between abundance changes and the consumption of an SVD (Pearson's correlation; $^{**}P < 0.01$; $^{*}P < 0.05$).

et al., 2012). This study showed that diet therapy reduced the relative abundance of *Enterobacteriaceae* and other bacterial groups associated with abnormalities in the gut microbiota, suggesting that a disappearance of pathobionts in this study might contribute to gut microbial homeostasis. This confirms the results of a study comparing the composition of the gut microbiota in children from Burkina Faso consuming a high-fibre diet with that of children from Europe consuming a Western-style diet (De Filippo *et al*., 2010), which suggests that the consumption of dietary fibre may inhibit the growth of pathobionts.

With a decrease in the pathobiont population, an SVD results in an increase in the population of commensal microbes. Subjects HA, HC, HD and HF showed an increase in the abundance of *Lachnospiraceae* and *Ruminococcaceae*, which correlated with the consumption of an SVD ($P < 0.05$); a similar pattern was observed in subjects HB and HE, although the significance was weak (Fig. 5). Unclassified *Erysipelotrichaceae* were also positively correlated with an SVD in subjects HD and HF ($P < 0.05$) (Fig. 5). Indeed, most of the OTUs within the *Lachnospiraceae* and *Ruminococcaceae* families showed that the populations of unclassified *Lachnospiraceae* and *Ruminococcaceae* were increased in subjects consuming an SVD. A previous study shows that *Clostridium* species belonging to Clostridium clusters XIVa and IV reduce intestinal inflammation by promoting the accumulation of regulatory T-cells in the colon (Atarashi *et al*., 2011). To determine the phylogenetic status of the OTUs identified in this study, we constructed a phylogenetic tree based on the V2 region of 16S rRNA gene sequences. The results showed that OTUs belonged to Clostridium clusters XIVa and IV (Fig. S5). Thus, it appears that the increased abundance of the *Lachnospiraceae*, *Ruminococcaceae* and *Erysipelotrichaceae* families induced by the consumption of an SVD may be associated with improved gut microbial homeostasis.

An increase in the abundance of *Prevotella* and *Bacteroides* was also observed in subjects HA, HB and HF, which was positively correlated with an SVD in subjects HA and HF ($P < 0.05$) (Fig. 5). *Prevotella* and *Bacteroides* are mainly responsible for the degradation of plant- and host-derived polysaccharides in the intestine (Wright *et al*., 2000; Martens *et al*., 2009); therefore, it appears that an SVD stimulates the growth of polysaccharide-degrading bacteria. Most of the OTUs assigned to the genus *Bacteroides* in subjects HC and HF were identified as *Bacteroides fragilis* (Table S4). Polysaccharide A produced by *B. fragilis* shows immunomodulatory characteristics in the colon (Round and Mazmanian, 2010); therefore, the selective increase in the *Prevotella* and *Bacteroides* populations consuming an SVD may also be associated with improved microbial homeostasis in the intestine.

An SVD increases the number of commensal bacteria in the gut, particularly *Bacteroides*, *Prevotella*, *Lachnospiraceae* and *Ruminococcaceae*, which can utilize the plant-derived polysaccharides as an energy source (Flint *et al*., 2008). A previous study shows that *Lachnospiraceae* can prevent infection by *Clostridium difficile* (Reeves *et al*., 2012). Another study shows that the gut microbiota in healthy individuals is enriched for *Lachnospiraceae*, *Ruminococcaceae* and *Erysipelotrichaceae* (Qin *et al*., 2012). Moreover, *B. fragilis* and *Clostridium* species of Clostridium clusters XIVa and IV induce anti-inflammatory effects in the colon by promoting the development of inducible $CD4^+Foxp3^+$ regulatory T-cells (Round and Mazmanian, 2010; Atarashi *et al*., 2011). Thus, the prebiotic effect of dietary fibre appears to increase resistance to colonization by pathobionts and to increase the population of commensal microbes.

Reduction of faecal lipocalin-2 and short-chain fatty acids by diet therapy

Given the disappearance of pathobionts from the intestine, one would expect to observe a reduction in intestinal inflammation in subjects. To address this question, we measured the concentration of faecal lipocalin-2 (Lcn-2), which is a sensitive biomarker of intestinal inflammation (Chassaing *et al*., 2012). The concentration of faecal Lcn-2 declined significantly between day 1 and day 28 in all subjects ($P < 0.05$) (Fig. 6A and Table S6), suggesting that promotion of microbial homeostasis by an SVD resulted in reduced intestinal inflammation. Changing the gut microbiota may have anti-inflammatory effects in the intestine. The marked decrease in faecal Lcn-2 levels noted in this study supports the concept of an immunosuppressive feedback mechanism; this anti-inflammatory effect may alleviate the symptoms associated with metabolic diseases (Musso *et al*., 2011) and contribute to an improvement in glucose tolerance and lipid metabolism observed in the study subjects.

Bacterial short-chain fatty acids (SCFAs) regulate host lipid metabolism (Turnbaugh *et al*., 2006). Therefore, we measured the levels of acetate, propionate and butyrate in faecal samples obtained on days 1, 14 and 28 using a solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME-GC-MS/MS). We noted a significant reduction in the concentrations of acetate and butyrate on day 28 compared with day 1 ($P < 0.05$) (Fig. 6B). Although the prebiotic effects associated with dietary fibre are thought to be primarily mediated by SCFAs, this study showed that an SVD did not induce SCFA production in the subjects. Bacteria-derived SCFAs are used as energy sources by colonocytes (Topping and Clifton, 2001), and act to regulate inflammatory responses (Maslowski *et al*., 2009) and suppress fat

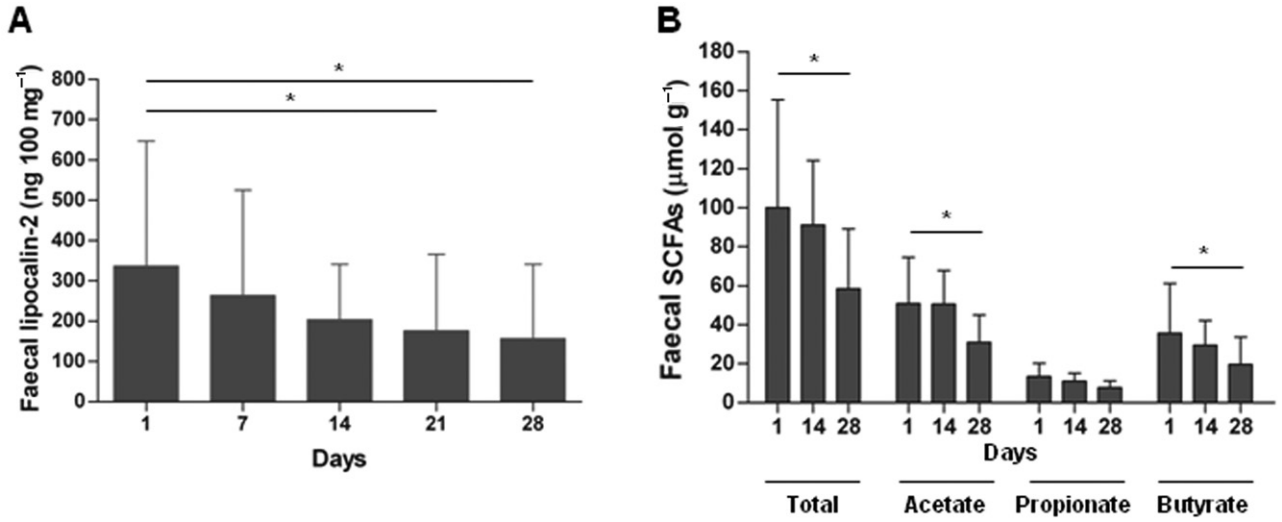

Fig. 6. Changes in faecal lipocalin-2 and short-chain fatty acid concentrations.
A. The concentration of faecal lipocalin-2 (a marker of intestinal inflammation) was monitored over time. The levels measured on day 1 and on day 28 (day 21 for subject HE) were compared using the paired *t*-test.
B. The concentrations of faecal short-chain fatty acids (acetate, propionate and butyrate) in faecal samples obtained on days 1, 14 and 28 were measured using SPME-GC-MS/MS. Asterisks indicate statistically significant differences ($P < 0.05$; paired *t*-test).

accumulation (Kimura *et al*., 2013) mediated by immune cells via G protein-coupled receptor 43 signalling. The immunosuppressive role of SCFAs is supported by the finding that patients with inflammatory bowel disease have low concentrations of faecal SCFAs (Huda-Faujan *et al*., 2010). By contrast, high concentrations of SCFAs are thought to contribute to obesity. SCFAs induce the release of peptide YY via GPR 41 signalling, leading to increased energy extraction from the diet (Samuel *et al*., 2008), and increased levels of SCFAs reflect increased energy extraction by the altered gut microbiota (Turnbaugh *et al*., 2006; Cho *et al*., 2012). Actually, obese individuals excrete large amounts of SCFAs in the faeces (Schwiertz *et al*., 2010). Improved insulin sensitivity is observed in individuals transplanted with gut microbiota containing a high abundance of butyrate-producing bacteria, but this does not correlate with the level of faecal SCFAs (Vrieze *et al*., 2012b). Despite this discrepancy, the low levels of faecal SCFAs measured in this study may be connected to the weight loss observed in the study subjects. Besides, low fermentable non-starch polysaccharides highly dominant in SVD might be affected in faecal SCFAs concentration (Hill, 1997).

Conclusion

Studying the human gut microbiota may provide useful insights into diet–gut microbiota–disease relationships. This study focused on diet therapy using an SVD by analysing metabolic biomarkers and anti-inflammatory responses, and showed that an increased intake of dietary fibre promoted microbial homeostasis by low *Firmicutes*-to-*Bacteroidetes* ratio, and a combination of an increase in the abundance of commensal bacteria and a decrease in the abundance of pathobionts in the intestine, resulting in improved metabolic and immunological parameters in patients with metabolic diseases. The diet–gut microbiota–disease relationships described in this study will make a significant contribution to the diagnosis and treatment of obesity and metabolic diseases.

Acknowledgements

This work was supported by a grant from the Mid-Career Researcher Program (2012-0008806) and NRF-2012-Forstering Core Leaders of the Future Basic Science Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology. M.-S. K. was supported by a grant from a Hi Seoul Science (humanities) Fellowship funded by the Seoul Scholarship Foundation. We thank Prof. Park Young-Seok in Kyung Hee University, Dr. Justin Kuczynski in University of Colorado and Tanya Yatsunenko in Washington University School of Medicine for bioinformatics' advice. We also thank the Jeju Center at the Korea Basic Science Institute for performing faecal SCFA analysis.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Changes in blood pressure in subjects consuming an SVD. Systolic and diastolic blood pressure was measured twice a day during the study (A–F) (HA, a; HB, b; HC, c; HD, d; HE, e; and HF, f). Circles indicate systolic blood pressure and squares indicate diastolic blood pressure.
Fig. S2. The dietary intervention induces changes of the gut microbiota over time. To determine whether an SVD causes the changes in the gut microbiota, the communities were compared the community of day 28 as the baseline with those of all other days. The data are based on unweighted UniFrac distance (A) and weighted UniFrac distance (B). The *P*-values

were calculated using Pearson's correlation. The *P*-values in parenthesis were derived from a linear regression.

Fig. S3. Principal coordinates analysis (PCoA) of the gut microbiota and gut enterotypes. Individual changes in the gut microbial communities (A) were defined according to unweighted UniFrac analysis. The enterotypes (B) were determined by cluster analysis using the partitioning around medoids method based on Jensen–Shannon divergence and visualized by between-class analysis. The genera that make the main contribution to a particular enterotype are indicated around each cluster.

Fig. S4. Quantification of the abundances of the *Enterobacteriaceae* family and the *Gammaproteobacteria* phylum. Using quantitative PCR analysis based on 16S rRNA gene sequences, the decrease in the abundances of the *Enterobacteriaceae* family (A) and the *Gammaproteobacteria* phylum (B) were observed in subject HA, HC, HE and HF (Pearson's correlation; $*P < 0.05$).

Fig. S5. Phylogeny of 16S rRNA gene sequences derived from unclassified *Lachnospiraceae* and *Ruminococcaceae*. The phylogenetic status of the OTUs (blue) assigned to unclassified *Lachnospiraceae* and *Ruminococcaceae* were determined by constructing a phylogenetic tree using the neighbor-joining method based on the V2 region sequences of the 16S rRNA genes. The sequences derived from colonic interfold microbes and from *Lachnospiraceae* isolates (red) (Nava *et al*., 2011; Reeves *et al*., 2012) were included in the phylogeny.

Table S1. Characteristics of the volunteers in this study.

Table S2. Menus of an SVD in the diet therapy.

Table S3. Nutrient components of an SVD [mean ± standard deviation (SD)].

Table S4. Alpha-diversity of the gut microbial communities of the subjects.

Table S5. The changes in the relative and absolute abundances of taxonomic groups mainly affected by an SVD.