Journal of Functional Foods 99 (2022) 105333

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Effect of *Ecklonia cava* polyphenol on adiposity reduction is associated with gut microbiota composition in subjects with abdominal obesity: A secondary analysis

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ARTICLE INFO

Keywords:
Association
Human clinical trial
Gut microbiota
Obesity
Phlorotannin
Functional profile prediction

ABSTRACT

A previous randomized controlled trial showed that *Ecklonia cava* polyphenol (EP) has the potential to reduce fat accumulation based on clinical outcomes and target gene expression analysis. Here, we investigated whether EP affects gut microbiota composition and impacts clinical outcomes. To this end, we analyzed 16S rRNA sequencing on fecal samples from subgroup subjects with abdominal obesity ($n = 20$/group). Compared with the placebo treatment, EP supplementation attenuated the increase of the *Firmicutes*-to-*Bacteroidetes* ratio and changed 18 genera composition, including genera related to obesity and fermentation of unabsorbed compounds. Furthermore, Spearman's correlation and Tax4Fun analysis demonstrated that alterations in genera are tightly associated with reducing adiposity via regulating oxidative stress. Together, these findings suggest that the gut microbiota-regulating effect might be a possible mechanism of EP-induced adiposity reduction in subjects with abdominal obesity.

1. Introduction

Accumulating evidence from epidemiological and clinical studies shows that obesity and its related chronic diseases can be prevented or managed by lifestyle interventions (Heymsfield & Wadden, 2017). Along with behavior therapy, various dietary interventions have been used to treat subjects with obesity. However, more and more research has recently focused on proposing functional foods as a complementary approach to reducing adiposity. Moreover, the recent advances in omics research offer opportunities to create a new paradigm for nutritional assessment and a new research area for functional foods (Angelakis et al., 2012; Marzullo et al., 2020; Shanahan et al., 2017), opening many possibilities. For example, a complex microbial ecosystem composed of at least 10^{14} bacterial cells can regulate host metabolism and homeostasis (Tilg & Kaser, 2011). Conversely, different dietary components can affect the gut microbiota balance differently, thus aggravating or improving the susceptibility to obesity risks (Duranti et al., 2017; Marzullo et al., 2020). Therefore, it is critical to understand the interactions

Abbreviations: ANCOM, analysis of the composition of microbiomes; BMI, body mass index; CAT, catalase; CLR Perm, centered log-ratio transformation and permutation logistic regression model; CONSORT, Consolidated Standards of Reporting Trials; DBP, diastolic blood pressure; F:B ratio, Firmicutes-to-Bacteroidetes ratio; FMI, fat mass index; GPx, glutathione peroxidase; HPLC, high-performance liquid chromatography; KEGG, Kyoto Encyclopedia of Genes and Genomes; KO, Kyoto Encyclopedia of Genes and Genomes Orthologus; LDL-cholesterol, low density lipoprotein cholesterol; HDL-cholesterol, high density lipoprotein cholesterol; MDA, malondialdehyde; OTU, operational taxonomic unit; Ox-LDL, oxidized-low-density lipoprotein; RFS, recommended food score; SBP, systolic blood pressure; SCFA, short-chain fatty acid; EP, *Ecklonia cava* polyphenol; SMI, skeletal muscle index; SOD, superoxide dismutase; ZIBSeq, zero-inflated beta regression; ZIG, zero-inflated Gaussian mixture model.

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https://doi.org/10.1016/j.jff.2022.105333
Received 13 May 2022; Received in revised form 28 September 2022; Accepted 7 November 2022
Available online 16 November 2022

among diet, microbiota, and host when looking for functional foods or bioactive ingredients to reduce the burden of obesity and related disease risks (Zhang et al., 2010).

Of all dietary components, plant foods seem most promising in modulating the host metabolism and gut microbiota composition due to an array of biologically active compounds with various functional properties (Ussar et al., 2015). Polyphenols have received particular attention as regulatory molecules activating the diverse genes and signaling pathways in obesity and crosstalk between gut microbiota and host metabolism (Most et al., 2017). *Ecklonia cava* is an edible marine brown alga abundant in the seas of central and southern Japan and South Korea. It contains unique polyphenols called phlorotannins that are structurally different from polyphenols found in land-based plants (Li et al., 2009). Previous studies, including our clinical trial, investigated the effect of *Ecklonia cava* polyphenol (EP) on body fat and oxidative stress by analyzing the clinical and targeted gene data in animals and humans (Abbas et al., 2022; Jeon et al., 2015; Lee et al., 2018). However, whether EP intervention can affect gut microbiota composition and whether alterations in gut microbiota composition can explain the effect of EP on reducing adiposity remain unknown.

Therefore, in the current study, we tested the hypothesis that daily EP supplementation might affect gut microbiota composition and, in turn, impact the effectiveness of EP against adiposity. To address this hypothesis, we performed 16S rRNA sequencing of fecal samples from subjects with abdominal obesity included in the previous clinical trial (Lee et al., 2018). Then, we investigated the relationship between EP-induced alterations in gut microbiota composition and clinical outcomes by correlation analysis and functional profile prediction.

2. Materials and methods

2.1. Test materials, subjects, and study design

The preparation of test materials, dosage information, and clinical trial details were reported in the previous publication (Lee et al., 2018). For this secondary analysis, out of 63 participants who completed the trial, we included 40 subjects (n = 20/group) who had abdominal obesity (visceral adipose tissue area $\geq$ 100 cm^2 or waist circumference $>$ 90 cm for men and $>$ 80 cm for women) and provided qualified fecal samples at baseline and endpoint. The study protocol was approved by the Institutional Review Board of Ewha Womans University (IRB No. 67–14) and registered prospectively at the WHO International Clinical Trials Registry Platform via Clinical Research Information Service in Korea (Registration number: KCT0001074).

2.2. DNA extraction and pyrosequencing

Fecal sample processing and pyrosequencing was performed at ChunLab, Inc. (Seoul, Korea). Briefly, total DNAs were extracted using the Fast DNA SPIN Kit for feces (MP Biomedicals, Santa Ana, CA, USA), and quality and quantity were measured using a NanoDrop ND-2000 (Thermo Scientific, Rockford, IL, USA). PCR amplification on the V1 − V3 region of the 16S rRNA gene was carried out with unique reverse barcoded primers, and the pyrosequencing was subsequently implemented with a 454 GS Junior Sequencing System (Roche, Branford, CT, USA).

2.3. 16S data processing

Raw reads were filtered by removing reads containing adaptor sequences, low-quality reads (Phred quality score $<$ 20), and reads shorter than 350 bp or longer than 550 bp. Chimeric sequences introduced by PCR amplification were identified and removed using VSEARCH with the SILVA gold database (Rognes et al., 2016). The remaining sequence reads were clustered into operational taxonomic units (OTUs) using VSEARCH with a de novo clustering algorithm under a threshold of 97 % sequence similarity, followed by taxonomy assignment with UCLUST (parallel_assign_taxonomy_uclust.py script on QIIME 2) under default parameters (Caporaso et al., 2010). OTUs containing one sequence in only one subject were excluded from further analysis.

2.4. Comparative analysis of abundance changes in gut microbiota

OTUs were filtered out from the dataset if the zero proportion was>99 %. Then, we performed seven statistical analyses on count data, including the Wilcoxon rank-sum test, DESeq2, edgeR, zero-inflated beta regression, zero-inflated Gaussian mixture model, analysis of the composition of microbiomes, and centered log-ratio transformation and permutation logistic regression model, to identify the alterations of the gut microbiome composition by EP treatment compared with the placebo treatment (Kim et al., 2020). Finally, we compiled a list of the differential genera identified as statistically significant by at least four statistical methods for further analysis. The correlations between differential genera and clinical parameters were analyzed using the Spearman rank correlation coefficient using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

2.5. Prediction of functional profiles of gut microbiota

Computational prediction of the functional capabilities was performed based on the 16S rRNA dataset using the Tax4Fun tool from MicrobiomeAnalyst (Asshauer et al., 2015). The Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthologus (KO) provided by Tax4Fun was analyzed using the Shotgun Data Profiling section in the Microbiome Analyst. The Wilcoxon rank-sum test was carried out by MetaboAnalyst version 5.0 (https://www.metaboanalyst.ca/) to compare differentially expressed KOs between the placebo and EP groups. The results were visualized with pathway analysis using Cytoscape version 3.7.2.

3. Results

3.1. Baseline characteristics

The Consolidated Standards of Reporting Trials diagram in Fig. 1 shows the flow from the subject enrollment to the secondary data analysis. For the secondary analysis, subgroup subjects who had abdominal obesity (n = 20/group) and provided fecal samples at the baseline and end of the trial were included. The baseline characteristics of the secondary study were not significantly different from the first study (**Supplementary Table S1**) and between placebo and EP groups except for the visceral adipose tissue area (Table 1). However, subjects included in the second study were all who met the following criteria: visceral adipose tissue area $>$ 100 cm^2 or waist circumference $>$ 90 cm for men and $>$ 80 cm for women.

3.2. Adiposity suppression effect

Consistent with the first trial results, EP supplementation effectively reduced adiposity and oxidative stress in subgroup subjects with central obesity (Table 2). Adiposity markers, including BMI, body weight, and fat mass, were significantly reduced in EP compared with placebo. Moreover, EP showed effectiveness in boosting the endogenous antioxidant responses, such as SOD and GPx, thus decreasing the ox-LDL level compared with placebo group.

3.3. Alterations in microbiota composition

A total of 1,936,293 high-quality sequences were obtained, delineating into 1,355 OTUs at a similarity level of 97 %. The α- and β-diversity analysis results demonstrated no significant differences between the two groups (**Supplementary** Fig. 1). However, we could find significant differences in microbiota composition between the two groups

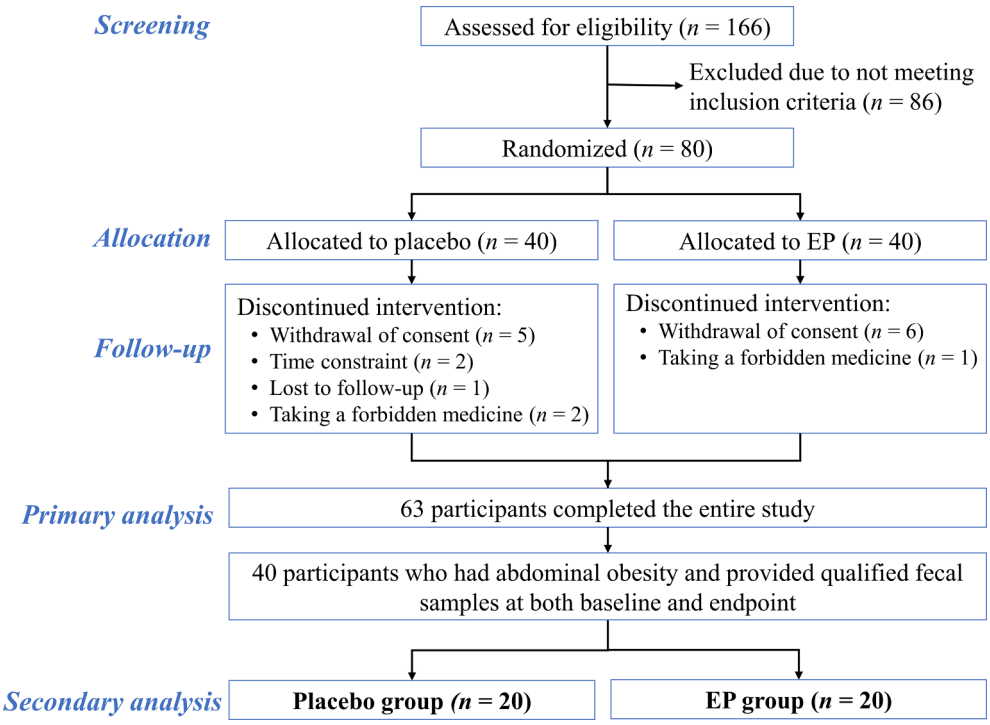


Fig. 1. CONSORT flow diagram of the secondary analysis study presented from the subject enrolment through data analysis. CONSORT, Consolidated Standards of Reporting Trials; EP, *Ecklonia cava* polyphenol.

Table 1
Baseline characteristics of the subjects included in the secondary analysis.

Variables	Placebo (n = 20)	EP (n = 20)	p-value
Age	39.5 ± 2.4	35.3 ± 1.9	0.180
Sex (male/female, n)	9/11	8/12	0.749
BMI (kg/m^2)	27.8 ± 0.3	27.6 ± 0.3	0.541
Waist circumference (cm)	90.5 ± 1.4	90.4 ± 1.0	0.952
Male	92.9 ± 1.5	93.4 ± 1.5	0.819
Female	88.5 ± 2.1	88.4 ± 1.2	0.954
Visceral adipose tissue area (cm^2)	144.1 ± 10.1	109.4 ± 8.6	0.013
Percentage body fat (%)	34.0 ± 1.3	33.5 ± 1.4	0.815
SBP (mmHg)	121.2 ± 2.0	118.5 ± 3.2	0.471
DBP (mmHg)	80.3 ± 1.7	82.4 ± 2.8	0.534
RFS (0–47)	22.8 ± 1.9	19.3 ± 2.3	0.244
Alcohol drinker, n (%)	12 (60.0)	17 (85.0)	0.177
Smoker, n (%)	1 (5.0)	3 (15.0)	0.565
Energy (kcal/day)	1513.3 ± 108.5	1631.9 ± 106.9	0.444
Carbohydrate (g/day)	219.2 ± 14.1	224.2 ± 13.0	0.795
Protein (g/day)	54.2 ± 5.1	58.3 ± 6.2	0.614
Fat (g/day)	46.2 ± 4.2	49.3 ± 5.2	0.656

Values are presented as the means ± standard error. Student's t-test or chi-square test was used to analyze the difference between groups. Nutrient intakes were estimated using a 3-day food record including one holiday/Sunday. EP, *Ecklonia cava* polyphenol; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; RFS, recommended food score.

at week 12 (Fig. 2**A**). The *Firmicutes*-to-*Bacteroidetes* (F:B) ratio significantly increased in placebo group, but the EP intervention attenuated this increase. As a result, there was a significant interaction between treatment and week, suggesting a significant difference in the F:B ratio between the two groups (Fig. 2**B**).

3.4. Differential microbiota changes between EP and control groups

We next sought interventions' effect on gut microbiota changes at the genus level. The Venn diagram in Fig. 3 depicts the number of genera selected as the differential between placebo and EP groups by 7 statistical methods. Eighteen genera were identified as differential markers for further analysis. *Methylobacterium* and *Terrisporobacter* were decreased most in the EP group, followed by *Microbacterium*, *Flavobacterium*, *Candidatus_Saccharimonas*, *Brevundimonas*, *Phreatobacter*, *Methanosaeta*, *Pseudoflavonifractor*, and *Blastococcus*. Conversely, *Butyricimonas* was the most prevalently enriched genus in the EP group than the placebo, followed by *Gordonibacter*, *Ruminococcaceae* UCG-004, *Coprococcus 2, Family XIII AD3011 group, uncultured (family* Peptococcaceae*), Eisenbergiella, and Desulfovibrio*.

3.5. Association between changes in gut microbiota and clinical outcomes

Spearman's rank correlation coefficient analysis in Fig. 4**A** revealed that 10 genera decreased in EP group positively correlated with adiposity and oxidative damage biomarkers but negatively correlated with antioxidant biomarkers (box 1), whereas 8 enriched genera showed the reverse condition (box 2). *Microbacterium* was positively associated with changes in BMI, body weight, fat mass, and FMI but negatively associated with SMI ($p < 0.05$). *Blastococcus* was positively associated with BMI and body weight, whereas *Ruminococcaceae* UCG-004 and *Butyricimonas* were negatively associated ($p < 0.05$). *Eisenbergiella*, *Gordonibacter*, and *Butyricimonas* were negatively associated with triglycerides ($p < 0.05$). Positive associations were found between *Candidatus Saccharimonas* and *Methylobacterium* and ox-LDL, whereas ox-LDL was negatively associated with *Desulfovibria* ($p < 0.05$). In addition, *Terrisporobacter* was negatively associated with changes in SOD and GPx activities, while *Ruminococcaceae* UCG-004 was positively associated with SOD ($p < 0.05$).

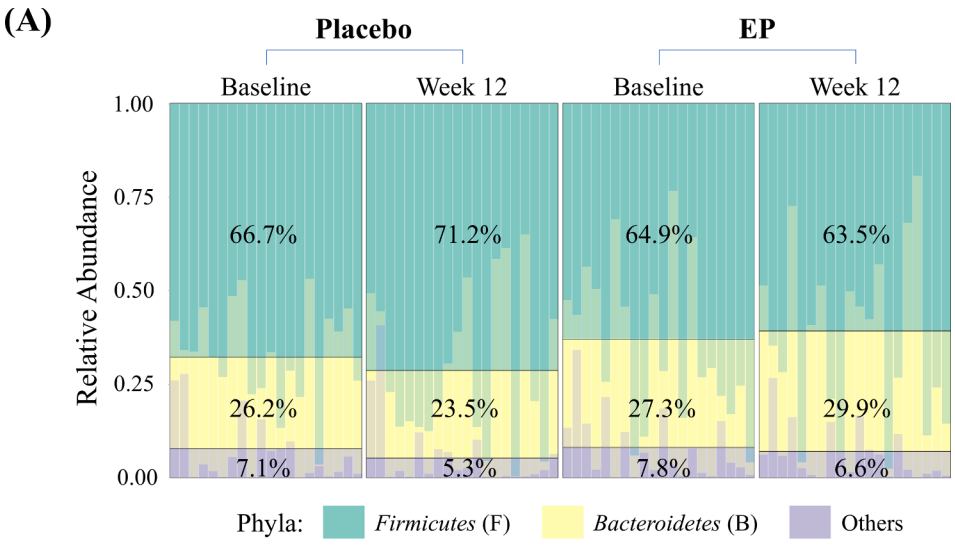
3.6. Prediction of the functional pathways of the gut microbiome

The Tax4Fun was performed to predict the functional profiles of gut microbiota. The differences in KEGG abundance in the two groups were evaluated using Wilcoxon rank-sum test. As shown in Fig. 4**B**, the most related KEGG pathway types were metabolism. KEGG analysis revealed two significantly enriched pathways in amino acid metabolism,

Table 2
Changes in anthropometric, metabolic, and oxidative stress parameters in subgroup subjects included in the secondary analysis.

Variables	Placebo (n = 20)		EP (n = 20)		β	p-value
	Baseline	Week 12	Baseline	Week 12		
Adiposity-related biomarkers						
BMI (kg/m^2)	27.8 ± 0.3	28.1 ± 0.3	27.6 ± 0.3	27.4 ± 0.3	−0.44	0.024
Body weight (kg)	77.2 ± 1.9	78.0 ± 2.0	79.4 ± 1.9	78.8 ± 2.0	−1.43	0.006
Waist circumference (cm)	90.5 ± 1.2	92.1 ± 1.6	90.4 ± 1.2	91.6 ± 1.6	−0.40	0.820
Hip circumference (cm)	102.0 ± 0.8	104.0 ± 0.8	103.1 ± 0.8	104.0 ± 0.8	−1.07	0.301
Fat mass (kg)	26.0 ± 0.9	26.5 ± 1.0	26.4 ± 0.9	25.7 ± 1.0	−1.24	0.045
SMI (%)	36.9 ± 0.9	36.7 ± 0.9	37.1 ± 0.9	37.5 ± 0.9	0.62	0.072
FMI (kg/m^2)	0.95 ± 0.04	0.97 ± 0.04	0.93 ± 0.04	0.90 ± 0.04	−0.04	0.061
Triglyceride (mg/dL)	141.7 ± 19.9	169.6 ± 19.2	105.8 ± 19.9	99.3 ± 19.2	−34.4	0.051
Total-cholesterol (mg/dL)	204.8 ± 7.9	207.2 ± 8.5	191.1 ± 7.9	187.0 ± 8.5	−6.50	0.321
LDL-cholesterol (mg/dL)	133.6 ± 6.8	136.0 ± 7.6	124.2 ± 6.8	119.8 ± 7.6	−6.75	0.297
HDL-cholesterol (mg/dL)	49.4 ± 2.2	51.6 ± 2.7	52.3 ± 2.2	55.5 ± 2.7	0.90	0.664
Oxidative stress-related biomarkers						
Plasma MDA (nmol/mL)	2.4 ± 0.1	2.4 ± 0.2	2.1 ± 0.1	2.2 ± 0.2	0.09	0.624
Urinary MDA (nmol/mL/g creatinine)	1.4 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	1.7 ± 0.2	−0.20	0.479
Ox-LDL (U/L)	42.8 ± 2.4	43.4 ± 2.3	39.5 ± 2.4	36.7 ± 2.3	−3.38	0.018
SOD (U/mL)	321.3 ± 20.1	316.6 ± 23.3	327.7 ± 20.1	351.6 ± 23.3	28.71	0.019
GPx (μmol/min/mL)	1.44 ± 0.12	1.42 ± 0.12	1.43 ± 0.12	1.66 ± 0.12	0.254	0.007
CAT (μmol/min/mL)	4.23 ± 0.20	4.38 ± 0.21	4.29 ± 0.20	4.55 ± 0.21	0.108	0.348

Values are presented as the LS means ± standard error. The β estimates and p-values were obtained for the treatment × week interaction using a linear mixed-effect model. EP, *Ecklonia cava* polyphenol; BMI, body mass index; SMI, skeletal muscle index (skeletal muscle mass ÷ body weight) × 100; FMI, fat mass index (fat mass ÷ height); LDL-cholesterol, low density lipoprotein cholesterol; HDL-cholesterol, high density lipoprotein cholesterol; MDA, malondialdehyde; Ox-LDL, oxidized-low-density lipoprotein; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase.



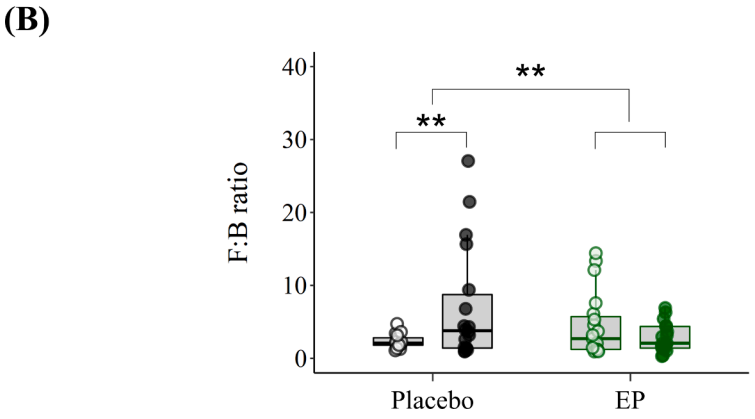
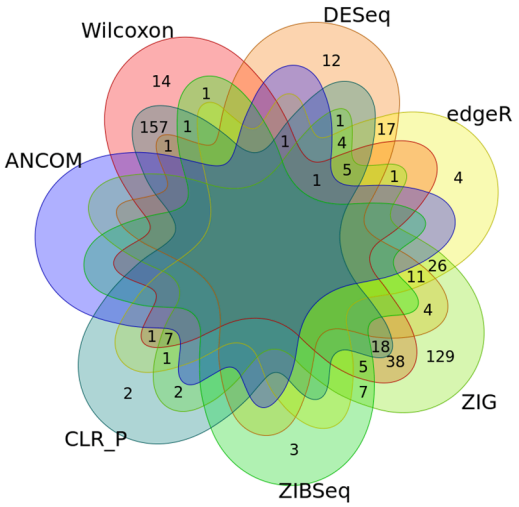


Fig. 2. Compositional alterations in bacterial abundance at the phylum level. (A) Stacked bar plots depict the average percentage and individual relative abundance of main phyla in the placebo and EP groups at baseline and week 12. (B) A boxed plot compared the *Firmicutes*-to-*Bacteroidetes* (F:B) ratios in the placebo (black) and EP groups (green) at baseline (empty circle) and week 12 (filled circle). The linear mixed-effect model was used to test the differences within and between groups. $^{**}p < 0.01$. EP, *Ecklonia cava* polyphenol; F:B ratio, *Firmicutes*-to-*Bacteroidetes* ratio. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



Genus	Wilcoxon	DESeq2	edgeR	ZIG	ZIBSeq	CLR perm	ANCOM
Brevundimonas	0.009	0.185	0.026	0.001	1.000	0.009	1
Methanosaeta	0.013	0.313	0.045	0.000	1.000	0.010	1
Phreatobacter	0.013	0.109	0.042	0.014	1.000	0.010	1
Blastococcus	0.008	0.089	0.009	0.033	1.000	0.006	1
Pseudoflavonifractor	0.009	0.037	0.001	0.023	0.677	0.006	1
Microbacterium	0.002	0.199	0.026	0.000	1.000	0.003	1
Flavobacterium	0.018	0.065	0.030	0.010	1.000	0.018	1
Candidatus Saccharimonas	0.006	0.005	0.037	0.003	0.588	0.008	1
Terrisporobacter	0.003	0.013	0.028	0.097	0.526	0.006	0
Methylobacterium	0.008	0.057	0.011	0.000	1.000	0.006	1
Desulfovibrio	0.414	0.045	0.004	0.000	0.725	0.038	1
Eisenbergiella	0.134	0.004	0.002	0.000	0.185	0.014	1
uncultured (Family Peptococcaceae)	0.121	0.044	0.015	0.000	0.289	0.027	1
Family XIII AD3011 group	0.026	0.008	0.041	0.021	0.112	0.033	1
Coprococcus 2	0.068	0.001	0.002	0.016	0.219	0.030	1
Ruminococcaceae UCG-004	0.002	0.003	0.005	0.005	0.056	0.004	1
Gordonibacter	0.018	0.019	0.022	0.000	0.161	0.008	1
Butyricimonas	0.001	0.025	0.003	0.000	0.137	0.000	0

Values are presented as the *p*-value. The *p*-values are obtained from 7 statistical methods (Wilcoxon rank sum test, EdgeR, DESeq2, ZIBSeq, ZIG, CLR perm, and ANCOM). The differential genera have the statistical significance of more than 4 among 7 statistical methods at $p<0.05$.

Fig. 3. Differentially abundant genera between the two groups. (A) Venn diagram depicts the number of differential genera selected from the comparison between placebo and EP groups by the seven statistical methods ($p < 0.05$). EP, *Ecklonia cava* polyphenol; ANCOM, analysis of the composition of microbiomes; CLR Perm, centered log-ratio transformation and permutation logistic regression model; ZIBSeq, zero-inflated beta regression; ZIG, zero-inflated Gaussian mixture model.

including the serine biosynthesis pathway (M00020) and the lysine degradation pathway (M00032). In addition, the ubiquinone biosynthesis pathway (M00128) in cofactor and vitamin metabolism and the anthranilate degradation pathway (M00637) in xenobiotics metabolism were also significantly enriched in EP than placebo group (Fig. 4**C**).

4. Discussion

Following the previous study, the current study improved the methodology to investigate the underlying mechanisms of EP supplementation in reducing adiposity by analyzing 16S rRNA sequencing in fecal samples. As expected, diet and exercise control with EP supplementation for 12 weeks effectively reduced adiposity and oxidative stress compared to the placebo group without EP. In addition, the 16S rRNA data provided novel evidence that EP intervention induced specific alterations in gut microbiota composition associated with clinical outcomes, supporting the hypothesis.

We first found that the F:B ratio did not differ at week 0 but differed substantially between the EP and placebo groups after a 12-week intervention. *Firmicutes* and *Bacteroidetes* were the two most predominant phyla in all mammals corresponding to over 90 % of the total microbiome. Although some studies have found no difference in the F:B ratio (Fernandes et al., 2014; Moreno-Indias et al., 2014), many studies have reported a positive association between the F:B ratio and obesity in humans and animals (Crovesy et al., 2020; Koliada et al., 2017; Ley et al., 2006), suggesting a link between alterations in microbiota and improvement in adiposity.

However, the diversity of genera was not different between the two groups. This phenomenon is commonly found in clinical trials of functional foods probably because the heterogeneity of healthy individuals may outweigh the intervention effect (Ye et al., 2020). Instead, specific bacterial shifts occurred at the genus level with the EP supplementation compared with the placebo group. The most prevalent and enriched genus was *Butyricimonas*. This genus is recognized as a butyrate producer with the potential to harvest energy from unabsorbed dietary carbohydrates, thus promoting weight loss in obesity (Ibrahim & Anishetty, 2012). *Gordonibacter*, a bacteria involved in ellagitannins metabolism (Selma et al., 2014), was also enriched following EP supplementation. Tindall *et al.* (Tindall et al., 2020) also reported a similar result by presenting the enrichment of this genus in response to a diet with walnuts compared with the matched control diet in individuals with cardiovascular risk factors.

In addition, the relative abundance of *Eisenbergiella* was increased by EP supplementation. A study by Chen *et al.* (Chen et al., 2021) demonstrated that supplementation of non-digestible carbohydrates increased *Eisenbergiella* abundance in healthy subjects. Similar findings have also been observed by Peng *et al.* (Peng et al., 2020), where *Eisenbergiella* abundance and its ability to produce short-chain fatty acid were promoted in C57BL/6 mice treated with the anthocyanin-rich extract. Collectively, these studies support our findings, implicating that the enrichment of these genera might have links to the fermentation of indigestible carbohydrates. EP contains a high concentration of phlorotannins, the high-molecular-weight components consisting of several phloroglucinol units (Catarino et al., 2017). The bioaccessibility index of phlorotannin-rich extracts was calculated between 2 % and 14 % in a simulated digestive system (Catarino et al., 2021). This low value suggests a potential prebiotic effect of EP phlorotannins in modulating gut microbiota. Corona *et al.* (Corona et al., 2016) demonstrated colonic fermentation of phlorotannins by reporting seven phlorotannin-derived metabolites using *in vitro* gastrointestinal digestion and fermentation system.

On the other hand, EP reduced *Terrisporobacter,* known as obesity-promoting bacteria (Zhang et al., 2020). *Candidatus Saccharimonas* also showed a decrease in the EP intervention group. Zhai *et al.* (Zhai et al., 2021) reported that the lipolysis-promoting effect of policosanol was associated with the decrease of *Candidatus Saccharimonas* in hyperlipidemic C57BL/6 mice. However, our results on the roles of *Coprococcus 2*, *Desulfovibrio*, *Flavobacterium*, and *Pseudoflavonifractor* were not following those reported in the literature (Lozano et al., 2022; Pisanu, 2021; Qu et al., 2019; Zhang et al., 2020). Spearman's correlation analysis also supported the involvement of the gut microbiome with the reductions in body fat and oxidative stress by showing correlations between the differential genera reduced (or increased) by EP intervention and the adiposity and oxidative stress-related parameters.

We further predicted the functional capabilities of microbial communities from 16S rRNA amplicon datasets using the Tax4Fun tool (Asshauer et al., 2015). The first prediction was related to enrichment of the two amino acid metabolism, including serine biosynthesis and lysine degradation. *De novo* serine synthesis is vital in normal mitochondrial function and cellular antioxidative capacity (Zhang et al., 2018). This prediction aligns with the clinical findings that EP alleviated adiposity concomitantly with improved SOD and GPx capacities. Meanwhile, lysine degradation provides an essential link in central carbon and energy metabolism within the mitochondrial matrix, producing acetyl-

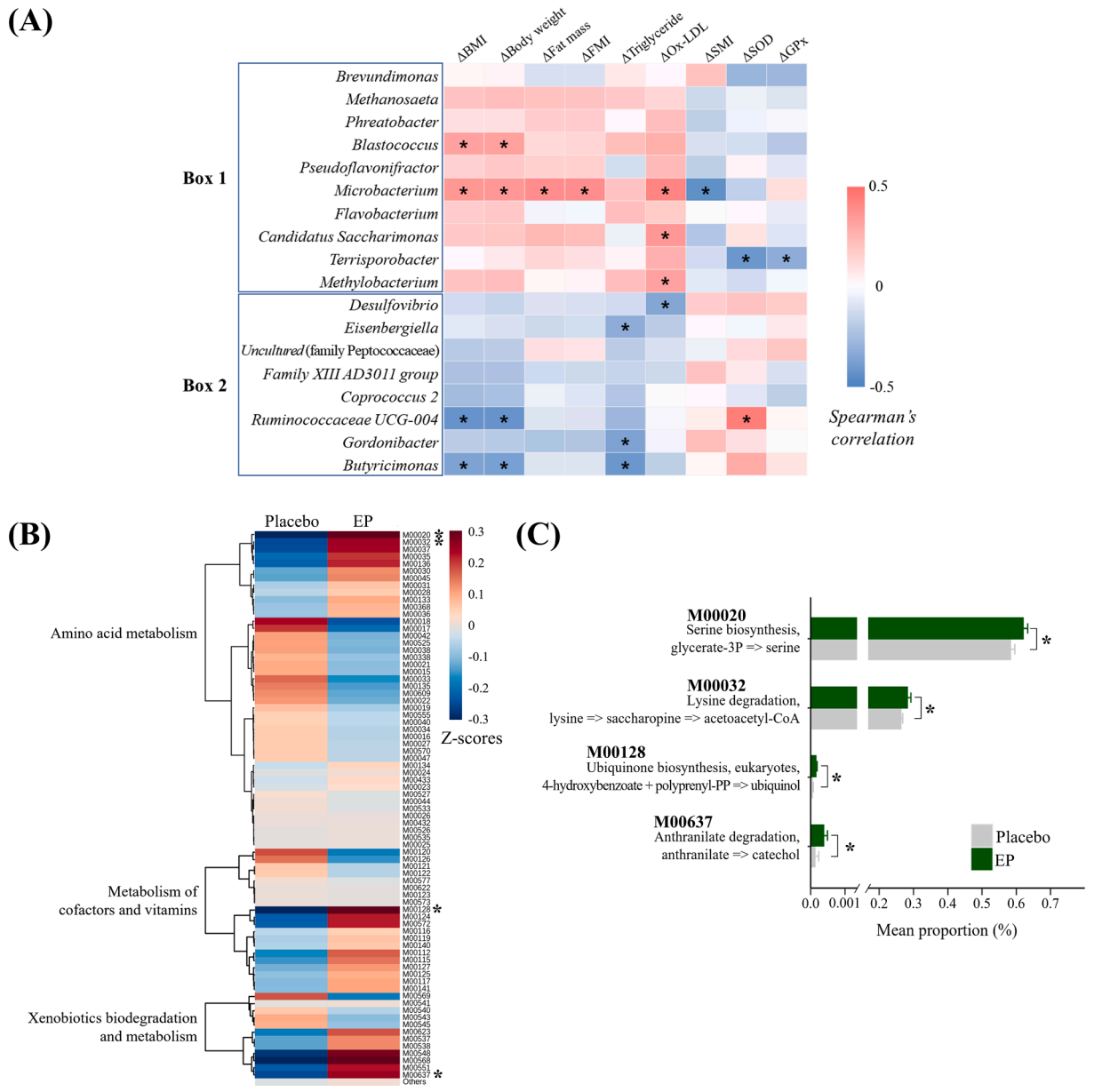


Fig. 4. Differences in functional profiles of bacterial communities between the placebo and EP groups at week 12. (A) Heatmap shows partial Spearman's correlation coefficients of differentially abundant genera with clinical outcomes. (B) Heatmap shows predicted gut microbiota function at KEGG levels 2 and 4. The abundance profiles were expressed by z-scores. The z-score was shown in blue color when the row abundance was lower than the mean and red color when the row abundance was higher than the mean. The asterisk indicates significantly different modules between the two groups. (C) Statistical results of the significantly differential modules based on the Wilcoxon rank-sum test. *$p < 0.05$. EP, *Ecklonia cava* polyphenol; BMI, body mass index; FMI, fat mass index; GPx, glutathione peroxidase; KEGG, Kyoto Encyclopedia of Genes and Genomes; Ox-LDL, oxidized-low-density lipoprotein; SMI, skeletal muscle index; SOD, superoxide dismutase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

coenzyme A (Knorr et al., 2018). This prediction is in line with the previous study where Lin *et al.* (Lin et al., 2012) stated that lysine acetylation regulated many metabolic enzymes, leading to increases in glycolysis, the tricarboxylic acid cycle, and fatty acid β-oxidation. The following prediction was about the enrichment of ubiquinone synthesis. Ubiquinone is a lipid-soluble compound involved in cellular energy production in the inner mitochondrial membrane (Chokchaiwong et al., 2018). Furthermore, Kaymak *et al.* reported that inhibition of ubiquinone synthesis could hamper electron transport by low oxygen availability and induce oxidative stress (Kaymak et al., 2020). These predictions suggest that the altered gut microbiota composition following EP supplementation might contribute to ameliorating adiposity *via* regulating oxidative stress. The last prediction was the stimulation of anthranilate degradation (Costaglioli et al., 2012). However, a thorough understanding of EP-induced enrichment of anthranilate degradation is still lacking.

Fig. 5 summarizes the overall findings as a correlation network of the differential clinical markers, genera, and functional features, generating 36 significant correlations (58.3 % positive and 41.7 % negative). EP supplementation promoted the growth of *Butyricimonas*, *Ruminococcaceae* UCG-004, *Eisenbergiella*, *Gordonibacter*, *Coprococcus* 2, and *Desulfovibrio*, which were associated with decreasing BMI, body weight, and fat mass and increasing SOD. Additionally, enrichments of *Butyricimonas* and *Ruminococcaceae* UCG-004 are directly associated with decreasing BMI and body weight and increasing SOD. Conversely, *Terrisporobacter*, *Methylobacterium*, *Candidatus Saccharimonas*, *Microbacterium*, and *Blastococcus* were less abundant in the EP group than in the placebo group. The decrease in the abundance of *Microbacterium* and *Blastococcus* was

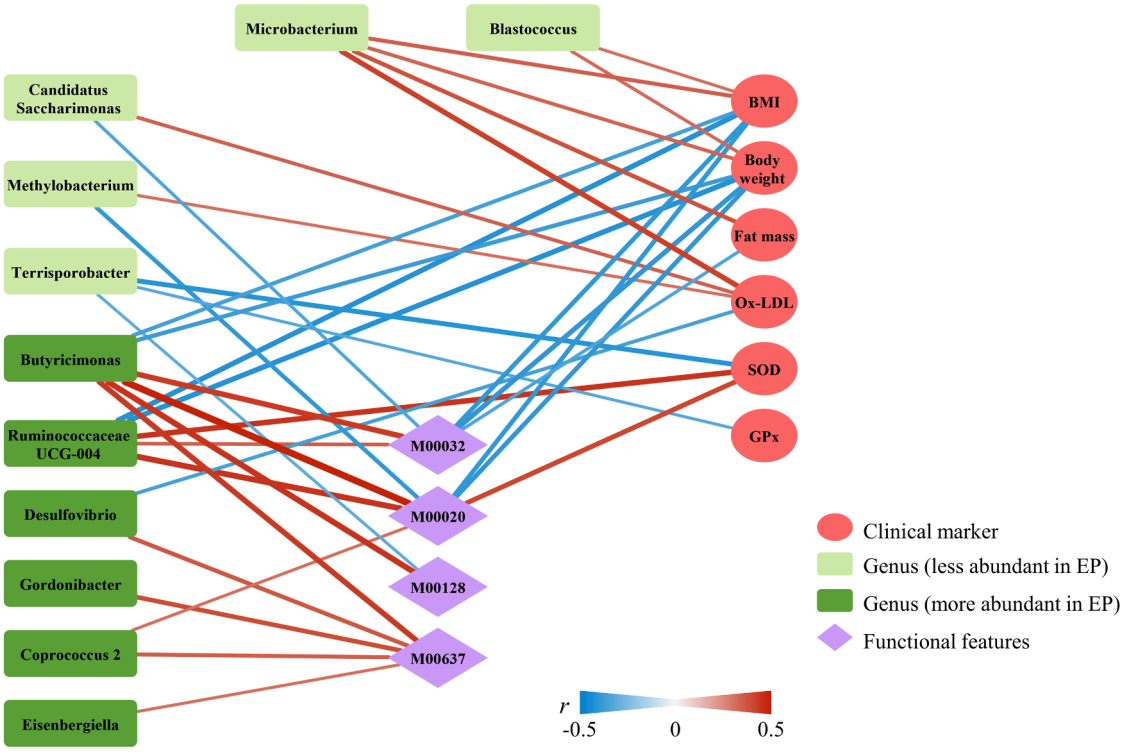


Fig. 5. Correlation network constructed among EP-induced differential clinical markers, bacterial genera, and microbial metabolic pathways. The network visualization was provided by Cytoscape version 3.7.2. The edge width and color (blue: negative and red: positive) are proportional to the correlation strength. EP, *Ecklonia cava* polyphenol; BMI, body mass index; GPx, glutathione peroxidase; M00032, lysine degradation module; M00020, serine biosynthesis module; M00128, ubiquinone biosynthesis module; M00637, anthranilate degradation module; Ox-LDL, oxidized-low-density lipoprotein; SOD, superoxide dismutase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

associated with decreasing BMI, body weight, fat mass, and ox-LDL. The decreases in the abundance of *Candidatus Saccharimonas, Methylobacterium*, and *Terrisporobacter* were associated with modulating adiposity and oxidative stress *via* enhancing lysine degradation, serine biosynthesis, and ubiquinone biosynthesis, respectively. Among all genera, *Butyricimonas* presented the broadest target scope (degree = 6), followed by *Ruminococcaceae* UCG-004 (degree = 5) and *Microbacterium* (degree = 4). The suggestions presented in this correlation network agree with the discussion in the above sections regarding the following perspectives: the beneficial effect of EP in adiposity might be associated with gut microbiota alterations related to (1) phlorotannin fermentation and (2) regulation of oxidative stress.

It is worth mentioning the limitations of this study. The first is about the small sample size that limits the generalization of our findings. The second limitation is that we speculated on the link between gut microbiota-mediated fermentation of indigestible carbohydrates and improved adiposity without measurement. Thus, we could not disclose the formation of phlorotannin metabolites generated by the biotransformation and bacterial metabolization in the colon. The third is that the sequencing methodology used in this study is outdated. Thus the results are not reproducible. The last limitation is the lack of consensus methods for differential abundance analysis of microbiomes. As each method provided different statistical significances, we used the majority voting strategy to identify significantly differentially abundant genera because choosing one specific method may impede employing multiple hypothesis testing. Further independent validations might be needed using mock-community metagenome samples to evaluate different methods for microbiome analysis. However, despite these limitations, the results from this study provided baseline information that will be useful for designing future studies to investigate this relationship comprehensively.

5. Conclusions

In summary, the data gathered by 16S rRNA sequencing of fecal samples and predictive functional analysis using Tax4Fun tool provided novel information regarding the effect of EP supplementation on the gut microbiota and their associations with reducing adiposity via the regulation of oxidative stress. Based on these unique interactions, we could suggest that the gut microbiota-regulating effect might be a possible mechanism of EP-induced adiposity reduction. However, since the limitations of the current study, further research is needed for a better understanding of this emerging science. Furthermore, a future study identifying microbiomes at least species-level would be more informative in future studies.

Ethics statement.

The study was carried out in accordance with the Helsinki Declaration, approved by the Institutional Review Board of Ewha Womans University (IRB No. 67–14) and registered prospectively at the WHO International Clinical Trials Registry Platform (ICTRP) via Clinical Research Information Service (CRIS) in Korea (Registration number: KCT0001074). All participants were informed about the risks and benefits of the study and provided written consent before the start of the intervention.

CRediT authorship contribution statement

Yu Sim Lee: Formal analysis, Visualization, Writing – original draft. **Seunghee Kang:** Formal analysis, Visualization, Writing – original draft. **Nayeon Kang:** Formal analysis, Writing – original draft. **Jaehong Yu:** Formal analysis, Visualization. **Taesung Park:** Conceptualization, Methodology. **Sunjae Lee:** Conceptualization, Methodology. **Oran Kwon:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Mendeley data: SeaPN_BM (DOI: 10.17632/cfwj5hxb3r.1)

Acknowledgments

We are thankful to all the study participants for the substantial time and effort they spent in contributing to our study. We also thank Botamedi, Inc. (Korea) for processing and providing the test materials used in both two groups.

Funding

This work was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF), funded by the Korea Ministry of Science & ICT (grant numbers: 2012M3A9C4048761 and 2013M3A9C4078158).

Research data

Mendeley data: SeaPN_BM (DOI: 10.17632/cfwj5hxb3r.1)

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2022.105333.

References

Abbas, M. A., Boby, N., Lee, E. B., Hong, J. H., & Park, S. C. (2022). Anti-Obesity Effects of Ecklonia cava Extract in High-Fat Diet-Induced Obese Rats. *Antioxidants (Basel), 11*(2). https://doi.org/10.3390/antiox11020310

Angelakis, E., Armougom, F., Million, M., & Raoult, D. (2012). The relationship between gut microbiota and weight gain in humans. *Future Microbiol, 7*(1), 91–109. https://doi.org/10.2217/fmb.11.142

Asshauer, K. P., Wemheuer, B., Daniel, R., & Meinicke, P. (2015). Tax4Fun: Predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics, 31*(17), 2882–2884. https://doi.org/10.1093/bioinformatics/btv287

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods, 7*(5), 335–336. https://doi.org/10.1038/nmeth.f.303

Catarino, M. D., Marcal, C., Bonifacio-Lopes, T., Campos, D., Mateus, N., Silva, A. M. S., … Cardoso, S. M. (2021). Impact of Phlorotannin Extracts from Fucus vesiculosus on Human Gut Microbiota. *Marine Drugs, 19*(7). https://doi.org/10.3390/md19070375

Catarino, M. D., Silva, A. M. S., & Cardoso, S. M. (2017). Fucaceae: A Source of Bioactive Phlorotannins. *International Journal of Molecular Sciences, 18*(6). https://doi.org/10.3390/ijms18061327

Chen, O., Sudakaran, S., Blonquist, T., Mah, E., Durkee, S., & Bellamine, A. (2021). Effect of arabinogalactan on the gut microbiome: A randomized, double-blind, placebo-controlled, crossover trial in healthy adults. *Nutrition, 90*, Article 111273. https://doi.org/10.1016/j.nut.2021.111273

Chokchaiwong, S., Kuo, Y. T., Lin, S. H., Hsu, Y. C., Hsu, S. P., Liu, Y. T., … Kao, S. H. (2018). Coenzyme Q10 serves to couple mitochondrial oxidative phosphorylation and fatty acid beta-oxidation, and attenuates NLRP3 inflammasome activation. *Free Radic Res, 52*(11–12), 1445–1455. https://doi.org/10.1080/10715762.2018.1500695

Corona, G., Ji, Y., Anegboonlap, P., Hotchkiss, S., Gill, C., Yaqoob, P., … Rowland, I. (2016). Gastrointestinal modifications and bioavailability of brown seaweed phlorotannins and effects on inflammatory markers. *British Journal of Nutrition, 115*(7), 1240–1253. https://doi.org/10.1017/S0007114516000210

Costaglioli, P., Barthe, C., Claverol, S., Brozel, V. S., Perrot, M., Crouzet, M., … Vilain, S. (2012). Evidence for the involvement of the anthranilate degradation pathway in Pseudomonas aeruginosa biofilm formation. *Microbiologyopen, 1*(3), 326–339. https://doi.org/10.1002/mbo3.33

Crovesy, L., Masterson, D., & Rosado, E. L. (2020). Profile of the gut microbiota of adults with obesity: A systematic review. *European Journal of Clinical Nutrition, 74*(9), 1251–1262. https://doi.org/10.1038/s41430-020-0607-6

Duranti, S., Ferrario, C., van Sinderen, D., Ventura, M., & Turroni, F. (2017). Obesity and microbiota: An example of an intricate relationship. *Genes & Nutrition, 12*(1), 18. https://doi.org/10.1186/s12263-017-0566-2

Fernandes, J., Su, W., Rahat-Rozenbloom, S., Wolever, T. M., & Comelli, E. M. (2014). Adiposity, gut microbiota and faecal short chain fatty acids are linked in adult humans. *Nutrition & Diabetes, 4*, e121.

Heymsfield, S. B., & Wadden, T. A. (2017). Mechanisms, Pathophysiology, and Management of Obesity. *New England Journal of Medicine, 376*(15), 1492. https://doi.org/10.1056/NEJMc1701944

Ibrahim, M., & Anishetty, S. (2012). A meta-metabolome network of carbohydrate metabolism: Interactions between gut microbiota and host. *Biochemical and Biophysical Research Communications, 428*(2), 278–284. https://doi.org/10.1016/j.bbrc.2012.10.045

Jeon, H. J., Choi, H. S., Lee, Y. J., Hwang, J. H., Lee, O. H., Seo, M. J., … Lee, B. Y. (2015). Seapolynol Extracted from Ecklonia cava Inhibits Adipocyte Differentiation in Vitro and Decreases Fat Accumulation in Vivo. *Molecules, 20*(12), 21715–21731. https://doi.org/10.3390/molecules201219796

Kaymak, I., Maier, C. R., Schmitz, W., Campbell, A. D., Dankworth, B., Ade, C. P., … Schulze, A. (2020). Mevalonate Pathway Provides Ubiquinone to Maintain Pyrimidine Synthesis and Survival in p53-Deficient Cancer Cells Exposed to Metabolic Stress. *Cancer Research, 80*(2), 189–203. https://doi.org/10.1158/0008-5472.CAN-19-0650

Kim, S. I., Kang, N., Leem, S., Yang, J., Jo, H., Lee, M., … Song, Y. S. (2020). Metagenomic Analysis of Serum Microbe-Derived Extracellular Vesicles and Diagnostic Models to Differentiate Ovarian Cancer and Benign Ovarian Tumor. *Cancers (Basel), 12*(5), 1309. https://doi.org/10.3390/cancers12051309

Knorr, S., Sinn, M., Galetskiy, D., Williams, R. M., Wang, C., Muller, N., … Hartig, J. S. (2018). Widespread bacterial lysine degradation proceeding via glutarate and L-2-hydroxyglutarate. *Nature Communications, 9*(1), 5071. https://doi.org/10.1038/s41467-018-07563-6

Koliada, A., Syzenko, G., Moseiko, V., Budovska, L., Puchkov, K., Perederiy, V., … Vaiserman, A. (2017). Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiology, 17*(1), 120. https://doi.org/10.1186/s12866-017-1027-1

Lee, H. J., Kwon, O., & Kim, J. Y. (2018). Supplementation of a polyphenol extract from Ecklonia cava reduces body fat, oxidative and inflammatory stress in overweight healthy subjects with abdominal obesity: A randomized, placebo-controlled, double-blind trial. *Journal of Functional Foods, 46*, 356–364. https://doi.org/10.1016/j.jff.2018.04.062

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Microbial ecology: Human gut microbes associated with obesity. *Nature, 444*(7122), 1022–1023. https://doi.org/10.1038/4441022a

Li, Y., Qian, Z. J., Ryu, B., Lee, S. H., Kim, M. M., & Kim, S. K. (2009). Chemical components and its antioxidant properties in vitro: An edible marine brown alga. *Ecklonia cava. Bioorg Med Chem, 17*(5), 1963–1973. https://doi.org/10.1016/j.bmc.2009.01.031

Lin, H., Su, X., & He, B. (2012). Protein lysine acylation and cysteine succination by intermediates of energy metabolism. *ACS Chemical Biology, 7*(6), 947–960. https://doi.org/10.1021/cb3001793

Lozano, C. P., Wilkens, L. R., Shvetsov, Y. B., Maskarinec, G., Park, S. Y., Shepherd, J. A., … Hullar, M. A. J. (2022). Associations of the Dietary Inflammatory Index with total adiposity and ectopic fat through the gut microbiota, LPS, and C-reactive protein in the Multiethnic Cohort-Adiposity Phenotype Study. *American Journal of Clinical Nutrition, 115*(5), 1344–1356. https://doi.org/10.1093/ajcn/nqab398

Marzullo, P., Di Renzo, L., Pugliese, G., De Siena, M., Barrea, L., Muscogiuri, G., Colao, A., Savastano, S., Obesity Programs of nutrition, E. R., & Assessment, G. (2020). From obesity through gut microbiota to cardiovascular diseases: a dangerous journey. *Int J Obes Suppl, 10*(1), 35-49. 10.1038/s41367-020-0017-1.

Moreno-Indias, I., Cardona, F., Tinahones, F. J., & Queipo-Ortuno, M. I. (2014). Impact of the gut microbiota on the development of obesity and type 2 diabetes mellitus. *Frontiers in Microbiology, 5*, 190. https://doi.org/10.3389/fmicb.2014.00190

Most, J., Penders, J., Lucchesi, M., Goossens, G. H., & Blaak, E. E. (2017). Gut microbiota composition in relation to the metabolic response to 12-week combined polyphenol supplementation in overweight men and women. *European Journal of Clinical Nutrition, 71*(9), 1040–1045. https://doi.org/10.1038/ejcn.2017.89

Peng, Y., Yan, Y., Wan, P., Dong, W., Huang, K., Ran, L., … Cao, Y. (2020). Effects of long-term intake of anthocyanins from Lycium ruthenicum Murray on the organism health and gut microbiota in vivo. *Food Research International, 130*, Article 108952. https://doi.org/10.1016/j.foodres.2019.108952

Pisanu, S. (2021). Gut microbiota alterations associated with obesity and impact of a weight-loss intervention based on a hypocaloric balanced diet.

Qu, L., Liu, Q., Zhang, Q., Tuo, X., Fan, D., Deng, J., & Yang, H. (2019). Kiwifruit seed oil prevents obesity by regulating inflammation, thermogenesis, and gut microbiota in high-fat diet-induced obese C57BL/6 mice. *Food and Chemical Toxicology, 125*, 85–94. https://doi.org/10.1016/j.fct.2018.12.046

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahe, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ, 4*, e2584.

Selma, M. V., Beltran, D., Garcia-Villalba, R., Espin, J. C., & Tomas-Barberan, F. A. (2014). Description of urolithin production capacity from ellagic acid of two human intestinal Gordonibacter species. *Food & Function, 5*(8), 1779–1784. https://doi.org/10.1039/c4fo00092g

Shanahan, F., van Sinderen, D., O'Toole, P. W., & Stanton, C. (2017). Feeding the microbiota: Transducer of nutrient signals for the host. *Gut, 66*(9), 1709–1717. https://doi.org/10.1136/gutjnl-2017-313872

Tilg, H., & Kaser, A. (2011). Gut microbiome, obesity, and metabolic dysfunction. *J Clin Invest, 121*(6), 2126–2132. https://doi.org/10.1172/JCI58109

Tindall, A. M., McLimans, C. J., Petersen, K. S., Kris-Etherton, P. M., & Lamendella, R. (2020). Walnuts and Vegetable Oils Containing Oleic Acid Differentially Affect the Gut Microbiota and Associations with Cardiovascular Risk Factors: Follow-up of a Randomized, Controlled, Feeding Trial in Adults at Risk for Cardiovascular Disease. *Journal of Nutrition, 150*(4), 806–817. https://doi.org/10.1093/jn/nxz289

Ussar, S., Griffin, N. W., Bezy, O., Fujisaka, S., Vienberg, S., Softic, S., … Kahn, C. R. (2015). Interactions between Gut Microbiota, Host Genetics and Diet Modulate the Predisposition to Obesity and Metabolic Syndrome. *Cell Metabolism, 22*(3), 516–530. https://doi.org/10.1016/j.cmet.2015.07.007

Ye, M., Sun, J., Chen, Y., Ren, Q., Li, Z., Zhao, Y., … Xue, H. (2020). Oatmeal induced gut microbiota alteration and its relationship with improved lipid profiles: A secondary analysis of a randomized clinical trial. *Nutr Metab (Lond), 17*, 85. https://doi.org/10.1186/s12986-020-00505-4

Zhai, Z., Liu, J., Niu, K. M., Lin, C., Tu, Y., Liu, Y., … Ouyang, K. (2021). Integrated Metagenomics and Metabolomics to Reveal the Effects of Policosanol on Modulating the Gut Microbiota and Lipid Metabolism in Hyperlipidemic C57BL/6 Mice. *Front Endocrinol (Lausanne), 12*, Article 722055. https://doi.org/10.3389/fendo.2021.722055

Zhang, C., Zhang, M., Wang, S., Han, R., Cao, Y., Hua, W., … Wei, C. (2010). Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *The ISME journal, 4*(2), 232–241.

Zhang, J., Yi, C., Han, J., Ming, T., Zhou, J., Lu, C., … Su, X. (2020). Novel high-docosahexaenoic-acid tuna oil supplementation modulates gut microbiota and alleviates obesity in high-fat diet mice. *Food Sci Nutr, 8*(12), 6513–6527. https://doi.org/10.1002/fsn3.1941

Zhang, T., Gillies, M. C., Madigan, M. C., Shen, W., Du, J., Grunert, U., … Zhu, L. (2018). Disruption of De Novo Serine Synthesis in Muller Cells Induced Mitochondrial Dysfunction and Aggravated Oxidative Damage. *Molecular Neurobiology, 55*(8), 7025–7037. https://doi.org/10.1007/s12035-017-0840-8