Gut microbiota composition in relation to the metabolic response to 12-week combined polyphenol supplementation in overweight men and women

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ORIGINAL ARTICLE

Gut microbiota composition in relation to the metabolic response to 12-week combined polyphenol supplementation in overweight men and women

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BACKGROUND/OBJECTIVES: The intestinal microbiota may have a profound impact on host metabolism. As evidence suggests that polyphenols affect substrate utilization, the present study aimed to investigate the effects of polyphenol supplementation on intestinal microbiota composition in humans. Furthermore, we examined whether (changes in) gut microbiota composition may determine the metabolic response to polyphenol supplementation.

SUBJECTS/METHODS: In this randomized, double-blind, placebo (PLA)-controlled trial, 37 overweight and obese men and women (18 males/19 females, 37.8 ± 1.6 years, body mass index: 29.6 ± 0.5 kg/m^2) received either epigallocatechin-3-gallate and resveratrol (EGCG+RES, 282 and 80 mg/day, respectively) or PLA for 12 weeks. Before and after intervention, feces samples were collected to determine microbiota composition. Fat oxidation was assessed by indirect calorimetry during a high-fat mixed meal test (2.6 MJ, 61 energy% fat) and skeletal muscle mitochondrial oxidative capacity by means of *ex vivo* respirometry on isolated skeletal muscle fibers. Body composition was measured by dual-energy X-ray absorptiometry.

RESULTS: Fecal abundance of Bacteroidetes was higher in men as compared with women, whereas other assessed bacterial taxa were comparable. EGCG+RES supplementation significantly decreased Bacteroidetes and tended to reduce *Faecalibacterium prausnitzii* in men ($P = 0.05$ and $P = 0.10$, respectively) but not in women ($P = 0.15$ and $P = 0.77$, respectively). Strikingly, baseline Bacteroidetes abundance was predictive for the EGCG+RES-induced increase in fat oxidation in men but not in women. Other bacterial genera and species were not affected by EGCG+RES supplementation.

CONCLUSIONS: We demonstrated that 12-week EGCG+RES supplementation affected the gut microbiota composition in men but not in women. Baseline microbiota composition determined the increase in fat oxidation after EGCG+RES supplementation in men.

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INTRODUCTION

The human intestinal microbiota consists of 10^{12}–10^{14} bacteria and may significantly impact health status.[1] Although antibiotic or fecal transplantation studies provide important proof-of-principle evidence for the impact of the microbiota on host metabolism,[2,3] dietary interventions may offer a more feasible approach to manipulate gut microbiota.[4]

Changes in macronutrient composition have been shown to affect the intestinal microbiota[5] and improve insulin sensitivity in patients with type 2 diabetes.[6] Interestingly, polyphenols may induce beneficial metabolic effects,[7–10] which may, at least partly, be mediated by alterations of the gut microbiota composition. For example, polyphenol-enriched products induced alterations in microbial composition and had beneficial effects on insulin sensitivity, ectopic fat storage and inflammation[11–14] in rodents. In line, supplementation of the polyphenols epigallocatechin-3-gallate (EGCG) and resveratrol (RES) has been shown to improve markers of insulin sensitivity, inflammation and fat oxidation in humans.[7,9,15] Although these effects have largely been attributed to direct effects on peripheral organs, importantly, however, our gut microbes may have an important role in the conversion of polyphenols to bioactive compounds.[16] Furthermore, polyphenols may modify the gut microbial composition, thereby acting as prebiotics.[17] Until now, studies that have examined the effects of polyphenols on gut microbiota composition and assessed the involvement of the intestinal microbiota in effects on peripheral metabolism are scarce.[18]

Here we aimed to investigate the effects of combined EGCG and RES supplementation for 12 weeks on gut microbiota composition in humans. Therefore, we measured fecal abundances of key microbial phyla and functional groups before and after 12-week supplementation with either EGCG+RES or placebo (PLA) in overweight and obese men and women. Second, we examined whether (changes in) microbiota composition may underlie the EGCG+RES-induced improvement in lipid oxidation and mitochondrial oxidative capacity in humans that we have previously found.[10]

MATERIALS AND METHODS

Study design

In this randomized, double-blind, PLA-controlled study, which was a substudy of a larger study designed to investigate the effects of polyphenol supplementation on insulin sensitivity, fat oxidation and skeletal muscle oxidative capacity,[10] subjects received a combination of EGCG and

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RES supplements (EGCG+RES; 282 and 80 mg/day, respectively) or PLA (partly hydrolyzed microcrystalline cellulose-filled supplements) for a period of 12 weeks. Body composition was determined by dual-energy X-ray absorptiometry (VitaK, Maastricht, The Netherlands).

Abundances of fecal microbiota were measured using stool samples. Sample collection and clinical measurements were performed before and in the last week of supplementation. Subjects were instructed to maintain their habitual lifestyle pattern throughout the study. Control visits were scheduled at weeks 2, 4 and 8 of intervention. In weeks 0, 4 and 12, subjects were asked to fill in a 3-day food intake record in order to assess dietary intake.

Subjects

Forty-two untrained (<3 h organized sports activities per week), weight-stable (<2 kg body weight change 3 months prior to inclusion), overweight/obese (body mass index >25 kg/m^2) but otherwise healthy Caucasian men and women (1:1) between 20 and 50 years with normal glucose tolerance and normal blood pressure were included in this study. Subjects were not allowed to use any antibiotics or medication/supplements that might interfere with insulin sensitivity and substrate metabolism for 3 months before entering the study. Daily intake of caffeine (<600 mg), green tea (<3 cups) and alcohol (<20 g) had to be limited. Detailed inclusion and exclusion criteria for study participants were published earlier.[10] All subjects gave written informed consent for participation in this study, which was reviewed and approved by the local Medical Ethical Committee of Maastricht University Medical Centre$^+$. All procedures were according to the Declaration of Helsinki.

Gut microbiota composition

DNA isolation. Metagenomic DNA was isolated as described previously.[19] Briefly, approximately 200 mg of feces was added to a 2-ml vial containing 0.5 g zirconia beads (0.1 mM) and 4 glass beads (3 mM) (BioSpec, Bartlesville, OK, USA) and 1.2 ml of PSP lysis buffer (Stractec Biomedical, Berlin, Germany). Samples were treated in a Magna Lyser at 5.5 ms in 3 cycles of 1 min and cooled on ice in between cycles. Subsequently, the DNA isolation was continued using the PSP SPIN Stool DNA Plus Kit as per the manufacturer's instructions and finally eluted in 200 µl.

Microbial analysis using real-time PCR assays. DNA from all fecal samples was subjected to real-time PCR assays for the enumeration of the bacterial phyla γ-Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes, as well as for the quantification of *Akkermansia muciniphila*, *Faecalibacterium prausnitizii*, sulfate-reducing bacteria, acetogenic bacteria and the archeon *Methanobrevibacter smithii* (primers and probes are listed in Table 1).

For the enumeration of γ-Proteobacteria, Actinobacteria, Bacteroidetes, *A. muciniphila*, *F. prausnitzii*, sulfate-reducing bacteria and acetogens, real-time detection of PCR products was conducted with SYBR Green I. The PCRs were conducted in a total volume of 25 µl, containing 1× iQ SYBR Green Supermix (Bio-Rad Laboratories Inc., Hercules, ON, Canada), 400 nM of both primers[20–24] and 2 µl of purified and 10-fold diluted target DNA. Amplification, melting curve analysis and detection were conducted with the MiQ-Single-Color real-time PCR Detection System (Bio-Rad).

The 5′-nuclease technique was used for the detection of Firmicutes and *M. smithii* as described previously.[25] PCRs were conducted in a total volume of 25 µl, containing 1×ABsolute qPCR Mix (ABgene, Hamburg, Germany), 200 nM of both primers and 200 nM TaqMan probe and 2 µl of purified and 10-fold diluted target DNA. The amplification and detection were conducted with an Applied Biosystems Prism 7900 sequence detection system (Applied Biosystems, Waltham, MA, USA). Serial dilutions of plasmid constructs containing the target sequences were used to create calibration curves for quantification.

Fat oxidation and ex vivo mitochondrial oxidative capacity

As described elsewhere,[10] a high-fat mixed meal test (2.6 MJ, 61.2 energy% fat) was performed to assess whole-body energy expenditure and fat oxidation before ($t=0$ min) and for 4 h after ingestion of the high-fat mixed meal test by means of indirect calorimetry, using an open-circuit ventilated hood system (Omnical, Maastricht University, Maastricht, The Netherlands). Mitochondrial oxidative capacity was measured by *ex vivo* high-resolution respirometry (Oroboros Instruments, Innsbruck, Austria) on permeabilized skeletal muscle fibers that were isolated from biopsies (*m. vastus lateralis)*, which were collected under fasting conditions under local anesthesia.[26]

Supplements

The supplements were commercially available and kindly provided by Pure Encapsulations Inc. (Sudbury, MA, USA). All capsules were manufactured, tested and checked in accordance to standards of EU GMP requirements.

EGCG capsules contained 94% EGCG (141 mg per capsule) and RES capsules 20% trans-resveratrol (40 mg per capsule). Two kinds of PLA capsules (microcrystalline cellulose) were used for blinding. Capsules were packed into white opaque boxes, labeled per subject without indication of the content. After completion of the study, returned capsules were counted for compliance.

Statistics

All data are expressed as mean ± s.e.m. Log10 DNA copies for a given microbial group/species per gram of wet weight feces were calculated for each stool sample from the Ct-values using the constructed calibration curves.

Analyses were performed by gender because of differences in bacterial composition. Differences between supplementation groups were analyzed using repeated-measures analysis of variance with time and supplementation as factors. Regression analysis was performed to assess relations between baseline abundances or intervention-induced changes ($\Delta_{\text{Week 12}-\text{Week 0}}$) in the abundance of microbial taxa and changes in fat

Table 1. Baseline subjects' characteristics

	Men (n = *18*)	*Women* (n = *19*)	P *(gender)*	*PLA* (n = *19*)	*EGCG+RES* (n = *18*)	P *(group)*
Age, years	40.2 ± 2.1	35.6 ± 2.4	0.17	39.5 ± 2.3	36.1 ± 2.3	0.30
Weight, kg	96.3 ± 3.5	83.2 ± 2.2	**0.00**	87.1 ± 2.9	92.2 ± 3.5	0.28
BMI, kg/m^2	30 ± 0.8	29.2 ± 0.6	0.46	29.3 ± 0.8	29.9 ± 0.6	0.50
Waist–hip-ratio	0.94 ± 0.02	0.81 ± 0.02	**0.00**	0.88 ± 0.02	0.87 ± 0.02	0.66
Diastolic BP, mm Hg	118 ± 2	113 ± 2	0.15	114 ± 3	117 ± 2	0.30
Systolic BP, mm Hg	77 ± 2	75 ± 2	0.59	77 ± 2	76 ± 2	0.83
Glucose, fasted, mmol/l	5.29 ± 0.05	5.02 ± 0.1	**0.03**	5.11 ± 0.08	5.19 ± 0.09	0.52
2-H glucose, mmol/l	5.27 ± 0.24	5.48 ± 0.25	0.55	5.41 ± 0.23	5.34 ± 0.25	0.84
HbA1c, %	5.16 ± 0.07	5.14 ± 0.06	0.79	5.17 ± 0.06	5.12 ± 0.06	0.57
Body fat, %	24.4 ± 0.9	36.6 ± 0.9	**0.00**	29.7 ± 1.9	31.6 ± 1.4	0.45
Adiponectin, mg/ml	6.42 ± 0.49	11.04 ± 0.76	**0.00**	8.84 ± 0.88	8.74 ± 0.82	0.93
Leptin, ng/ml	10.8 ± 1.5	30.3 ± 3	**0.00**	21.7 ± 4.1	19.8 ± 2.4	0.68
Interleukin-6, pg/ml	0.79 ± 0.14	0.88 ± 0.14	0.63	0.89 ± 0.17	0.77 ± 0.07	0.52
Interleukin-8, pg/ml	10.9 ± 0.7	8.1 ± 0.7	**0.01**	9.1 ± 0.6	9.8 ± 0.9	0.55
TNF-α, pg/ml	3.3 ± 0.22	2.3 ± 0.08	**0.00**	2.88 ± 0.22	2.69 ± 0.18	0.49

Abbreviations: BMI, body mass index; BP, blood pressure; EGCG+RES, epigallocatechin-3-gallate and resveratrol; 2-H glucose, plasma glucose concentration 2 h after oral glucose tolerance test; HbA1c, glycated hemoglobin A1c; PLA, placebo; TNF-α, tumor necrosis factor-α; *P*, *P*-value for statistical difference between men and women (P (gender)) and PLA versus EGCG+RES (P (group)), respectively. Values are given as mean ± s.e.m. Bold values are statistically significant (all $P<0.05$).

oxidation and muscle mitochondrial oxidative capacity in the EGCG+RES group (men, $n=7$; women, $n=7$). Microbial abundances and EGCG+RES-induced changes in microbial abundances were used as independent variable and changes in fat oxidation and mitochondrial oxidative capacity as dependent variables. Variables were normally distributed and the mean of the dependent variable ((week 0+week 12)/2) was included in the model to correct for regression to the mean. Statistics was carried out using SPSS 19.0 (IBM Corporation, Armonk, NY, USA) for Macintosh. $P<0.05$ of the interaction term (time × supplementation) and for the standardized β within a regression term was considered as statistically significant.

RESULTS

Subjects characteristics

Forty-two overweight and obese but otherwise healthy men and women volunteered to participate in this study, of which four subjects (three men, one women) dropped out (one went traveling abroad, one was re-employed at full-time job, one did not comply with supplementation and one substantially changed diet and/or physical activity pattern). In addition, one female subject was excluded from analyses because of reported diarrhea. Characteristics of the 37 subjects who completed the study are summarized in Table 1. There were no significant differences between the EGCG+RES and PLA groups with respect to baseline characteristics. Men had higher fasting plasma glucose and inflammatory cytokine levels (Table 1), but leptin and adiponectin concentrations were, as expected, significantly lower.

Compliance was confirmed by counting returned supplements and increased plasma concentrations of EGCG, RES and dihydro-RES after the intervention in the EGCG+RES group as reported earlier.[10] Energy intake and macronutrient composition were not significantly changed in the EGCG+RES and PLA groups over time.

Table 2. Baseline abundances of bacterial phyla and species

	Men	*Women*	P
γ-Proteobacteria, log10	11.29 ± 0.11	10.93 ± 0.14	0.05
Actinobacteria, log10	11.5 ± 0.08	11.55 ± 0.12	0.77
Akkermansia muciniphila, log10	8.77 ± 0.45	9.71 ± 0.36	0.11
Firmicutes, log10	12.33 ± 0.06	12.34 ± 0.06	0.83
Bacteroidetes, log10	13.21 ± 0.07	12.88 ± 0.06	**0.00**
Methanobrevibacter smithii, log10	9.38 ± 0.33	8.95 ± 0.36	0.40
Faecalibacterium prausnitzii, log10	12.03 ± 0.09	11.98 ± 0.1	0.71
Sulfate-reducing bacteria, log10	10.54 ± 0.27	10.15 ± 0.41	0.43
Acetogen bacteria, log10	10.83 ± 0.06	10.88 ± 0.06	0.58
B/F ratio	9.4 ± 1.4	3.8 ± 0.5	**0.00**
γ-Proteobacteria, %	1.4 ± 0.3	1.3 ± 0.2	0.61
Actinobacteria, %	2.5 ± 0.6	6 ± 1.6	**0.05**
Akkermansia muciniphila, %	0.2 ± 0.1	0.7 ± 0.4	0.17
Firmicutes, %	12.5 ± 1.6	22.7 ± 2	**0.00**
Bacteroidetes, %	83.3 ± 2	69.3 ± 2.9	**0.00**

Abbreviation: B/F ratio, Bacteroidetes/Firmicutes ratio. Absolute (log10) and relative (%) abundances of bacterial phyla and species in 1 g wet weight of fecal samples. *P*-value for statistical difference between gender. Values are given as mean ± s.e.m. Bold values are statistically significant (all $P<0.05$).

Gender differences in microbiota composition

Fecal abundances of microbial groups were different between men and women (Table 2). Absolute abundances of γ-Proteobacteria ($P=0.05$) and Bacteroidetes ($P<0.01$) were higher in men as compared with women, while other phyla and species were comparable. The relative abundance of Bacteroidetes, expressed as the percentage of the five dominant phyla, was higher in men than in women ($P<0.01$), while Firmicutes ($P<0.01$) and Actinobacteria ($P=0.04$) abundance was relatively lower in men (Table 2).

EGCG+RES supplementation decreased bacteroidetes abundance in men but not in women

In men, EGCG+RES supplementation significantly reduced the abundance of Bacteroidetes ($P=0.05$) and tended to decrease *F. prausnitizii* abundance as compared with PLA ($P=0.10$, Table 3). However, these changes were not observed in women (Table 4). The abundance of Firmicutes, Actinobacteria, γ-Proteobacteria and *A. muciniphila* (phylum Verrucomicrobiae), sulfate-reducing bacteria, acetogenic bacteria and the archeon *M. smithii* were not significantly affected by the intervention neither in men (Table 3) nor in women (Table 4).

Table 3. The effects of 12-week EGCG+RES supplementation on the abundance of bacterial phyla and species as compared with PLA in men

Men	*PLA* (n = *10*)		*EGCG+RES* (n = *8*)		P
	Week 0	*Week 12*	*Week 0*	*Week 12*	
γ-Proteobacteria, log10	11.31 ± 0.16	11.2 ± 0.09	11.26 ± 0.13	10.95 ± 0.23	0.49
Actinobacteria, log10	11.51 ± 0.1	11.44 ± 0.07	11.5 ± 0.13	11.32 ± 0.2	0.59
Akkermansia muciniphila, log10	0.38 ± 0.23	0.11 ± 0.07	0.04 ± 0.03	0.01 ± 0.01	0.59
Firmicutes, log10	12.31 ± 0.09	12.33 ± 0.07	12.35 ± 0.07	12.17 ± 0.15	0.28
Bacteroidetes, log10	13.18 ± 0.09	13.19 ± 0.07	13.25 ± 0.11	12.94 ± 0.14	**0.05**
Methanobrevibacter smithii, log10	9.31 ± 0.44	8.8 ± 0.42	9.56 ± 0.58	8.57 ± 0.72	0.75
Faecalibacterium prausnitzii, log10	11.99 ± 0.13	12.02 ± 0.13	12.08 ± 0.12	11.64 ± 0.3	0.10
Sulfate-reducing bacteria, log10	10.7 ± 0.24	10.57 ± 0.25	10.34 ± 0.54	9.38 ± 0.88	0.14
Acetogen bacteria, log10	10.76 ± 0.1	10.77 ± 0.06	10.92 ± 0.06	10.82 ± 0.16	0.47
B/F ratio	8.8 ± 1.6	8.9 ± 1.5	10.1 ± 2.7	6.4 ± 0.9	0.22
γ-Proteobacteria, %	1.7 ± 0.4	1.1 ± 0.2	1.1 ± 0.3	1.2 ± 0.4	0.30
Actinobacteria, %	2.8 ± 1	1.7 ± 0.3	2 ± 0.5	2.8 ± 0.7	0.19
Akkermansia muciniphila, %	0.4 ± 0.2	0.1 ± 0.1	0 ± 0	0 ± 0	0.37
Firmicutes, %	12.6 ± 2.1	13.5 ± 2.9	12.5 ± 2.7	14.5 ± 1.8	0.79
Bacteroidetes, %	82.5 ± 2.9	83.6 ± 3.2	84.3 ± 2.9	81.5 ± 1.8	0.38

Abbreviation: B/F ratio, Bacteroidetes/Firmicutes ratio. Absolute (log10) and relative (%) abundances of bacterial phyla and species in 1 g wet weight of fecal samples. *P*-value for statistical significance for time × treatment interactions. Values are given as mean ± s.e.m. Bold values are statistically significant (all $P<0.05$).

Table 4. The effects of 12-week EGCG+RES supplementation on the abundance of bacterial phyla and species as compared with PLA in women

Women	*PLA* (n = *9*)		*EGCG+RES* (n = *10*)		P
	Week 0	*Week 12*	*Week 0*	*Week 12*	
γ-Proteobacteria, log10	11.09 ± 0.08	11.17 ± 0.08	10.78 ± 0.26	10.99 ± 0.16	0.71
Actinobacteria, log10	11.61 ± 0.22	11.42 ± 0.14	11.48 ± 0.13	11.39 ± 0.12	0.70
Akkermansia muciniphila, log10	9.36 ± 0.63	9.2 ± 0.54	10.02 ± 0.39	9.94 ± 0.36	0.91
Firmicutes, log10	12.43 ± 0.07	12.32 ± 0.1	12.27 ± 0.1	12.22 ± 0.06	0.66
Bacteroidetes, log10	12.82 ± 0.08	12.96 ± 0.07	12.95 ± 0.1	12.87 ± 0.05	0.15
Methanobrevibacter smithii, log10	9.81 ± 0.11	9.7 ± 0.11	8.66 ± 0.41	8.25 ± 0.79	0.93
Faecalibacterium prausnitzii, log10	11.92 ± 0.17	11.84 ± 0.19	12.03 ± 0.13	11.91 ± 0.09	0.77
Sulfate-reducing bacteria, log10	10.76 ± 0.1	10.76 ± 0.08	9.6 ± 0.74	10.07 ± 0.52	0.38
Acetogen bacteria, log10	10.91 ± 0.1	10.85 ± 0.08	10.86 ± 0.09	10.78 ± 0.07	0.71
B/F ratio	2.7 ± 0.5	4.7 ± 0.7	4.8 ± 0.8	4.9 ± 0.6	0.70
γ-Proteobacteria, %	1.3 ± 0.2	1.3 ± 0.1	1.2 ± 0.4	1.2 ± 0.3	0.91
Actinobacteria, %	7.6 ± 2.8	3.3 ± 1.2	4.3 ± 1.2	4 ± 1.7	0.66
Akkermansia muciniphila, %	0.5 ± 0.3	0.2 ± 0.1	1 ± 0.6	0.6 ± 0.4	0.15
Firmicutes, %	26.9 ± 2.7	18.6 ± 2.2	18.6 ± 2.4	17.7 ± 2	0.93
Bacteroidetes, %	63.7 ± 4.4	76.6 ± 2.5	74.9 ± 2.8	76.5 ± 2.2	0.77

Abbreviation: B/F ratio, Bacteroidetes/Firmicutes ratio. Absolute (log10) and relative (%) abundances of bacterial phyla and species in 1 g wet weight of fecal samples. *P*-value for statistical significance for time × treatment interactions. Values are given as mean ± s.e.m.

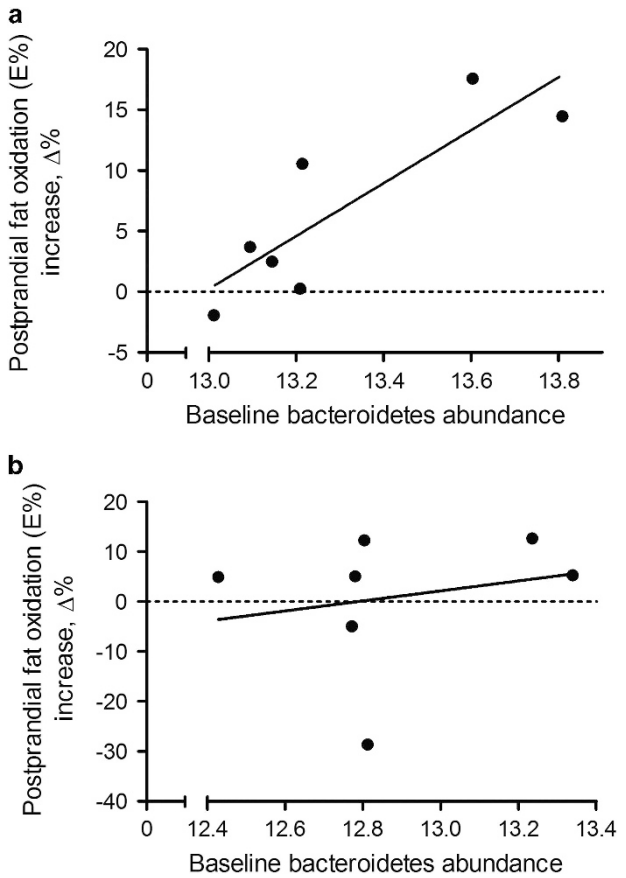

Figure 1. Correlation between baseline Bacteroidetes abundance and the EGCG+RES-induced increase in postprandial fat oxidation. Baseline Bacteroidetes abundance significantly correlated with (**a**) the increase in postprandial fat oxidation in men ($n = 7$, $P = 0.03$) but (**b**) not in women ($n = 7$, $P = 0.68$). The increase in fat oxidation is expressed as $\Delta_{\text{Week 12 – Week 0}}$, calculated from the relative contribution of fat oxidation to total energy expenditure (%).

Fat oxidation and mitochondrial oxidative capacity

The effects of EGCG+RES supplementation on fat oxidation and mitochondrial oxidative capacity have been reported previously for the total group of participants.[10] Briefly, fat oxidation, expressed as the percentage of energy expenditure, was increased in the EGCG+RES group as compared with PLA during fasting ($P = 0.03$) and postprandial conditions after consumption of a high-fat mixed meal (2.6 MJ, 61 energy% fat, $P = 0.02$). In line, skeletal muscle mitochondrial oxidative capacity, assessed using *ex vivo* high-resolution respirometry, was increased after EGCG +RES supplementation versus PLA ($P = 0.01$).

Relation between (changes in) microbiota composition and fat oxidation following EGCG+RES supplementation

Strikingly, baseline Bacteroidetes abundance was significantly correlated with the EGCG+RES-induced increase in postprandial fat oxidation (area under the receiver-operating characteristic curve (AUC)) ($r = 0.855$, $P = 0.01$, Figure 1a) in men. This correlation remained significant after adjustment for mean fat oxidation (dependent variable: $\Delta_{\text{(week 12 – week 0)}}$ fat oxidation (AUC), independent variables: Bacteroidetes $\text{abundance}_{\text{week 0}}$, Std. $\beta = 0.865$, $P = 0.03$; $\text{mean}_{\text{(week 0, week 12)}}$ fat oxidation (AUC), Std. $\beta = -0.057$, $P = 0.84$). Moreover, the Bacteroidetes/Firmicutes ratio was significantly correlated with postprandial fat oxidation ($r = 0.813$, $P = 0.03$). The abundance of Firmicutes was not related to changes in fat oxidation ($r = -0.191$, $P = 0.68$). Other bacterial groups showed no significant association with fat oxidation (data not shown). The EGCG+RES-induced reduction in the abundance of Bacteroidetes and *F. prausnitzii* in men was, however, not related to the observed changes in fat oxidation und oxidative capacity following EGCG+RES supplementation as compared with PLA. Likewise, no significant relationship was found between (changes in) gut microbiota composition and skeletal muscle mitochondrial oxidative capacity.

In women, no significant associations were observed between (changes in) gut microbiota composition and EGCG+RES-induced effects on fasting and postprandial fat oxidation (Figure 1b for baseline Bacteroidetes and postprandial fat oxidation).

DISCUSSION

Dietary polyphenols have been associated with a variety of health benefits related to chronic metabolic and inflammatory diseases such as obesity, diabetes and cardiovascular diseases.[7,9,15] Accumulating evidence indicates that the gut microbiome influences metabolic health,[2,3] but very few studies have examined the effects of dietary interventions on the gut microbiota. Interestingly, polyphenol supplementation may affect gut microbiota composition and, consequently, metabolic health.[18,27] Here we report that men show a pronounced higher abundance of Bacteroidetes as compared with women. Furthermore, 12 weeks of EGCG+RES supplementation decreased the fecal abundance of Bacteroidetes and tended to reduce *F. prausnitzii* in men, whereas no alterations were found in women. Interestingly, our data indicate that baseline Bacteroidetes abundance seems to be involved in the EGCG+RES-induced increase in fat oxidation in men but not in women.

Previous studies have demonstrated gender differences in gut microbiota composition in humans,[4,28] which may at least partly be explained by differences in sex hormones.[29,30] Alternatively, early-life acquisition (use of antibiotics)[31] and lifestyle (local environment, eating and defecation pattern)[32,33] may underlie inter-individual variation and may also contribute to gender-specific microbiota composition. The present study, therefore, examined the effects of prolonged polyphenol supplementation on gut microbiota composition in a gender-specific manner. Interestingly, we found that EGCG+RES supplementation reduced the abundance of Bacteroidetes but not Firmicutes in men, which resulted in a decreased Bacteroidetes/Firmicutes ratio following 12-week EGCG+RES supplementation. In line with our findings, polyphenols seem to have antimicrobial characteristics,[34] most likely because of their chelating properties on iron, which is an important oligo-element for heme-utilizing bacteria.[35] In contrast to most previous studies (reviewed in Duenas *et al.*[17] and Cardona *et al.*[27]), we did not observe any polyphenol-induced prebiotic effects on Firmicutes abundance in overweight and obese humans.

Next we investigated whether microbial species and phyla relate to host metabolism, as has previously been suggested.[4,18,28,32,36] Intriguingly, we found that a high abundance of Bacteroidetes at baseline was correlated with a more pronounced EGCG+RES-induced increase in postprandial fat oxidation in men.

Only 5–10% of the ingested polyphenols are absorbed in the small intestine,[27] and the majority is transferred to the colon where they are subjected to microbial metabolism.[37] Interestingly, the produced polyphenolic metabolites show a high interindividual variability, which may be dependent on the gut microbiota.[38] These metabolites seem to have distinct and/or synergistic effects on peripheral tissues.[39,40] Bacteroidetes contain more glycan-degrading enzymes[41] and may therefore facilitate diffusion of polyphenols and/or related metabolites through the intestinal barrier, thereby enhancing bioavailability. Although our data should be interpreted with caution, it is tempting to postulate that a high baseline abundance of Bacteroidetes might allow a more pronounced improvement in fat oxidation following polyphenol supplementation. In fact, it has been proposed earlier that metabolic health status might determine the effect of polyphenol supplementation on host metabolism.[42,43] Bacteroidetes abundance appears to be related to markers of impaired metabolic health.[44] Moreover, Bacteroidetes abundance was related to low fecal short-chain fatty acid concentrations,[45] which may stimulate fat oxidation in overweight humans.[46] Noteworthy, in the present study, a higher abundance of Bacteroidetes in men was paralleled by a more unhealthy metabolic profile (increased fasting glucose and inflammatory markers) as compared with women, which might support this notion.

Changes in microbial composition after pharmacological interventions have been related to changes in host physiology.[2,3] In line, polyphenol supplementation in rodents reduced adipose tissue mass and ectopic fat accumulation, insulin resistance and inflammation, and these improvements were related to changes in microbiota composition.[11–13] In the present study, however, we did not find significant correlations between the changes in gut microbiota composition and other metabolic parameters beside fat oxidation after EGCG+RES supplementation. Queipo-Ortuño *et al.*[18] have reported previously that effects of red wine polyphenol supplementation on blood pressure, plasma triacylglycerol, cholesterol and C-reactive protein concentrations were linked to changes in microbial composition. Thus the ability of microbiota to mediate effects on physiological parameters clearly warrants further investigation.

The present findings in women should be interpreted with some caution, as we did not obtain information on the phase of the menstrual cycle. Moreover, the use of oral contraceptives (11 out of the 19 female participants) may have influenced substrate metabolism[47] and intestinal microbiota composition, as has already been reported for vaginal microbiota.[48] Finally, although determination of phyla abundances has been used as valuable indicator of the impact of microbiota on substrate metabolism,[36] a detailed microbial phenotyping using state-of-the-art next-generation sequencing may provide additional insight into relevant bacterial species in future studies.[49] Importantly, within the phyla Bacteroidetes, different genera, Bacteroides and Prevotella, do have different metabolic traits and define a host's enterotype.[5,50]

In conclusion, we demonstrated that overweight and obese men and women differ in their intestinal microbiota and in their susceptibility to intervention effects. Whereas EGCG+RES supplementation for 12 weeks reduced abundance of Bacteroidetes in men, no effects were observed in women. Furthermore, Bacteroidetes abundance at baseline was a significant predictor for the EGCG+RES-induced increase in fat oxidation in men. Future studies are warranted to further explore gender differences in gut microbiota composition and its relationship with metabolic outcome after interventions. Furthermore, state-of-the-art sequencing techniques to determine microbiota composition should be combined with metabolomics approaches to assess polyphenol metabolite profiles to obtain better insight into interindividual variability in the response to polyphenol supplementation on metabolic health in humans.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

JM, GHG and EEB designed the study. JM performed the experiments and analyzed the data. JM wrote the manuscript. JM, JP, ML, GHG and EEB revised the content of the manuscript and approved the manuscript for publication.

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