



Distinct effects of supplementation with resistant starch and polydextrose on plasma and faecal bile acid profile and associations with gut microbiota: a randomised, controlled intervention in healthy participants

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Abstract

Purpose Dietary fibre may influence bile acid (BA) metabolism via interactions with gut microbiota. We hypothesised that dietary fibres with distinct fermentative properties, resistant starch (RS) and polydextrose (PD), would differentially alter BA profiles in plasma and faeces through gut microbiota-mediated mechanisms.

Methods BA profiles were analysed by ultra-performance liquid chromatography mass spectrometry in plasma ($n=74$) and faeces ($n=50$) from a double-blind, randomised, placebo-controlled 2×2 factorial trial. Healthy participants consumed 23 g/day Hi-maize[®]260 (type 2 RS) and/or 12 g/day Litesse[®]Ultra[™] (PD) for 50 days. The intervention effects of RS and PD on BA profile were investigated using general linear models and beta regression models. Genus abundances derived from 16 S rRNA gene sequencing were used to investigate fibre-specific microbial correlations with BA profiles.

Results Supplementation with RS, but not PD, increased a range of conjugated BAs and deoxycholic acid (FDR<0.05). Concentrations of taurochenodeoxycholic acid (FDR=0.027) and taurine conjugated BAs (FDR=0.049) in plasma correlated positively with *Akkermansia* abundance in response to RS. Although neither RS nor PD altered BA concentrations in faeces, RS decreased ($p=0.032$) and PD increased ($p=0.012$) faecal proportions of primary BAs. PD reduced secondary BA transformation ratios ($p<0.05$), along with shifts in related microbial associations. There were negative correlations between plasma primary conjugated BAs and faecal secondary BAs in response to RS specifically ($p<0.05$).

Conclusion RS increased plasma BAs, particularly conjugated BAs, whereas PD reduced faecal secondary BA transformation. The distinct impacts of RS and PD on BA profiles and fibre-specific microbial associations may underlie their differential metabolic effects.

Trail registration The DISC Study was registered with <https://clinicaltrials.gov/> (Identifier NCT01214681) in 2010.

Keywords Bile acid · Resistant starch · Polydextrose · Gut microbiota

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Introduction

Bile acids (BAs) are synthesised in the liver from cholesterol and released into the duodenum via the common bile duct, where they function as surfactant molecules to facilitate lipid digestion. Most BAs are reabsorbed into the hepatic portal vein and enter hepatocytes, completing the entero-hepatic circulation [1]. BAs absorbed from the gut, that are not taken up by the liver, are detected in the systemic circulation, while unabsorbed BAs are excreted in faeces [2]. Beyond their role in digestion, BAs serve as signalling molecules that regulate cardiometabolic homeostasis and gastrointestinal function through activation of key receptors such as the Farnesoid X Receptor (FXR) and the G protein-coupled bile acid receptor 1 (TGR5) [1]. Disrupted BA metabolism, characterised by altered BA concentrations in blood and faeces, has been associated with increased risk of various diseases [1].

Dietary fibre has modulatory effects on BA metabolism in humans [3, 4]. Early studies focused primarily on how fibre may protect against colorectal cancer (CRC) by reducing secondary BAs such as deoxycholic acid (DCA) and lithocholic acid (LCA), which are potential risk factors for CRC development [5]. Intervention studies showed that various sources of fibre, such as vegetables [6, 7] and cereal fibre [7–10] increased faecal bulk and lowered secondary BA formation. However, certain soluble and viscous fibres, such as pectin [11] and guar gum [8] have been associated with increased concentrations and excretion of faecal BAs. These findings show that dietary fibres of different physiochemical and fermentation properties may have distinct effects on BA metabolism. The mechanisms underlying these fibre-specific effects may be owing to differential modulation of microbial activity by fibres with varied fermentability [12, 13]. This is particularly relevant to the regulation of BA metabolism, given that BAs undergo multiple microbial transformations in the large intestine, including deconjugation, 7 α / β -dehydroxylation, oxidation and epimerisation, all of which contribute to BA diversification in the gut [14].

Beyond faecal BAs, circulating BAs have been reported to be associated with the risk of diseases, such as type 2 diabetes and CRC [15–18]. However, current studies investigating the relationship between dietary fibre and circulating BAs are limited and have yielded inconsistent results in healthy people or patients. Byrd et al. reported inverse cross-sectional correlations between dietary fibre intake and circulating concentrations of multiple secondary and glycine and taurine conjugated BAs in a large health cohort [19]. In contrast, another cross-sectional analysis found that concentrations of serum conjugated BAs were higher in vegans compared with omnivores [20]. Heterogeneity also exists in intervention studies. A crossover feeding trial

found that a whole-grain diet, compared with a refined-grain diet, increased serum conjugated BAs in healthy adults [21]. However, circulating BA concentrations did not change following high-fibre, high-fruit and vegetable, and low-fat diet interventions [22]. We hypothesise that these discrepancies may be attributed to differences in the types and sources of dietary fibre.

Resistant starch (RS) has been long reported to improve large bowel health, largely due to its high fermentability with beneficial impacts on gut microbiota and metabolites, such as short-chain fatty acids [23]. In contrast, polydextrose (PD) is a type of dietary fibre that is slowly and incompletely fermentable [24]. Both RS and PD are commonly used as food ingredients [23, 24]. Current evidence comparing the effect of highly and slowly fermentable dietary fibres on BA profile remains limited. The current study aimed to use biological samples and gut microbiota data from the Dietary Intervention, Stem cells and Colorectal Cancer (DISC) Study to test the hypothesis that RS and PD have distinct effects on plasma and faecal BA profiles in healthy individuals, which may exhibit fibre-specific associations with gut microbiota.

Methods

Intervention study

This study analysed BA profiles in plasma and faecal samples from the DISC Study [25] (ClinicalTrials.gov NCT01214681). Full details of study design have been published [25–27]. In brief, the DISC Study is a double-blind, randomised, placebo-controlled dietary intervention in which 75 healthy participants were supplemented with RS and/or PD, or respective placebo, for 50 days in a 2 $\times$ 2 factorial design. Participants were randomised to one of four intervention groups: (A) 23 g Amioca starch (RS placebo) + 12 g maltodextrin (PD placebo); (B) 23 g Amioca starch + 12 g Litesse®Ultra™ (90% PD, w/w); (C) 23 g Hi-maize®260 (53% type 2 RS, w/w) + 12 g maltodextrin; (D) 23 g Hi-maize®260 + 12 g Litesse®Ultra™ [25]. The dose of type 2 RS and PD (~ 12 g/day) was determined based on the average daily fibre intake (~ 20 g/day) in the UK [28], with supplementation designed to increase total intake to approximately the recommended 30 g/day. All participants provided informed written consent. Participants were asked to maintain their habitual diet throughout the study. Anthropometric, dietary and lifestyle data and biological samples were collected as described previously [25–27]. Habitual dietary intake was assessed at baseline using a food frequency questionnaire adapted from the EPIC-Norfolk study [26]. Plasma and faecal samples were collected at baseline

(day 0) and post-intervention (day 50). Baseline plasma samples from the participants who underwent colonoscopy were fasted as required for endoscopy, whereas those from participants who had flexible sigmoidoscopy were unfasted. There was no difference of the number of fasted baseline samples distributed across four groups. All post-intervention plasma samples were unfasted.

Gut microbiota 16s rRNA sequencing

Faecal microbiota were analysed using 16 S rRNA gene sequencing as previously described [27]. Briefly, DNA was extracted from 250 mg stool samples using the DNeasy® PowerLyzer® PowerSoil® kit (Qiagen, UK) following the manufacturer's instructions. The variable region 4 (V4) of the 16 S rRNA gene was amplified and sequenced by NU-OMICS (Northumbria University, UK) according to the Schloss wet-lab MiSeq SOP (Kozich et al., 2013).

Plasma and faecal bile acid measurement

Plasma BAs were extracted as described previously with adaption [29]. In brief, 80 µl plasma were diluted with 720 µl of ice-cold methanol and incubated in dry ice for 15 min. After centrifugation (16,000rcf, 5 min), the supernatant (600–650 µl) was filtered using a 0.45µm PTFE filter and then evaporated (HT-6, Genevac). The dried extract was reconstituted with 25µL of methanol ($\geq 99.8\%$, ACS reagent, Sigma) with addition of 15 µl of glycochenodeoxycholic acid (GCDCA)-d4 and 15 µl of cholic acid (CA)-d4 at 5ppm each. BAs were extracted from faeces using an adaptation of the method of Zawadzki et al. [30]. Wet faecal samples (20–30 mg) were weighed and added with 720 µl of ice-cold MeOH. The mixture was homogenised using TissueLyser (LT, Qiagen). After incubation on dry ice for 1.5 h, the sample was centrifuged, filtered and evaporated. Dried extract was reconstituted with 500µL of methanol. After vortexing, 50µL of 500µL methanol solution was transferred into a glass vial and 30 µl of an internal standard mixture, containing GCDCA-d4, chenodeoxycholic acid (CDCA)-d4, DCA-d4 and LCA-d4 at 5ppm each was added. Faecal samples were weighed, dried in an oven to constant weight, and water content was calculated from weight loss.

BA profiling was performed using ultra-performance liquid chromatography mass spectrometry (Waters Acquity UPLC system and Xevo TQ-S Cronos mass spectrometer, Waters) controlled by MassLynx 4.1 software. Study samples were randomised into experimental batches, with paired baseline and post-intervention samples from each participant analysed within the same batch but in a random order. Quality control (QC) samples, prepared by pooling equal aliquots of study samples, were run with study samples in

all batches to monitor inter-batch variation. The limits of quantification (LOQ) were defined by signal-to-noise (SN) ratios of ≥ 10 . BAs with more than 30% of samples with concentrations below the LOQ were excluded from quantification. Eleven plasma BAs including CA, CDCA, DCA, GCDCA, glycocholic acid (GCA), taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA), glycodeoxycholic acid (GDCA), taurodeoxycholic acid (TDCA), glycolithocholic acid (GLCA), glyoursodeoxycholic acid (GUDCA), and ten faecal BAs including CA, CDCA, GCDCA, DCA, LCA, isolithocholic acid (isoLCA), hyodeoxycholic acid (HDCA), 3-ketolithocholic acid (3-keto LCA), 3-ketodeoxycholic acid (3-keto DCA), 12-ketolithocholic acid (12-keto LCA) were quantified. Concentrations below the LOQ were imputed as half the minimum detected value of a given BA [18, 31]. Inter-batch variations (CV) of BAs were between 10.96% and 20.46%, except for plasma glycolithocholic acid (GLCA) (CV = 43.79%) and faecal CDCA (CV = 26.12%). See supplementary methods for full details of calibration sample preparation, QC sample preparation, LC-MS/MS conditions, and analytical performance.

Statistical analysis

Individual BAs were categorised and summed based on chemical structure (conjugated/unconjugated/keto BAs) or the sequence of BA biotransformation by gut microbiota (primary/secondary BAs), as detailed in Supplementary Table 1. BA concentrations showed right-skewed distributions and were log transformed for analysis of intervention effects and partial correlation.

Analysis of intervention effects

The general linear model was used to investigate the main intervention effects of RS and PD, as well as the interaction effect (RS * PD) on post-intervention concentrations of individual BAs and BA categories. The model was adjusted for the corresponding baseline BA concentrations, age, BMI, sex, smoking status, habitual intake of alcohol, energy and fibre, endoscopy procedure (that adjusted for baseline fasting status), and experimental LC-MS/MS experiment batch. Least squares means (LSMs) for the main effect were estimated using the *emmeans* function and *p*-values were derived from the contrast of LSMs. For BA proportions and secondary BA transformation ratios DCA/(DCA+CA) and LCA/(LCA+CDCA), which fall within the (0,1) interval, beta regression fitted with a logit link function (*betareg* R package) was used. The same approach was also applied to analyse faecal water content.

Analysis of partial correlations

Fibre-specific microbial associations with significant BA outcomes at baseline and post-intervention were evaluated among participants who received RS, i.e. RS+ participants, or those who received PD, i.e. PD+ participants. Significant BA outcomes included plasma conjugated BA concentrations to be analysed for RS specific microbial correlations, and faecal primary BA proportions as well as secondary BA transformation ratios for PD specific microbial correlations. A total of 45 taxa at the genus level were included in the analysis, selected based on having a relative abundance greater than 0.01% in at least 50% of samples. To compute pairwise partial correlations, we firstly fitted regression models to adjust covariates for raw relative abundances of taxa and BA outcomes, respectively. Spearman correlations were then performed on the residuals derived from BA models and taxa models. Correlations were adjusted with age, BMI, sex, smoking status, habitual intake of alcohol, energy and fibre, endoscopy procedure, experiment batch and intervention effects (only for post-intervention correlations), i.e., PD adjustment in RS+ participants and vice versa. Details of the statistical approach for partial correlation analysis are provided in Supplementary Table 2. The same approach was also applied to analyse partial correlations between plasma and faecal BA concentrations in response to RS and PD.

The Benjamini–Hochberg method was applied to control the false discovery rate (FDR) for multiple testing on individual BAs in the intervention effect analysis, and on 45 microbial correlations for each BA outcome. Unadjusted *p*-values were used to evaluate the significance of pre-defined outcomes, including concentrations and proportions of BA categories and secondary BA transformation ratios. Statistical significance was defined as either a raw *p*-value or FDR adjusted *p*-value (denoted as ‘FDR’) < 0.05, where applicable. All statistical analyses and data visualization were performed using R version 4.4.1.

Results

Baseline participant characteristics and BA profile

Plasma BA profiles were obtained from 74 participants with paired samples collected at baseline and post-intervention. The average age and BMI of these participants were 52.5 ± 12.3 years and 30.0 ± 5.3 kg/m². There were no differences in demographic characteristics, macronutrient intake (Supplementary Table 3) or plasma BA concentrations (Supplementary Table 5) between RS–vs. RS+ or

PD–vs. PD+ participants. RS+ participants had a higher plasma GLCA proportion than RS– participants ($p=0.018$, and Supplementary Table 6).

Paired faecal samples were available for 50 participants. There were no differences at baseline in demographic characteristics and macronutrient intake between treatment groups (Supplementary Table 4). However, compared with RS– participants at baseline, RS+ participants had lower concentrations of faecal isoLCA, HDCA and 12-keto LCA ($p<0.05$, Supplementary Table 7) and higher proportions of faecal CA, CDCA and GCDCA ($p<0.05$, Supplementary Table 8). PD+ participants had higher proportions of faecal LCA and lower proportion of faecal 12-keto LCA compared with PD– participants at baseline ($p<0.05$, Supplementary Table 8).

Effects of RS and PD on post-intervention concentrations and proportions of BA in plasma

The post-intervention concentration of total BAs in plasma significantly increased in RS+ participants compared with RS– participants ($p=0.003$) (Table 1). RS+ participants consistently had higher concentrations of primary BAs ($p=0.025$), secondary BAs ($p=0.0004$), glycine conjugated BAs ($p=0.002$) and taurine conjugated BAs ($p=0.003$), while the concentrations of unconjugated BAs remained unchanged. For individual BAs, elevated GCA, TCA, GCDCA, TCDCA, GDCA, TDCA, GLCA and GUDCA, as well as DCA concentrations were observed in RS+ compared with RS– participants (all, FDR < 0.05). Regarding BA proportions, increased proportions of glycine conjugated BAs ($p=0.032$) and decreased proportions of unconjugated BAs ($p=0.018$) were observed in RS+ participants compared with RS– participants (Supplementary Table 9). In contrast, PD supplementation did not affect concentrations or proportions of plasma BAs (Table 1). No interaction effects between RS and PD on BA concentrations were detected. There was a significant interaction effect on the proportion of taurine conjugated BAs in plasma (p for RS*PD interaction = 0.022, Supplementary Table 9). Additionally, DCA/(DCA+CA) ratio in plasma was unchanged with RS or PD supplementation (Table 3).

Effects of RS and PD on post-intervention concentrations and proportions of BA in faeces

There were no effects of RS and PD supplementation on BA concentrations in wet faeces (all, FDR > 0.05, Supplementary Table 10), although PD nominally decreased the concentration of faecal 12-keto LCA ($p=0.023$). Faecal water content was not altered by RS and PD (Supplementary Table 11). Regarding BA proportions, RS

Table 1 Effects of supplementation with RS and PD on post-intervention concentrations (nmol/L) of BAs in plasma ^a

BAs/ BA category ^c	Effect of RS			Effect of PD			<i>P</i> value interaction effect
	RS- (<i>n</i> =40)	RS+ (<i>n</i> =34)	<i>P</i> value ^b	PD- (<i>n</i> =37)	PD+ (<i>n</i> =37)	<i>P</i> value ^b	
Total BAs	1847.19 (246.48)	3112.37 (448.85)	0.003	2440.53 (335.73)	2355.69 (333.6)	0.836	0.625
Primary BAs	1230.8 (188.07)	1926.37 (323.43)	0.025	1563.03 (248.66)	1516.91 (247.2)	0.879	0.469
Secondary BAs	556.33 (81.77)	1110.13 (171.48)	0.000	859.73 (127.32)	718.37 (111.78)	0.336	0.710
Glycine conjugated BAs	942 (147.33)	1789.14 (307.8)	0.002	1329.45 (215.75)	1267.73 (212.36)	0.816	0.450
Taurine conjugated BAs	118.29 (16.63)	212.22 (33.12)	0.003	159.46 (23.24)	157.44 (23.87)	0.946	0.172
Unconjugated BAs	670.9 (118.98)	847.34 (151.91)	0.258	812.33 (146.57)	699.81 (124.84)	0.477	0.422
CA	115.9 (33.17)	120.15 (36.17)	0.907	114.93 (33.86)	121.17 (35.82)	0.867	0.646
GCA	137.35 (27.49)	263.15 (55.45)	0.013*	194.99 (40.02)	185.35 (38.61)	0.844	0.550
TCA	24.02 (3.63)	43.62 (6.82)	0.003*	32.03 (4.89)	32.71 (5.13)	0.917	0.116
CDCA	204.33 (48.34)	237.62 (59.89)	0.586	253.07 (62.94)	191.86 (46.58)	0.328	0.657
GCDCA	522.79 (83.05)	902.82 (159.55)	0.011*	700.4 (116.26)	673.87 (114.93)	0.853	0.392
TCDCA	67.84 (9.33)	106.36 (16.71)	0.019*	85.51 (12.26)	84.39 (12.74)	0.943	0.126
DCA	257.78 (39.26)	377.39 (56.99)	0.039*	364.14 (54.88)	267.16 (41.25)	0.097	0.993
GDCA	172.54 (37.3)	419.37 (98.71)	0.002**	279.24 (61.85)	259.12 (60.29)	0.786	0.516
TDCA	22.52 (4.15)	57.14 (11.4)	0.000**	37.97 (7.2)	33.89 (6.64)	0.638	0.270
GLCA	17.33 (2.68)	29.9 (4.22)	0.004*	25.43 (3.77)	20.38 (2.99)	0.228	0.710
GUDCA	67.4 (10.12)	108.62 (17.62)	0.013*	90.04 (13.73)	81.31 (13.17)	0.591	0.845

a. Data are presented as LSMs and (standard error) from analysing using general linear models for the effects of RS or PD supplementation on post-intervention BA concentrations (log transformed). Models were adjusted for the corresponding baseline BA concentrations (log transformed), age, sex, BMI, smoking status, habitual intake of alcohol, energy and fibre, endoscopy procedure and experiment batches

b. *P* values for the effect of RS and PD were derived from the contrast of LSMs. FDR correction using Benjamini-Hochberg procedure was applied on 11 tests of concentrations of individual plasma BAs. ***P* value after FDR<0.01.**P* value after FDR<0.05

c. CA, Cholic acid; GCA, Glycocholic acid; TCA, Taurocholic acid; CDCA, Chenodeoxycholic acid; GCDCA, Glycochenodeoxycholic acid; TCDCA, Taurochenodeoxycholic acid; DCA, Deoxycholic acid; GDCA, Glycodeoxycholic acid; TDCA, Taurodeoxycholic acid; GLCA, Glycolithocholic acid; GUDCA, Glycoursodeoxycholic acid

supplementation significantly decreased the proportion of faecal primary BAs ($p=0.032$), while PD supplementation significantly enhanced this proportion ($p=0.012$, Table 2). For individual BAs, RS nominally decreased CA proportion ($p<0.05$, $0.05<\text{FDR}<0.1$). PD significantly increased CA proportion ($\text{FDR}=0.021$), and nominally decreased the proportion of isoLCA, 3-keto DCA and 12-keto LCA (all, $p<0.05$, $0.05<\text{FDR}<0.1$). No interaction effects between RS and PD on BA concentrations or proportions in faeces were detected. Additionally, PD supplementation lowered DCA/(DCA+CA) ($p=0.029$) and LCA/(LCA+CDCA) ($p=0.031$) in faeces, whereas RS supplementation had no effect on these two ratios (Table 3).

Associations between plasma BAs and faecal BAs in response to RS and PD supplementation

We hypothesised that the distinct effect of RS and PD on plasma and faecal BAs may lead to differential inter-matrix correlations between plasma BAs and faecal BAs. Partial correlation analysis showed widespread negative correlations between faecal BAs and plasma BAs after RS supplementation (Fig. 1a), particularly for faecal secondary BAs

with plasma primary BAs and conjugated BAs ($p<0.01$). These negative correlations were not observed at baseline (Supplementary Fig. 1a). In contrast, the concentrations of plasma BAs and faecal BAs were not associated after PD supplementation (Fig. 1b).

Associations between gut microbiota and plasma conjugated BAs in response to RS supplementation

A wide range of bacteria in the human gut express bile salt hydrolase (BSH), which deconjugates glycine or taurine conjugated BAs to liberate unconjugated BAs [14]. Consequently, we hypothesised that RS supplementation would alter the microbial associations of plasma conjugated BAs, specifically showing negative correlations between these BAs and BA-deconjugating bacteria such as *Bacteroides*, *Lactobacillus*, and *Bifidobacterium* after RS supplementation [14]. However, partial correlation analysis showed no negative correlations between these three genera and conjugated BAs at baseline or post intervention (Supplementary Table 12). Notably, in RS+ participants post intervention, the abundance of *Akkermansia* showed strong positive correlations with taurine primary conjugated BAs ($\rho = 0.61$,

Table 2 Effects of supplementation with RS and PD on post-intervention proportions of BAs (%) in faeces ^a

BAs/ BA category ^c	Effect of RS			Effect of PD			P value interaction effect
	RS- (n=24)	RS+ (n=26)	P value ^b	PD- (n=22)	PD+ (n=28)	P value ^b	
Primary BAs	2.95 (0.63)	1.66 (0.4)	0.032	1.51 (0.38)	3.1 (0.66)	0.012	0.269
Secondary BAs	79.41 (2.06)	80.78 (1.9)	0.602	81.69 (2.04)	78.49 (2.01)	0.244	0.169
Keto BAs	15.87 (1.35)	17.15 (1.21)	0.454	17.32 (1.4)	15.71 (1.18)	0.352	0.434
CA	0.85 (0.18)	0.41 (0.1)	0.008	0.34 (0.08)	0.92 (0.2)	0.002*	0.989
CDCA	1.39 (0.32)	0.99 (0.25)	0.207	0.91 (0.25)	1.47 (0.32)	0.084	0.061
GCDCA	0.19 (0.03)	0.17 (0.02)	0.547	0.15 (0.03)	0.2 (0.02)	0.166	0.138
DCA	29.42 (2.47)	26.75 (2.12)	0.387	29.06 (2.45)	27.11 (2.06)	0.51	0.275
LCA	37.77 (2.38)	41.72 (2.29)	0.215	40.36 (2.4)	39.12 (2.22)	0.69	0.467
IsoLCA	6.22 (0.69)	7.62 (0.71)	0.155	8.1 (0.78)	5.73 (0.56)	0.011	0.482
HDCA	5.1 (0.48)	4.87 (0.41)	0.705	5.36 (0.49)	4.61 (0.36)	0.194	0.534
3-keto DCA	0.89 (0.09)	0.85 (0.07)	0.723	0.98 (0.09)	0.76 (0.06)	0.025	0.051
3-keto LCA	4.13 (0.62)	3.81 (0.54)	0.662	3.85 (0.59)	4.09 (0.58)	0.754	0.786
12-keto LCA	10.69 (0.87)	12.14 (0.77)	0.186	12.55 (0.92)	10.28 (0.71)	0.04	0.329

a. Data are present as estimated marginal means and (standard error) from analysing using beta regression models for the effects of RS or PD supplementation on post-intervention BA proportions. Models were adjusted for the corresponding baseline BA proportions, age, sex, BMI, smoking status, habitual intake of alcohol, energy and fibre, endoscopy procedure and experiment batches

b. P values for the effect of RS and PD were derived from the contrast of estimated marginal means. FDR correction using Benjamini-Hochberg procedure was applied on 10 tests of proportions of individual faecal BAs. *P value after FDR < 0.05

c. CA, Cholic acid; CDCA, Chenodeoxycholic acid; GCDCA, Glycochenodeoxycholic acid; DCA, Deoxycholic acid; isoLCA, Isolithocholic acid; HDCA, Hyodeoxycholic acid; LCA, Lithocholic acid; 3-keto LCA, 3-ketolithocholic acid; 3-keto DCA, 3-ketodeoxycholic acid; 12-keto LCA, 12-ketolithocholic acid

Table 3 Effects of supplementation with RS and PD on post-intervention secondary BA transformation ratios in plasma and faeces ^a

Secondary BA transformation ratios ^c	Effect of RS			Effect of PD			P value interaction effect
	RS-	RS+	P value ^b	PD-	PD+	P value ^b	
Plasma DCA / (DCA+CA)	0.66 (0.03)	0.73 (0.03)	0.127	0.73 (0.03)	0.67 (0.03)	0.170	0.338
Faecal DCA / (DCA+CA)	0.96 (0.01)	0.97 (0.01)	0.161	0.97 (0.01)	0.95 (0.01)	0.029*	0.269
Faecal LCA / (LCA+CDCA)	0.96 (0.01)	0.97 (0.01)	0.069	0.98 (0.01)	0.96 (0.01)	0.031*	0.126

a. Data are present as estimated marginal means and (standard error) from analysing using beta regression models for the effects of RS or PD supplementation on post-intervention secondary BA transformation ratios. Models were adjusted for the corresponding baseline ratios, age, sex, BMI, smoking status, habitual intake of alcohol, energy and fibre, endoscopy procedure and experiment batches

b. P values for the effect of RS and PD were derived from the contrast of estimated marginal means. *P value < 0.05

c. CA, Cholic acid; CDCA, Chenodeoxycholic acid; DCA, Deoxycholic acid; LCA, Lithocholic acid

FDR = 0.049), including TCDCA ($\rho = 0.64$, FDR = 0.027) and TCA ($\rho = 0.61$, FDR = 0.051) (Fig. 2a and c). Additionally, *Akkermansia* was nominally positively correlated with TDCA and several glycine conjugated BAs ($p < 0.05$, Supplementary Table 13). These post-intervention positive associations between *Akkermansia* and plasma conjugated BAs were not observed at baseline (Supplementary Table 13).

Associations between gut microbiota and faecal secondary BA transformation in response to PD supplementation

The microbial transformation of primary BAs to secondary BAs via 7 α -dehydroxylation is primarily mediated by *Clostridium* species [32]. In participants following PD supplementation, we hypothesised that the microbial associations of faecal secondary BA transformation ratios would be altered,

particularly with *Clostridium* species. Partial correlation analysis revealed that PD supplementation substantially changed the microbial correlations with DCA/(DCA + CA) and LCA/(LCA+CDCA). At baseline, DCA/(DCA + CA) correlated positively with *Clostridium XIVa* and *Barnesiella*, and negatively with *Parasutterella* (FDR < 0.05, Fig. 2d), mirroring their correlation with CA proportion (Fig. 2e). However, these associations were no longer present after PD supplementation. Similarly, LCA/(LCA+CDCA) was positively correlated with *Barnesiella* and negatively correlated with *Parasutterella* at baseline (FDR < 0.05, Fig. 2g), but these associations were no longer present after PD supplementation. Instead, post-intervention LCA/(LCA+CDCA) tended to be negatively associated with *Bifidobacterium* ($\rho = -0.54$, FDR = 0.088) and *Lachnospiraceae_UC* ($\rho = -0.52$, FDR = 0.088), and positively associated with *Alistipes* ($\rho = 0.55$, FDR = 0.088). These associations partially reflected their relationships with LCA (Fig. 2i).

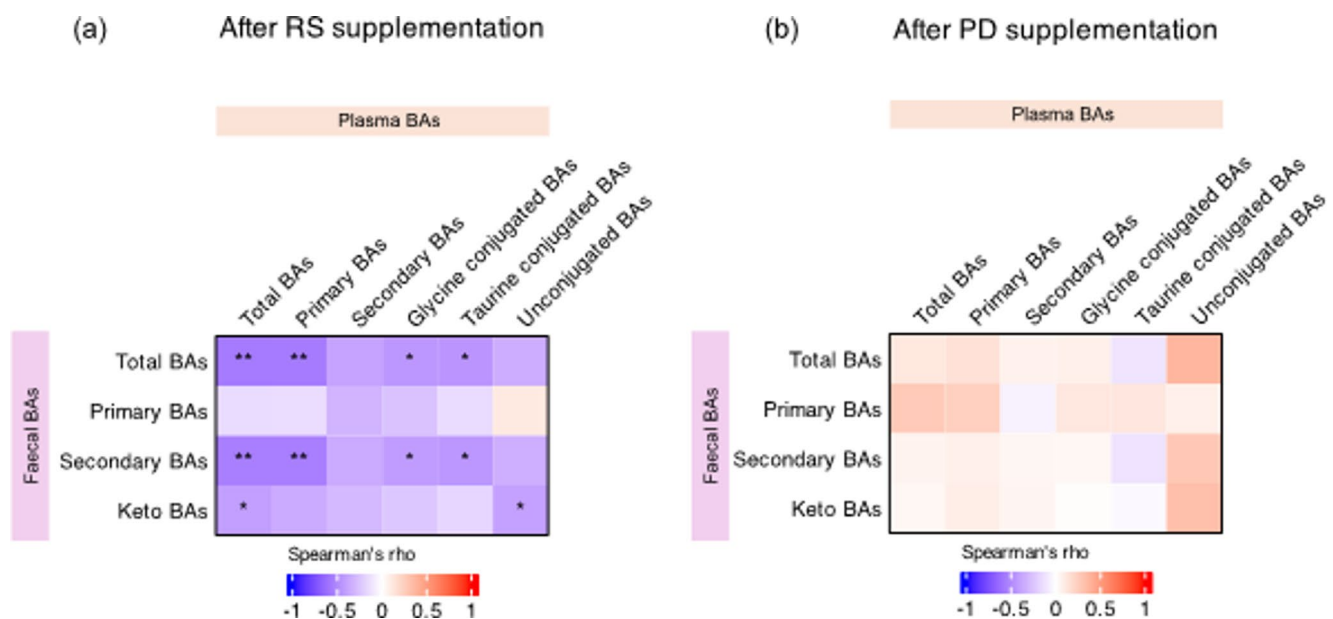


Fig. 1 Partial spearman correlations between concentrations of BAs in plasma and faeces **a** after RS supplementation (RS+ participants, $n=25$) and **b** after PD supplementation (PD+ participants, $n=27$). Spearman correlations were adjusted for treatment (PD adjustment in

RS+ participants and vice versa), age, BMI, sex, smoking status, habitual intake of alcohol, energy and fibre, experimental batch. *Unadjusted p value < 0.05 , **Unadjusted p value < 0.01

Discussion

We observed that supplementation with RS, but not PD, significantly increased circulating BA concentrations, with increases in conjugated BAs including GCA, GCDCA, GDCA, GLCA, GUDCA, TCA, TCDCA, TDCA, and DCA. Concentrations of taurine conjugated BAs were correlated positively with *Akkermansia* abundance after RS supplementation. Neither RS nor PD altered BA concentrations in wet faeces, but RS decreased and PD increased the proportion of faecal primary BAs. Moreover, PD specifically reduced secondary BA transformation ratios DCA/(DCA+CA) and LCA/(LCA+CDCA). The specific microbiota correlated with these ratios shifted following PD supplementation.

To date, few studies have reported the effects of RS and PD on circulating BAs and the findings were inconsistent. Bush et al. found that supplementation with resistant potato starch containing 2.1 g/day RS2 for 4 weeks in healthy participants lowered taurine conjugated BAs in serum with no changes in total BAs or in glycine conjugated BAs [33]. We speculate that the findings from Bush et al. differed from those in our study because of a lower RS2 dose and a shorter intervention duration. In contrast, Li et al. reported that supplementation with 40 g RS2/day for 8 weeks increased the concentrations of serum GDCA, GCA, DCA, 7-keto-lithocholic acid and TDCA in overweight/obese participants [34]. Similarly, Dhakal et al. reported increased circulating conjugated BAs and DCA in participants with metabolic

syndrome supplemented with 12 g/day RS4 for 12 weeks [35]. The intervention intensity of these studies, including ours, was ranked as Bush et al. (2.1 g/day RS2 for 4 weeks) $<$ the DISC Study (12 g/day RS2 for 7 weeks) $<$ Dhakal et al. (12 g/day RS4 for 12 weeks) and Li et al. (40 g/day RS2 for 8 weeks). This suggests that higher RS exposure is more likely to increase circulating BAs, potentially independent of metabolic status. Further human studies are warranted to investigate circulating BA responses to different dose of RS supplementation across metabolically healthy and unhealthy populations. Regarding PD, we are aware of only one human study that reported no effect of supplementation with 12 g/day PD in healthy participants with obesity for 6 months on plasma BAs [36]. This trial used the same type and dose of PD as in our study, supporting our finding of no effects of PD on circulating BAs.

Most conjugated BAs are actively reabsorbed in the terminal ileum, while a smaller portion escapes reabsorption and reaches the colon, where gut microbiota expressing BSH catalyse deconjugation [14]. Therefore, we initially hypothesised that RS increases circulating conjugated BAs by reducing microbial deconjugation. However, the current study did not identify negative correlations between plasma conjugated BAs and the commonly reported BA-deconjugating bacteria including *Bacteroides*, *Lactobacillus*, and *Bifidobacterium* in response to RS. This suggests that increased plasma conjugated BA in response to RS was not directly related to the abundance of individual BA-deconjugating bacteria in faeces. One possible explanation is that

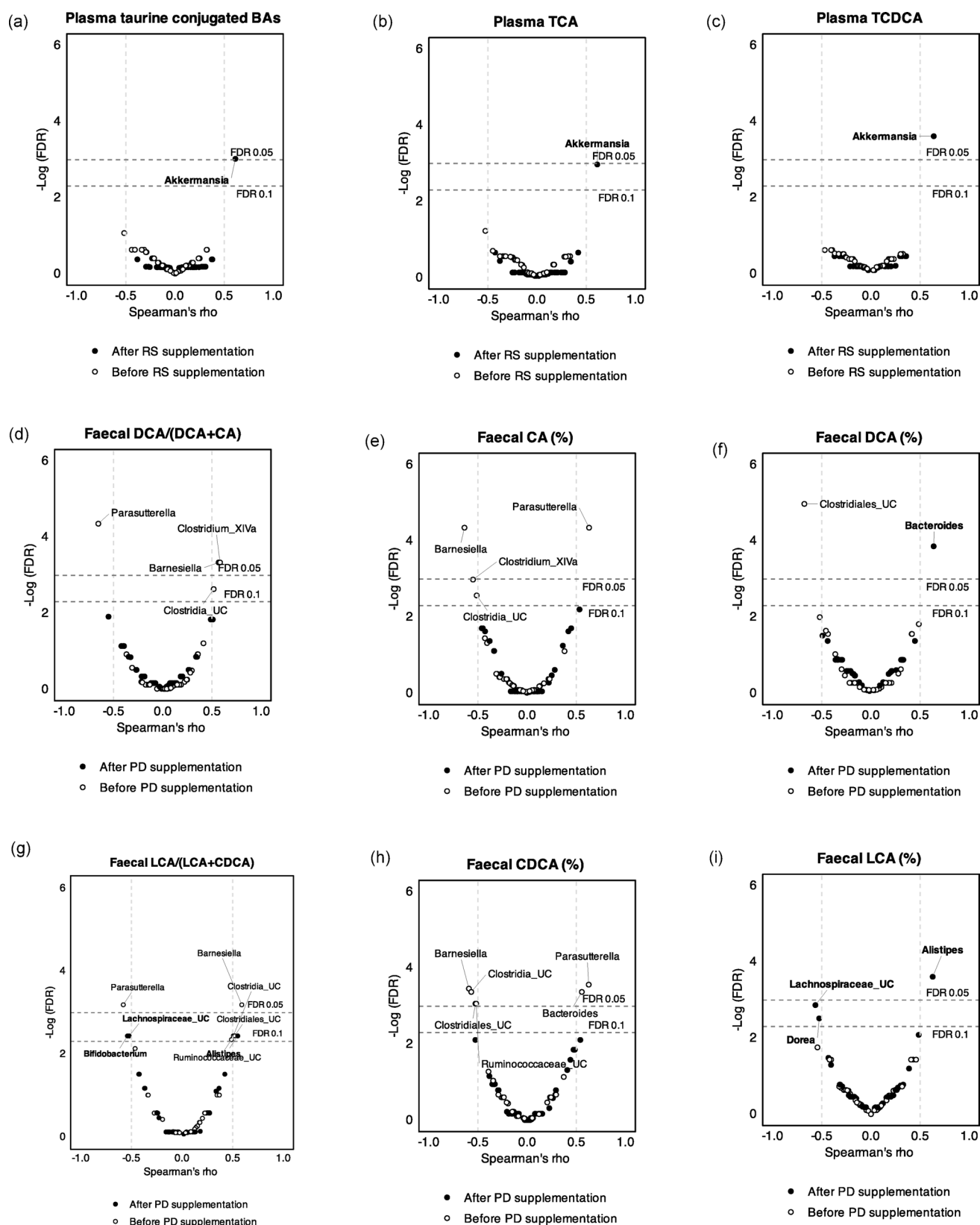


Fig. 2 Partial spearman correlations between the relative abundance of genus abundance and (a–c) concentrations of plasma BAs in response to RS (RS+ participants, $n=26$) and (d–i) proportions or ratios of faecal BAs in response to PD (PD+ participants, $n=26$). FDR correction

was applied on 45 genera for each BA outcome. **Abbreviations:** TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid

~ 95% of conjugated BAs are reabsorbed in the terminal ileum before reaching the large intestine [37], thereby possibly weakening the associations with faecal gut microbiota. Thus, it may be important to investigate the impact of the ileal microbiota on circulating conjugated BAs. Nevertheless, microbial BSH activity and BA deconjugation profile were reported to be similar between the proximal colon and faeces [38, 39], suggesting that faecal microbiota may still provide relevant insights. Another potential reason is that deconjugation likely reflects activity across a broader microbial community rather than specific taxa, as a large group of bacterial species have BSH-expressing ability [40]. Supporting this concept, Li et al. reported that RS supplementation reduced faecal metagenomic expression of BSH, accompanied by increased circulating conjugated BAs [34]. In addition to the commonly reported BA-deconjugating bacteria, we observed positive correlations between taurine conjugated BAs and *Akkermansia* in response to RS. *Akkermansia* is not typically considered a BA-metabolising bacteria, but animal studies have reported that *Akkermansia* influenced the BA pool and increased taurine conjugated BAs in plasma [41, 42]. Taurine conjugated BAs have specifically greater agonist potency for the TGR5 receptor [43]. Whether the observed associations between taurine conjugated BAs and *Akkermansia* are involved with alternations in TGR5 activation with RS supplementation warrants further investigation.

In addition to the microbial factor, another hypothesis for increased circulating BAs induced by RS is that RS may enhance intestinal reabsorption of conjugated BAs into the portal vein and/or reducing hepatic BA uptake. Supporting this concept, our correlation analysis between plasma BAs and faecal BAs suggests that increases in plasma BAs were associated with reallocation of BAs from faeces in response to RS. Animal evidence has shown that RS stimulated ileal absorption of conjugated BAs [44] and downregulated gene expression of hepatic BA uptake transporters [45]. However, it remains unclear whether RS directly, or RS-derived metabolites, e.g., SCFAs, regulate transporter-mediated flux of conjugated BAs. Furthermore, viscosity of fibres is considered as an important factor influencing intestinal BA reabsorption [4]. Higher viscosity contributes to BA-binding of dietary fibre in the intestine, which is associated with interrupted intestinal BA absorption and increased faecal BA excretion [4]. Unfortunately, because we do not have data on faecal daily output, we do not know whether RS and PD affected faecal BA excretion. However, both RS and PD are both non-viscous fibres, therefore, their BA-binding ability in the intestine and contribution to faecal BA excretion is not expected to differ substantially. Additionally, some animal evidence showed that RS influenced hepatic BA synthesis [45] and conjugation [46]. BA biosynthesis is

negatively regulated by BAs across gut-liver axis via FXR signalling [1]. Whether RS-induced increases in circulating BAs reflect an enlarged BA pool or contribute to negative feedback regulation on BA synthesis warrants further investigation.

Although RS supplementation markedly increased BA concentrations in plasma, neither RS nor PD altered total or individual BA concentrations in wet faeces. Faecal water content was not altered by RS and PD, therefore, faecal BA concentrations measured in wet faeces were not expected to be affected by this variation. This aligns with the finding from Dhakal et al., who also observed increased plasma BAs with RS4 supplementation without changes in BA concentrations analysed in wet faeces [35]. In contrast, other studies have reported that the supplementation of RS [47–49] and PD [50] reduced secondary BAs in dry faeces and in faecal water. When assessing molar proportions of BAs, PD increased faecal proportion of primary BAs and decreased the ratios of DCA/(DCA + CA) and LCA/(LCA + CDCA). In addition, baseline positive associations between 7 α -dehydroxylating bacteria, such as *Clostridium* clusters XIVa and secondary BA transformation ratios were shifted after PD supplementation. This suggests that the reduction of PD on secondary BA transformation was associated with BA-dehydroxylating bacteria. This notion is supported by decreased faecal proportions of isoLCA, 3-keto DCA, and 12-keto LCA, which are derived from LCA and DCA through microbial oxidation and epimerization, largely mediated by *Clostridium* species [51]. On contrary, RS decreased the proportion of primary BAs in faeces, and its effect on increasing the LCA/(LCA+CDCA) ratio approached significance. A plausible explanation for the divergent effects of RS and PD lies in their fermentation kinetics: RS is rapidly fermented in the proximal colon, while PD is more slowly fermented, leading to SCFA accumulation primarily in the distal colon. As BA-dehydroxylating bacteria prefer an alkaline luminal environment approaching the distal colon [52], the acidification caused by distal SCFAs accumulation from PD may suppress their growth. Supporting this, we observed negative correlations between LCA/(LCA + CDCA) and SCFA-producing genera, *Bifidobacterium*, *Lachnospiraceae_UC*. These genera are not directly involved in 7 α -dehydroxylation but may indirectly inhibit this process through luminal acidification [53].

Further research is needed to investigate the health implication of RS-induced increases in circulating conjugated BAs and DCA. A human trial has shown that ileo-colonic delivery of conjugated BAs improved glucose homeostasis via colonic GLP-1-producing enteroendocrine cells, potentially mediated by TGR5 activation [54]. Whether increased circulating conjugated BAs by RS confers similar benefits warrants further investigation, particularly in pre-diabetic

individuals. In addition, prospective studies reported that circulating DCA and conjugated BAs were associated with increased CRC risk [15, 18]. Although DCA is considered a carcinogenic metabolite in the context of a high-fat diet [55], it remains unclear whether long-term RS-induced increases in these circulating BAs elevate CRC risk in healthy individuals or individuals with high CRC risk.

A strength of this study is that we compared two different types of dietary fibre at matched doses and durations, minimising confounding from dosage and intervention duration. Additionally, simultaneous quantification of BAs in plasma and faeces allows an integrated view of BA dynamics across intestinal and systemic compartments. The use of unfasted post-intervention plasma samples reflects real-life metabolic responses. To account for individual variation of habitual diet, we included habitual intake of alcohol, energy and fibre as covariates in the statistical models. A limitation is that this study is a secondary analysis without a priori sample size calculation, which may limit statistical power. A further limitation is that GCDCA was the only conjugated BA in faeces that was above LOQ, restricting our ability to explore links between conjugated BAs in plasma and faeces.

Conclusion

In conclusion, RS and PD supplementation in healthy individuals had distinct effects on BA profiles in plasma and faeces. RS supplementation increased plasma BAs, particularly conjugated BAs. Taurine primary conjugated BAs showed positive correlations with *Akkermansia* in response to RS. In contrast, PD supplementation decreased secondary BA transformation ratios DCA/(DCA+CA) and LCA/(LCA+CDCA) in faeces, which may be associated with inhibited microbial 7 α -dehydroxylation activity. These fibre-specific effects on BA profile and microbial associations likely reflect the different fermentation properties of RS and PD. Further studies are needed to evaluate the health consequences of RS-induced increases in circulating BAs, particularly in relation to glucose metabolism and CRC risk. Investigation of BA metabolism, particularly circulating BA profiles, warrants extension to other dietary fibres with varying viscosity and fermentability.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00394-026-04060-1>.

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Data availability The data supporting the conclusion of this article is published on Figshare <https://doi.org/10.6084/m9.figshare.32117815>.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval for the DISC Study was granted by the Newcastle and North Tyneside Research Ethics Committee on 10th December 2009 (REC No. 09/H0907/77) and Caldicott approval for the storage of data was provided by the Northumbria NHS Foundation Trust (C1792). The study has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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