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Impact of polyphenols from black tea and red wine/grape juice on a gut model microbiome

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ABSTRACT

Food and beverage products derived from fruit and vegetables contain polyphenols, which have been associated with various health benefits. Polyphenols may influence health through direct uptake in the intestine but also upon interaction with the gut microbiota for example by modification of the microbial composition or by conversion of the polyphenols to further bioactive compounds. So far, there are limited studies of complex polyphenols on the human gut microbiota especially using modern molecular technologies. Most studies investigating effects of dietary polyphenols have focused on single molecules or bacterial strains. In the current study, an *in vitro* gut microbial ecosystem, namely simulator of the intestinal microbial ecosystem (SHIME), was challenged with either a black tea or a red wine grape extract (RWGE), both containing complex dietary polyphenol mixtures. Within the context of the model system, the effects of these interventions on both microbial numbers and composition as well as metabolite levels were assessed. Antimicrobial effects, largely confined to unculturable members of the ecosystem, were revealed by complementary microbiological techniques. Pyrosequencing analysis showed a shift in the Firmicutes:Bacteroidetes ratio for both interventions. Black tea stimulated *Klebsiella*, enterococci and *Akkermansia* and reduced bifidobacteria, *B. coccoides*, *Anaeroglobus* and *Victivallis*. RWGE promoted growth of *Klebsiella, Alistipes*, *Cloacibacillus*, *Victivallis* and *Akkermansia* while bifidobacteria, *B. coccoides, Anaeroglobus, Subdoligranulum* and *Bacteroides* were decreased.
The study shows that these complex polyphenols in the context of a model system can modulate select members of the human gut microbiota. These members represent novel targets of polyphenol degrading or resistant microbes to be validated under physiological conditions *in vivo* and further investigated for polyphenol metabolism or resistance mechanisms.

1. Introduction

Polyphenolic compounds are abundantly present in foods such as tea and wine and their dietary intake has been associated with health benefits related to cardiovascular function, modulating various parameters such as, vascular and platelet function, blood pressure and the plasma lipid profile, with modulation of oxidative stress, inflammation, and endothelial function as accompanying polyphenolic targets (van Duynhoven et al., 2010). In addition, a recent population level study linked high consumption of black tea to a low prevalence of diabetes (Beresniak, Duru, Berger, & Bremond-Gignac, 2012). Both bioconversion of complex polyphenols by the gut microbiota to more active forms as well as alteration of the abundance or activity of bacterial populations in the intestine may be involved in delivering these benefits since the bioavailability of polyphenol molecules as present in the diet is low (Crozier, Jaganath, & Clifford, 2009). After ingestion, some polyphenolic compounds can be absorbed by the gut epithelium, whereas the majority and particularly the more complex structures pass to the large intestine, where they are metabolized by the colonic microbiota (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). To a limited extent, biotransformation of smaller molecules such as tea catechins may also occur in the small intestine (Schantz, Erk, & Richling, 2010). Therefore, potential positive effects of polyphenol-rich foods might be dependent on biotransformation into more bioavailable forms by intestinal bacteria potentially modulating related health benefits (van Duynhoven et al., 2010).

Gut community composition and resilience can be related to healthy or diseased conditions, such as obesity and inflammatory bowel disease, recently reviewed by Lozupone, Stombaugh, Gordon, Jansson, and Knight (2012). Therefore, shifts in the microbial

Abbreviations: SHIME, simulator of the intestinal microbial ecosystem; DGGE, denaturing gradient gel electrophoresis; qPCR, quantitative PCR; SCFA, short chain fatty acid; RWGE, red wine grape extract.

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abundance due to the effect of polyphenols on the gut microbial community may also play a role in modulation of health. So far, a limited number of intestinal bacterial species have been identified to be specifically capable of degrading particular polyphenolic substrates (Braune, Engst, & Blaut, 2005; Schoefer, Mohan, Schwiertz, Braune, & Blaut, 2003). Even fewer studies have investigated the effect of dietary polyphenols on the complex gut microbial community, which showed that polyphenols as well as their microbial breakdown products can stimulate or inhibit specific bacterial populations that are present in the human intestine (Lee, Jenner, Low, & Lee, 2006; Tzounis et al., 2008). For example, using a batch-culture fermentation system inoculated with fecal bacteria reflective of the distal colon, the flavonol catechin was found to significantly increase the growth of the *Blautia coccoides–Eubacterium rectale* group, *Bifidobacterium* spp. and *Escherichia coli*, while the *Clostridium histolyticum* group was inhibited (Tzounis et al., 2008). In most of these studies, single polyphenolic compounds were investigated, which is not representative as the composition of dietary polyphenols in different foods is very complex. For example, red wine contains proanthocyanidins, anthocyanins, catechins and some phenolic acids and flavonols, whereas black tea is primarily composed of thearubigins, theaflavins, catechins, flavonols and phenolic acids (Gonthier et al., 2003; Luczaj & Skrzydlewska, 2005). Moreover, most studies so far have limited microbiological analysis to the main 5–6 groups of intestinal bacteria while a complex polyphenol dietary intervention analyzed using generic approaches like DGGE with fragment sequencing have demonstrated changes in microbial species other than those identified using a single polyphenol challenge (Queipo-Ortuño et al., 2012).

In this study, the influence of a bolus dose and a 2-week continuous administration of complex dietary polyphenols from black tea or red wine grape extract (RWGE) on the colonic microbiota was assessed using the SHIME model. This validated model comprises stomach and small intestinal sections for predigestion of food as well as vessels simulating the ascending, transcending and descending parts of the human colon, allowing to assess changes in colonic areas that are very challenging to access in a human intervention. In this case, the Twin-SHIME system involving two models run in parallel was inoculated with the same fecal sample for direct comparison of the effect of the two polyphenol types. A combination of microbiological analyses including cultivation, PCR-denaturing gradient gel electrophoresis, quantitative PCR and high throughput pyrosequencing of the 16S ribosomal RNA gene were applied to characterize microbial community changes. This work aims to improve our knowledge of interactions between these complex dietary polyphenol mixtures and the gut microbiota in view of potential health effects.

2. Materials and methods

2.1. Twin-SHIME experiment with complex polyphenols

The RWGE was composed of two parts of the red wine extract Provinols (Seppic, France) and one part of MegaNatural™ Rubired grape juice extract (Polyphenolics, USA). The tea extract was prepared by spray-drying Lipton Yellow Label black tea (Unilever, The Netherlands). The RWGE contained ca. 2% carbohydrates, 1% fiber and 6% protein and 80% total polyphenols of which 13% were identified, anthocyanins (9%) and catechins and procyadin di/trimers (3%). The unidentified polyphenolic fraction consisted primarily of oligomeric proanthocyanidins (Van Dorsten et al., 2010). The tea extract contained, besides uncharacterized amounts of carbohydrates and protein, ca. 5% caffeine and 44% total polyphenols of which 4% were catechins, 2% flavonols, 2% gallic acid and 0.5% theaflavins. The unidentified polyphenolic fraction is generally considered to be largely consisting of thearubigins (Mulder, Rietveld, & van Amelsvoort, 2005).

Two parallel SHIME models (Fig. 1A), inoculated with the same fecal sample from one healthy human volunteer as described earlier (Possemiers, Verthe, Uyttendaele, & Verstraete, 2004) and detailed in supplementary file S1, received either a supplementation with the tea extract or RWGE. Total fermentation time was more than 8 weeks with multiple sampling moments starting after steady state I, i.e. 3.5 weeks after inoculation (Fig. 1B). Steady state I was followed by 1 week of baseline measurements. Then, a single dose of the polyphenol extracts (1000 mg polyphenols) was applied once. Starting 1 week later, polyphenol extracts (1000 mg polyphenols/day) were administered 3× daily to the system through addition to the SHIME feeding medium for a two-week continuous period. Finally, a two-week washout period was included at the end of the experiment. Fluid from the three SHIME vessels representing the colon ascendens (CA), transversum (CT) and descendens (CD) collected at regular time points was used for both microbial as well as metabolite analyses. Samples for DNA isolation and metabolite analysis were stored at −20 °C. At least once per week, microbiological analyses were performed by selective enumeration.

2.2. Selective bacterial enumeration

The number of colony forming units/ml SHIME fluid was determined by plating serial dilutions of samples of fluid in physiological salt solution on selective culture media mainly as described earlier (Possemiers et al., 2004). Numbers of total aerobes and total anaerobes were determined by plating on Brain Heart Infusion agar and incubation at 37 °C for 24 h aerobically or 72 h anaerobically, respectively. LAMVAB Agar with microaerophilic incubation at 37 °C for 72 h was used to determine the number of lactobacilli. Fecal coliforms, enterococci and staphylococci were enumerated aerobically using MacConkey agar at 37 °C for 24 h, *Enterococcus* agar and Mannitol Salt agar both at 37 °C for 48 h as selective media, respectively.

2.3. DNA extraction

Bacterial DNA was extracted as described earlier (Possemiers et al., 2004), using CTAB (hexadecyl trimethyl ammonium bromide) buffer, bead-beating/phenol-chloroform extraction and precipitation by polyethyleneglycol (PEG-6000). All samples were dissolved in PCR water, checked by agarose electrophoresis and stored at −20 °C until further use. The concentration and quality of the samples were assessed using a Nanodrop.

2.4. Quantitative PCR

Bacterial numbers were quantified by qPCR using absolute quantification on an Applied Biosystems 7500 Real-time PCR machine using Power SYBR Green I PCR Master Mix (Applied Biosystems). Primers used for *Bifidobacterium*, *Bacteroides fragilis* group and, *Blautia coccoides* group were described by Matsuki et al. (Matsuki, Watanabe, Fujimoto, Takada, & Tanaka, 2004), primers for total bacteria by Nadkarni et al. (Nadkarni, Martin, Jacques, & Hunter, 2002). The amplification program consisted of 1 cycle of 94 °C for 5 min followed by 40 cycles of 94 °C for 20 s, 55 °C (total bacteria, *B. fragilis* group and *B. coccoides* group) or 60 °C (*Bifidobacterium*) for 20 s, and 60 °C for 1 min and a dissociation cycle. Bacterial DNA isolated from the SHIME samples was diluted 1:100 to avoid PCR inhibition. The number of cell equivalents ml^{-1} was calculated by dividing the DNA concentration, determined from a reference curve, by the weight of the reference DNA (Malinen, Kassinen, Rinttila, & Palva, 2003). We assumed 1 chromosome or 16S rDNA copy per cell. The genome weight was calculated by multiplying the base pair weight (607.4 g mol^{-1}) with the DNA size, divided by the Avogadro number (6.02E + 23).