Investigation of the impact of black chokeberry polyphenols in different matrices on the human gut microbiota using the in vitro model of the large intestine (TIM-2)

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Mild intermittent hypoxia exposure alters gut microbiota composition in men with overweight and obesity

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RESEARCH ARTICLE

Abstract

Results from high altitude studies in humans and controlled animal experiments suggest that hypoxia exposure induces alterations in gut microbiota composition, which may in turn affect host metabolism. However, well-controlled studies investigating the effects of normobaric hypoxia exposure on gut microbiota composition in humans are lacking. The aim of this study was to explore the impact of mild intermittent hypoxia (MIH) exposure on gut microbiota composition in men with overweight and/or obesity. We performed a randomised, single-blind crossover study, in which participants were exposed to MIH (FiO_2: 15%, 3×2 h per day) and normoxia (FiO_2: 21%) for seven consecutive days. Following the MIH and normoxia exposure regimens, faecal samples were collected for determination of faecal microbiota composition using 16S rRNA gene-amplicon sequencing in the morning of day 8. Paired faecal samples were available for five individuals. Furthermore, tissue-specific insulin sensitivity was determined using the gold-standard two-step hyperinsulinemic-euglycemic clamp. MIH did not affect microbial alpha and beta-diversity but reduced the relative abundance of *Christensenellaceae* and *Clostridiaceae* bacterial families. MIH significantly increased the abundances of obligate anaerobic bacterial genera including *Fusicatenibacter*, *Butyricicoccus* and *Holdemania*, whilst reducing *Christensenellaceae* R-7 group and *Clostridium sensu stricto* 1, although these findings were not statistically significant after correction for multiple testing. Furthermore, MIH-induced alterations in abundances of several genera were associated with changes in metabolic parameters such as adipose and peripheral insulin sensitivity, plasma levels of insulin, fatty acids, triacylglycerol and lactate, and substrate oxidation. In conclusion, we demonstrate for the first time that MIH exposure induces modest effects on faecal microbiota composition in humans, shifting several bacterial families and genera towards higher abundances of anaerobic butyrate-producing bacteria. Moreover, MIH-induced effects on faecal microbial composition were associated with parameters related to glucose and lipid homeostasis, supporting a link between MIH-induced alterations in faecal microbiota composition and host metabolism.

The study was registered at the Netherlands Trial Register: NL7120/NTR7325.

Keywords: mild intermittent hypoxia, gut microbiota, anaerobic bacteria, metabolism, obesity

1. Introduction

The gastrointestinal microbiota consists of a highly complex composition, which has been demonstrated to affect brain function (Rogers *et al.*, 2016), immune defence (Belkaid and Hand, 2014), and host metabolism (Martin *et al.*, 2019). Disturbances in gut microbiota composition (that is, microbiome dysbiosis), might contribute to altered substrate metabolism and energy expenditure, and may affect inflammatory processes as well as metabolism in the

liver, skeletal muscle (SM) and adipose tissue (AT) (Canfora *et al.*, 2019). Thus, microbial dysbiosis might contribute to several chronic diseases such as the inflammatory bowel diseases Crohn's disease and ulcerative colitis, cardiovascular diseases and type 2 diabetes, and may contribute to the pro-inflammatory profile of these diseases (Gerard and Vidal, 2019; Khan *et al.*, 2019). Since the gut microbiota may play a key role in metabolic health, recent studies focus on strategies to modulate the gut microbiome, with the aim to prevent or alleviate the progression of cardiometabolic diseases and other conditions such as *Clostridioides difficile* infection, inflammatory bowel disease and hepatological encephalopathy (Allegretti *et al.*, 2019; Markowiak and Slizewska, 2017).

Intriguingly, the oxygenation of the different layers of the intestines may affect the gut microbiota composition. The vascularisation within the different layers of the intestines differs considerably, which is illustrated by a rather unique oxygenation profile. The oxygen tension (pO_2) within the intestines reduces drastically moving across the longitudinal axis, with highest reported pO_2 levels at the proximal level of the intestinal tract, the duodenum (32 mmHg), decreased levels in the ascending colon (11 mmHg), and lowest levels in the sigmoid colon (3 mmHg) in mice (He *et al.*, 1999; Zheng *et al.*, 2015). Furthermore, pO_2 levels have been determined in the human intestines, more specifically in the terminal ileum (34 mmHg), cecum (30 mmHg) and sigmoid colon (39 mmHg) (Sheridan *et al.*, 1990). Notably, these pO_2 levels have been measured at the serosal side, whereas oxygen tension seems lower at the luminal side (Zheng *et al.*, 2015). Interestingly, rats acutely exposed to hypoxia (15% O_2) showed reductions in both serosal and mucosal pO_2 (Suski *et al.*, 1997), providing proof-of-principle that hypoxia exposure is able to modulate intestinal pO_2.

Thus far, only a few human and animal studies have examined the effects of hypoxia exposure on gut microbiota composition. Mice exposed to severe, intermittent hypoxia (5% O_2, 20s per cycle, 360 cycles per day) for 6 weeks, demonstrated increased α- and β-diversity (Moreno-Indias *et al.*, 2015). More specifically, intermittent hypoxia exposure, which reduced pO_2 in close vicinity of the intestinal epithelium (Moreno-Indias *et al.*, 2015; Suski *et al.*, 1997), increased the abundance of *Firmicutes*, whilst reducing the abundance of *Bacteriodetes* and *Proteobacteria* phyla (Moreno-Indias *et al.*, 2015). In addition, rats exposed to chronic, severe intermittent (hypobaric) hypoxia (5,000 m, 6 h per day for 28 days) demonstrated increased abundance of the genera *Lactobacillus*, *Prevotella* and *Methylotenera*, whereas the *02d06* genus within the *Clostridiaceae* family was decreased subsequent to exposure (Tian *et al.*, 2018). Furthermore, a decrease in the *Firmicutes* to *Bacteriodetes* ratio was found in the latter study, which may at least partially underlie the observed improvements in glucose and lipid metabolism (Tian *et al.*, 2018). Remarkably, high altitude exposure (4,300 m for 22 days) increased *Prevotella* abundance in healthy men (Karl *et al.*, 2018). These findings in healthy men are in agreement with previous animal studies, demonstrating increased abundance of mainly obligate anaerobes following hypoxia exposure (Mazel, 2019). Taken together, intermittent hypoxia exposure appears to affect gut microbiota composition, which may contribute to hypoxia-mediated effects on host metabolism. However, controlled human studies that investigated the effects of normobaric hypoxia exposure on the gut microbiota composition, and related effects on host metabolism, are lacking.

Therefore, in the present randomised, single-blind crossover study, we exposed men with overweight and obesity to mild intermittent hypoxia (MIH; FiO_2: 15%, 3×2 h per day) and normoxia (FiO_2: 21%) for seven consecutive days, to determine for the first time the effects of MIH exposure on gut microbiota composition in a well-controlled, laboratory setting. Furthermore, we examined whether hypoxia-induced effects on gut microbiota composition were associated with alterations in host metabolism.

2. Materials and methods

Study design

The design of this study has been described in detail previously (Van Meijel *et al.*, 2021). Briefly, twelve participants enrolled in this randomised, single-blind, crossover study were exposed to normobaric MIH (15% O_2) and normobaric normoxia (21% O_2) for 7 consecutive days (3 cycles of 2 h/d with 1 h of normoxia exposure between hypoxic cycles), separated by a 3-6 week wash-out period. As described previously, hypoxia exposure was performed in an in-house manufactured airtight clinical room with the capability to accurately adjust oxygen availability at the Metabolic Research Unit Maastricht (Maastricht University, the Netherlands). The oxygen level was set and maintained at 15.0±0.2% for the hypoxia exposure regimen. (Participants were blinded for the exposure regimen (hypoxia or normoxia) (Van Meijel *et al.,* 2021)). Systemic oxygen saturation levels were continuously monitored throughout the exposure regimens by pulse oximetry (Nellcore N-595 Pulse oximeter, Nellcor, Pleasanton, CA, USA). At day 6 of the exposure regimens, AT and SM pO_2 were determined using an optochemical measurement system for continuous monitoring of tissue pO_2, as described previously (Goossens *et al.*, 2011). At day 7, a high-fat mixed meal test was performed (2.6 MJ, consisting of 61 E% fat, 33 E% carbohydrates and 6 E% protein). Following the exposure regimens, faecal samples were collected for determination of faecal microbiota composition using 16S rRNA gene amplicon sequencing in the morning of day 8 after an overnight fast. Unfortunately, paired faecal samples (collected both after MIH and normoxia exposure) were

available for only 5 out of the 12 original study participants due to difficulties in collecting a faecal sample at the right time. In addition, a two-step hyperinsulinemic-euglycemic clamp was performed at day 8, under normoxic conditions, to determine hepatic, adipose tissue and peripheral insulin sensitivity. Participants were kept under energy-balanced conditions throughout the study. The diet was adjusted individually to match the energy requirements and maintain an energy balance throughout the study. Based on the estimated daily energy expenditure (basal metabolic rate [BMR; Ventilated Hood, Omnical, Maastricht University, Maastricht, the Netherlands] multiplied by activity score of 1.55), subjects received a standardised diet consisting of 50% carbohydrate, 35% fat, and 15% protein to maintain a stable body weight throughout the study.

The study, registered at Netherlands Trial Register (NL7120/NTR7325), was performed according to the Declaration of Helsinki (revised version, October 2008, Seoul, South Korea) and was approved by the Medical-Ethical Committee of Maastricht University. All subjects gave their written informed consent before participation in the study.

Microbiota analysis

Genomic DNA extraction was performed using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research, Leiden, the Netherlands) according to the manufacturer's instructions. 16S rRNA gene amplicon libraries for Illumina 2×300 bp paired end sequencing were generated and sequenced on an Illumina MiSeq (Illumina, Eindhoven, the Netherlands), according to the standard Illumina protocols. Briefly, barcoded amplicons from the V3-V4 region of 16S rRNA genes were generated using a 2-step PCR. For this, 10-25 ng genomic DNA was used as the template for the first PCR using the 341F (50-CCTACGGGNGGCWGCAG-30) and the 785R (50-GACTACHVGGGTATCTAATCC-30) primers appended with Illumina adaptor sequences in a total volume of 50 µl. PCR products were purified (QIAquick PCR Purification Kit, Qiagen, Venlo, the Netherlands). Subsequently, the size of the PCR products was checked on a fragment analyser (Advanced Analytical, Ankeny, IA, USA) and quantified by fluorometric analyses. These purified PCR products were used for the second PCR in combination with sample-specific unique barcoded primers (Nextera XT index kit, Illumina). Subsequently, PCR products were purified, checked on a Fragment analyser and quantified as described above. Then samples were multiplexed, clustered and sequenced on an Illumina MiSeq. The raw data were analysed with the Illumina CASAVA pipeline (v1.8.3; Illumina) with demultiplexing based on the unique sample-specific barcodes. Sequences were converted into FASTQ files using BCL2FASTQ pipeline version 1.8.3. The quality cut was applied based on the quality level of the Phred (Phred quality score). The Quantitative Insights Into Microbial Ecology (QIIME) software package (1.9.0) was used for microbial analyses (Caporaso *et al.*, 2010). The sequences were classified using Greengenes (version 13.8) as a reference 16S rRNA gene database.

Statistical analysis

The effects of MIH exposure on alpha-diversity indices were analysed using Wilcoxon-signed rank tests using SPSS version 24 (IBM, Armonk, NY, USA), whereas effects on beta-diversity indices were tested using nonparametric permutational multivariate analysis of variance (PERMANOVA) with 999 permutations (Anderson, 2017) in RStudio. In addition, to compare MIH-induced effects on microbiota composition, relative abundances of taxa were tested using nonparametric Wilcoxon-signed rank tests, and Benjamini-Hochberg FDR correction for multiple testing was also applied. Data are expressed as mean ± standard error of the mean. A P-value of <0.05 was considered statistically significant. Correlation analysis between changes in the relative abundances (Δrelative abundances: MIH relative abundance – normoxia relative abundance) and alterations in metabolic parameters (Δmetabolic parameter: MIH metabolic parameter – normoxia metabolic parameter) was performed using Spearman's rank-order correlation with multiple testing correction using the software package R (3.5.0) (R-Core team, http://www.R-project.org/).

3. Results

Participant characteristics

Characteristics of study participants in which stool samples were collected (n=5 paired samples) are depicted in Table 1. By design, participants had a body mass index ≥28 kg/m^2 and the homeostatic model assessment for insulin resistance (HOMA-IR) ≥2.2.

MIH exposure alters gut microbiota composition

Samples were rarefied to 10,000 sequences before determining alpha-diversity (within-sample diversity) and beta-diversity (inter-sample similarity) indices. The rarefaction curves of observed operational taxonomic units (OTUs) was characterised by a plateau phase around 6,500 reads. Shannon index (Figure 1A, P=0.374) and Faith's phylogenetic diversity (Figure 1B, P=0.929) were not different after seven days of MIH exposure relative to normoxia exposure. In addition, the observed OTUs (Figure 1C, P=0.536) and the evenness index (Figure 1D, P=0.624) were not different between MIH and normoxia exposure, suggesting that alpha-diversity was not affected by MIH exposure. In addition, unweighted (P=0.973) and weighted Unifrac (P=0.508), Jaccard distance (P=0.975) and Bray-Curtis dissimilarity (P=0.886) indices were not significantly

Table 1. Participant characteristics at baseline.[1]

Parameter		Reference values (range)
Age (y)	62±2	
BMI (kg/m^2)	30.1±1.1	18.5-25.0
Hemoglobin (mmol/l)	9.4±0.4	8.5-11.0
Creatinine (µmol/l)	89±3.7	45-100
ALAT (U/l)	29±6.7	<50
HbA_{1c} (%)	5.6±0.2	4.0-6.0
Fasting glucose (mmol/l)	5.9±0.3	<5.6
2h-glucose (mmol/l)	5.6±1.0	<7.8
HOMA-IR	4.0±0.6	

[1] BMI = body mass index; ALAT = alanine aminotransferase; HbA_{1c} = glycated haemoglobin; 2h-glucose = glucose concentration after 2 h oral glucose tolerance test; HOMA-IR = homeostatic model assessment for insulin resistance.

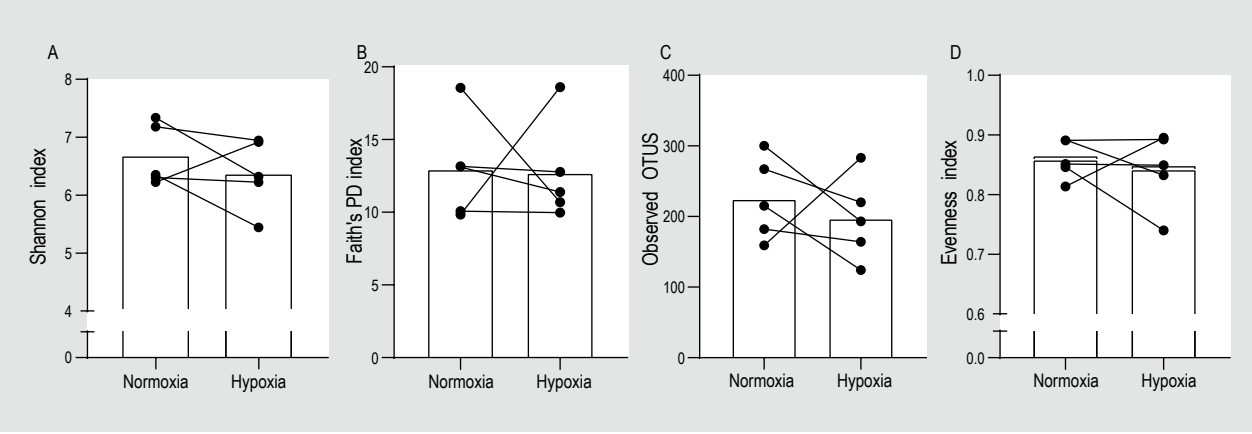

Figure 1. The effect of mild intermittent hypoxia compared to normoxia exposure on alpha-diversity indices. Alpha-diversity, determined by (A) Shannon index, (B) observed OTUS, (C) Faith's phylogenetic diversity and (D) evenness indices. The line and whiskers represent the median, and the lowest/highest values, respectively. Individual samples are plotted as circles (n=5 paired samples). Statistical analysis was performed using nonparametric related-samples Wilcoxon-signed rank tests.

different between MIH and normoxia exposure, indicating that MIH did not alter gut microbial β-diversity.

Furthermore, we found that MIH exposure for seven days did not significantly alter the relative abundances of taxonomical phyla, classes and orders. In addition, the *Firmicutes*/*Bacteriodetes* ratio was not altered (P=0.500) by MIH exposure. However, MIH significantly reduced relative abundances of the families *Clostridiaceae* (1.6% MIH versus 4.7% normoxia, P=0.043) and *Christensenellaceae* (1.0% MIH versus 2.1% normoxia, P=0.043). At genus level, relative abundances of *Clostridium sensu stricto 1* (1.6% MIH versus 4.7% normoxia, P=0.043, Figure 2A) and *Christensenellaceae* R-7 group (1.0% MIH versus 1.9% normoxia, P=0.043, Figure 2B) were significantly reduced by MIH, whereas *Fusicatenibacter* (1.3% MIH versus 0.9% normoxia, P=0.043, Figure 2C), *Butyricicoccus* (1.7% MIH versus 0.7% normoxia, P=0.043, Figure 2D) and *Holdemania* (0.06% MIH versus 0.02% normoxia, P=0.043, Figure 2E) were significantly increased subsequent to MIH exposure. These differences were non-significant after correction for multiple testing.

MIH-induced changes in levels of genera are associated with host metabolism

MIH-induced alterations (Δ) in the abundance of several genera were strongly associated with MIH-differences in several metabolic parameters (Figure 3, Supplementary Figure S1). Δ*Barnesiella* was inversely associated with ΔAT insulin sensitivity ($q=1.53\times10^{-21}$). Moreover, Δ*Butyricimonas* ($q=1.08\times10^{-22}$), Δ*Odoribacter* ($q=1.08\times10^{-22}$), Δ*Parabacteroides* ($q=1.08\times10^{-22}$) and Δ*Ruminococcus torques* group ($q=1.08\times10^{-22}$) were

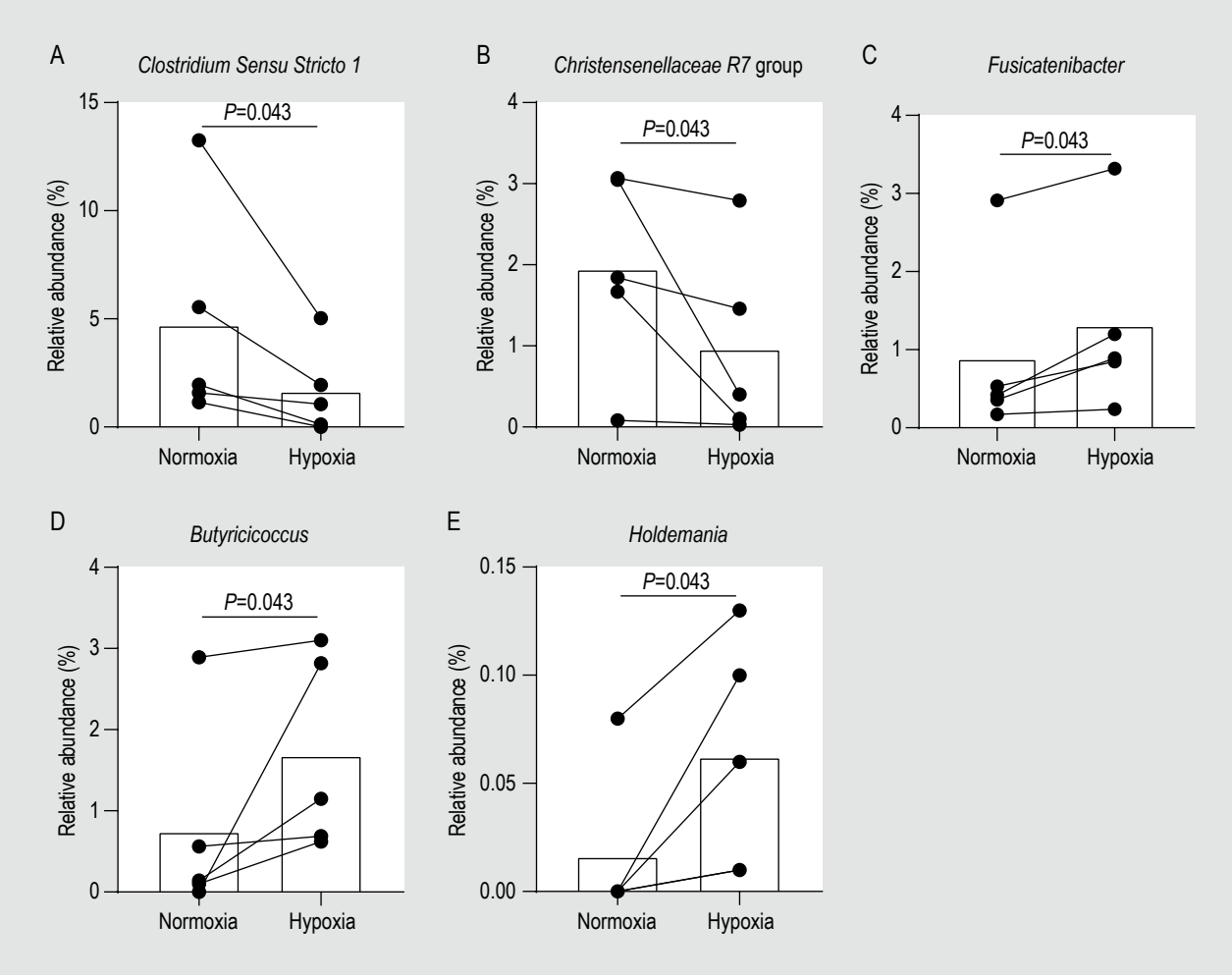

Figure 2. Relative abundances of genera affected by mild intermittent hypoxia exposure. Relative abundances of (A) *Clostridium sensu stricto* 1 and (B) *Christensenellaceae* R7 group genera were significantly reduced, whereas (C) *Fusicatenibacter*, (D) *Butyricicoccus* and (E) *Holdemania* genera were significantly increased subsequent to mild intermittent hypoxia exposure as compared to normoxia. The *P*-values are based on related-samples Wilcoxon-signed rank tests on paired samples (n=5), without false discovery rate (FDR) correction.

positively associated with Δperipheral insulin sensitivity. Furthermore, Δ*Alistipes* (q=5.4×10^{-22}) and Δ*Coprococcus 2* (q=5.4×10^{-22}) were associated with Δfasting and Δpostprandial triglycerides, respectively. In addition, Δ*Christensenellaceae* R-7 group (q=5.09×10^{-22}) and Δ*Marvinbryantia* (q=5.09×10^{-22}) were inversely, whereas Δ*Holdemania* (q=5.09×10^{-22}) was positively associated with Δpostprandial systemic free fatty acid (FFA) concentrations. Δ*Caproiciproducens* (q=5.4×10^{-22}) was associated with Δpostprandial plasma lactate levels. Δ*Eubacterium rectale* group (q=5.4×10^{-22}) was positively, whereas Δ*Ruminococcaceae* UGC 014 (q=7.64×10^{-22}) was inversely associated with Δfasting plasma insulin levels. Lastly, Δ*Ruminococcacceae* UGC 010 was positively associated with Δpostprandial carbohydrate oxidation (q=5.4×10^{-22}), whereas it inversely associated with Δfat oxidation (q=1.53×10^{-21}).

4. Discussion

To the best of our knowledge, the present randomised, single-blind crossover trial is the first well-controlled study investigating the effects of normobaric MIH exposure on faecal microbiota composition in overweight and/or obese humans. Here, we report that MIH has slight effects on gut microbial composition, with no effect on alpha- and beta-diversity indices. Interestingly, our findings indicate that MIH-induced alterations in relative abundances of faecal microbiota composition on genus level are associated with several alterations in metabolic outcome parameters, including AT and peripheral insulin sensitivity, as well as fasting and postprandial metabolite plasma levels in overweight and obese men.

In contrast to previous findings, we found no alterations in microbial composition on phylum, order and class levels.

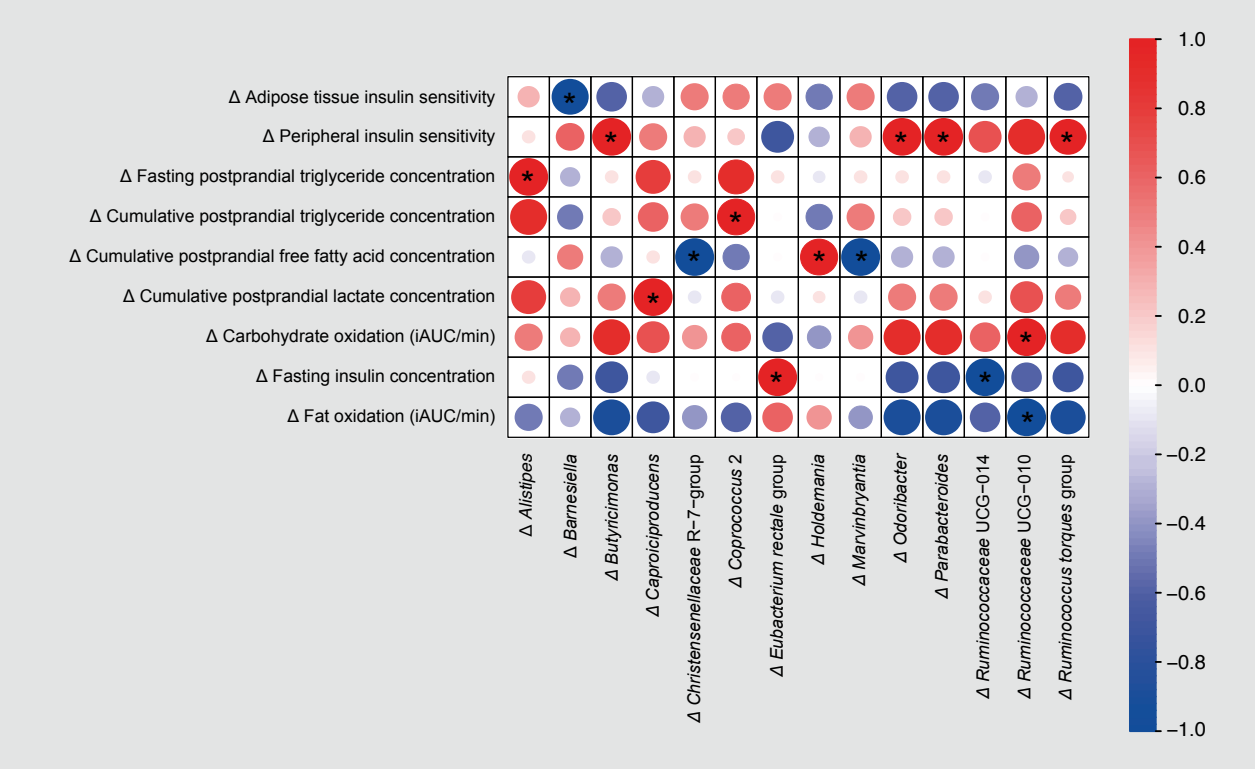

Figure 3. Heatmap of associations between mild intermittent hypoxia-induced alterations in the abundance of several genera and mild intermittent hypoxia-induced change in several metabolic parameters. Correlation analysis between changes in the relative abundances (Δrelative abundances: mild intermittent hypoxia (MIH) relative abundance – normoxia relative abundance) and alterations in metabolic parameters (Δmetabolic parameter: MIH metabolic parameter – normoxia metabolic parameter) was performed using Spearman's rank-order correlation with multiple testing correction. Asterisks indicate statistically significant correlation.

Indeed, animal studies demonstrated that intermittent hypoxia exposure, albeit under very severe and hypobaric conditions, decreased *Firmicutes*, whilst increasing *Bacteriodetes* (Moreno-Indias *et al.*, 2015; Tian *et al.*, 2018) and hence a reduced *Firmicutes*/*Bacteriodetes* ratio (Tian *et al.*, 2018). Recently, it has been demonstrated that Tibetan highlanders, who are chronically exposed to hypobaric hypoxia, show reductions in *Prevotella* and *Bacteroides* genera compared to sea level residents (Li and Zhao, 2015). In the present study, MIH seemed to evoke a reduction in relative abundance on family and genus level of *Christensenelleceae* and *Clostridiaceae*-1, with concomitant decreased abundances of *Clostridium sensu stricto*-1 and *Christensenelleceae* R-7 group. Our results are partially in contrast with previous findings, demonstrating increased *Christensenelleceae* abundance in healthy males when exposed to high altitude hypoxia (4,300 m) (Karl *et al.*, 2018). Interestingly, however, *Christensenelleceae* R-7 group was previously found to be negatively associated with very low density lipoproteins, small-sized high density lipoprotein (HDL)-particles and triglycerides within medium-sized HDL particles (Vojinovic *et al.*, 2019), whereas *Clostridium sensu stricto*-1 was associated with large-sized HDL particles (Vojinovic *et al.*, 2019). In line with these findings, we found a relationship of these families and genera with parameters of lipid metabolism, showing an inverse association between *Clostridiaceae*-1 and *Christensenellaceae* and (*Christensenellaceae* R-7 group) and fasting and postprandial systemic FFA concentrations, respectively.

Moreover, we found that MIH increased the relative abundances of the obligate anaerobic genera *Holdemania*, *Butyricicoccus* and *Fusicatenibacter*. These findings are in agreement with previous studies in humans and rodents, demonstrating that hypoxia increased the abundance of anaerobic bacteria in the gut (Li and Zhao, 2015; Mazel, 2019; Moreno-Indias *et al.*, 2015). Indeed, high altitude exposure has been demonstrated to increase abundance of *Holdemania* (Karl *et al.*, 2018). In addition, *Holdemania*, *Butyricicoccus* and *Fusicatenibacter* are butyrate-producers, which plays an important role in gut homeostasis by ensuring mucus production and maintaining tight-junction integrity (Peng *et al.*, 2009).

MIH-induced effects on faecal microbiota composition might affect host metabolism. Indeed, we found that MIH-induced alterations in the abundances of *Buytiricimonas*,

Odoribacter, *Parabacteroides* and *Ruminococcus torques* group were significantly associated with peripheral insulin sensitivity. In agreement with our findings, *Butyricimonas* and *Odoribacter*, both strictly anaerobic butyrate-producers with anti-inflammatory effects, were inversely correlated with glucose levels in people with morbid obesity (Moreno-Indias *et al.*, 2016), and positively associated with Matsuda index in non-diabetic humans (Yamashita *et al.*, 2019). Previous studies demonstrate that the *Ruminococcus torques* group was decreased after diabetes remission (Murphy *et al.*, 2017). Yet, *Ruminococcaceae* are able to generate short-chain fatty acids, enhancing energy metabolism, attenuating inflammatory processes and decreasing gut permeability (Moreno-Indias *et al.*, 2016). Interestingly, we found that *Ruminococcaceae* UGC-014 was inversely associated with plasma insulin levels, whereas UGC-010 was positively associated with carbohydrate oxidation, and negatively with fat oxidation. Furthermore, *Caproiciproducens* was positively associated with MIH-induced lactate formation, suggesting a contribution of gut-derived lactate production to circulating lactate levels. Alternatively, MIH-induced peripheral lactate production may induce intestinal caproic acid generation via microbial chain elongation (reverse β-oxidation) (Contreras-Davila *et al.*, 2020; Zhu *et al.*, 2017). Finally, we found an inverse association between MIH-induced alteration in the relative abundance of *Barnesiella* and changes in AT insulin sensitivity. Interestingly, *Barnesiella* was found to be less abundant in native highlanders, who are chronically exposed to hypobaric hypoxia, compared to sea level residents (Li and Zhao, 2015). In line, in mice fed an anti-diabetogenic diet (gluten-free diet), the abundance of *Barnesiella* decreased with concomitant reduced incidence of hyperglycaemia (Marietta *et al.*, 2013). Taken together, our findings together with previous studies might support a putative role for *Barnesiella* in the modulation of insulin sensitivity in humans.

Although the present randomised, single-blind crossover study is the first well-controlled study to investigate the effects of MIH on faecal gut microbiota composition in humans, several limitations need to be taken into account. Firstly, our study population consisted of men with overweight/obesity and mild impairment in glucose homeostasis. Therefore, the effects of MIH exposure on faecal microbial composition in other subgroups of the population such as those with a different metabolic status or women remain to be elucidated. Furthermore, study participants were exposed to MIH for a relatively short duration (1 week, 42 h mild hypoxia exposure in total) compared to previous animal studies (4-6 weeks) (Moreno-Indias *et al.*, 2015; Tian *et al.*, 2018) and observational studies in humans (high-altitude natives). Therefore, the impact of the severity as well as duration of hypoxia exposure on gut microbial composition requires further investigation. Finally, the present data should be interpreted with caution due to the small sample size and hence limited statistical power. Thus, further studies are warranted to confirm the present findings.

In conclusion, the present randomised, single-blind crossover study indicates for the first time that normobaric MIH exposure has slight effects on faecal microbial composition in men with overweight and obesity, inducing changes in several bacterial families and genera such as a shift towards higher abundances of anaerobic butyrate-producing bacteria. Furthermore, we found that MIH-induced alterations in gut microbial composition are associated with changes in several parameters related to glucose and lipid homeostasis. Together, our findings suggest that hypoxia-induced alterations in host metabolism might at least partly be mediated by changes in gut microbiota composition in humans. The present findings warrant future studies to investigate the effects of the severity, duration as well as frequency of hypoxia exposure on gut microbiota composition and functionality in humans, and examine the relationship between hypoxia-induced changes in the gut microbiota and host metabolism in more detail.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/BM2021.0159.

Figure S1. Correlation analysis of mild intermittent hypoxia induced alterations in relative abundance of genera and alterations in metabolic outcome parameters.

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Conflict of interest

Koen Venema is editor-in-chief of Beneficial Microbes. He had no influence in the review process and decision making on this manuscript. The other authors declare no conflict of interest.

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