Genetically determined hypertensive phenotype affects gut microbiota composition, but not vice versa

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**Objectives:** Research suggests reciprocal crosstalk between the host and gut bacteria. This study evaluated the interaction between gut microbiota and arterial blood pressure (BP) in rats.

**Methods:** Continuous telemetry recordings of BP were started in 7-week-old normotensive Wistar–Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). Two weeks later, half of the WKY and SHR were subjected to cross-transplantation of fecal matter, with stools harvested from either WKY or SHR and BP measurements until the age of 14 weeks. The composition of gut bacteria was assessed through analysis of the bacterial 16S ribosomal RNA gene sequence. The concentration of microbiota-derived metabolites was evaluated using HPLC-MS.

**Results:** There was a significant difference between WKY and SHR in the composition of gut bacteria at the start and end of the study. This was accompanied by significant histological differences in the colon. SHR, but not WKY, showed a gradual increase in BP throughout the experiment. For both WKY and SHR, there was no significant difference in BP or metabolic parameters between animals receiving fecal transplantation from either SHR or WKY.

**Conclusion:** Genetically induced hypertension in SHR is associated with alterations in the composition of gut bacteria and histological morphology of the colon. An interstrain fecal transplant does not affect BP and does not produce long-term changes in gut bacteria composition. We propose that the impact of the host genotype and/or phenotype on the gut bacteria may be greater than the impact of the gut bacteria on the host BP.

**Keywords:** blood pressure, fecal microbiota transplantation, hypertension, intestines, microbiota

INTRODUCTION

The gut microbiome is a diverse ecosystem that evolves alongside the host and plays an essential role in numerous physiological processes [1,2]. In the past few years, it has become increasingly apparent that gastrointestinal and extraintestinal diseases are associated with alterations in gut bacteria composition, a condition known as dysbiosis [3]. It is important to note that the definition of dysbiosis is broad. There is currently no consensus as to what constitutes a ‘physiological’ or ‘healthy’ composition of gut microbiota. This is because the composition of gut bacteria depends on numerous factors, such as demographic characteristics, diet, mode of delivery, and others [4–6].

Alterations in gut microbiota have been associated with obesity [7], colitis [8], type 2 diabetes mellitus [9], and cardiovascular disease (CVD), including hypertension [2,10,11]. The mechanisms responsible for these associations are not clear. Specifically, it remains to be determined whether changes in gut bacteria composition are primary or secondary to these systemic disorders. Hence, the association between gut dysbiosis and systemic diseases represents a typical example of the chicken or the egg causality dilemma.

It has recently been postulated that alterations in the composition of gut bacteria may trigger the development of hypertension [11]. However, it is also possible that alterations in the gut bacteria composition are secondary to the...
hypertension-induced changes occurring in bacterial habitat (i.e. in the intestines of hypertensive animals). In this regard, our previous work [12] and the work of others [13,14] have shown that there are significant functional and morphological disturbances in the gut of hypertensive rats [12–14].

This study aimed to evaluate the interaction between blood pressure (BP) and gut bacteria in rats in light of the current lack of consensus.

METHODS

Animals and group characteristics

Studies were performed on male Wistar–Kyoto (WKY, n = 21) and spontaneously hypertensive (SHR, n = 21) rats. The rats were obtained from the Central Laboratory of Experimental Animals, Medical University of Warsaw (Warsaw, Poland), housed in groups of three to four per opaque plastic cage (60 cm × 38 cm × 19.6 cm, L × W × H) with pine chip bedding in an air-conditioned room (22 ± 2°C, 55 ± 5% relative humidity) under a 12-h light/12-h dark cycle (lights on at 9.00 a.m.). The animals had unlimited access to tap water and standard rat chow (Labofeed B, Morawski, Poland, the nutritional composition is described in the Supplementary Digital Content, Table S1, http://links.lww.com/HJH/B639). The study’s experimental design was approved by the 2nd Local Ethics Committee for Experiments on Animals at the Warsaw University of Life Sciences, Warsaw, Poland (Certificate of approval No. WAW2/082/2018). All protocols used were in accordance with the guidelines published in the European directive 2010/63/EU on the protection of animals used for scientific purposes.

Experimental design

Seven-week-old rats were randomly divided into either transplant donors or transplant recipients. Transplant recipients were implanted with telemetry probes for continuous telemetry hemodynamic recordings in WKY (n = 14) and SHR (n = 14). After 2 weeks, transplant recipients were transplanted with stools (colon content) from 14-week-old either WKY (n = 7) or SHR (n = 7) transplant donors. Specifically, four experimental groups were included in this study, that is, WKY implanted with stools from either WKY or SHR (WKY(wky) (n = 7) and WKY(shr) (n = 7)) and SHR implanted with stools from either SHR or WKY (SHR(shr) (n = 7) and SHR(wky) (n = 7)), (Fig. 1).

Surgical preparation

All surgical procedures were performed under general anesthesia with ketamine at 100 mg/kg (Bioketan, Vetoquinol Biowet, Poland) and xylazine at 10 mg/kg of body weight (Xylapan, Vetoquinol Biowet, Poland) administered intraperitoneally. After surgery, the animals were given an intramuscular injection of benzathine penicillin 30 000IU (Debecylina, Polfa Tarchomin, Poland).

Hemodynamic measurements

The rats were anesthetized, as mentioned before. Surgical implantation of the arterial catheter connected to the telemetry transmitter was performed (see Surgical implementation of the telemetric transmitter, Supplementary Digital Content 1, http://links.lww.com/HJH/B639).

Five days after the catheter implantation, hemodynamic measurements began. Using DSITM Dataquest ATR equipment (Data Sciences International, St. Paul, Minnesota,
The NGS Reads were evaluated based on their quality by Bioinformatics (San Diego, USA), http://links.lww.com/HJH/B639) designed by Illumina.

16S Metagenomic Library Supplementary Digital Content 1, http://links.lww.com/HJH/B639).

25 ng of DNA was used. Amplification of the V3–V4 region of the 16S rRNA gene was performed according to the 16S Metagenomic Sequencing Library Preparation protocol (see 16S Metagenomic Library Supplementary Digital Content 1, http://links.lww.com/HJH/B639) designed by Illumina (San Diego, USA).

To generate NGS (next-generation sequencing) libraries, 25 ng of DNA was used. Amplification of the V3–V4 region of the 16S rRNA gene was performed according to the 16S Metagenomic Sequencing Library Preparation protocol (see 16S Metagenomic Library Supplementary Digital Content 1, http://links.lww.com/HJH/B639) designed by Illumina (San Diego, USA).

Bioinformatics

The NGS Reads were evaluated based on their quality measured by phred quality score (>30) using the FastQC software. Subsequent analyses were performed using specific software (see Bioinformatics, Supplementary Digital Content 1, http://links.lww.com/HJH/B639), as described previously [15–20].

Metabolic cage experiments

At the end of the experiment, the rats were maintained for 2 days in metabolic cages to evaluate their 24-h fluid and energy balance.

On day 52, the rats were terminally anesthetized with urethane (1.5 g/kg body weight intraperitoneally, Sigma-Aldrich), followed by a collection of their colons and blood from the right ventricle of the heart for further analysis.

Evaluation of microbiota-derived short-chain fatty acids

Short-chain fatty acids (SCFAs; acetic, butyric and valeric acids) were evaluated using a high-performance liquid chromatography–tandem mass spectrometry method (HPLC-MS) as we have previously described [12,21].

Biochemical analysis

Biochemical analysis of plasma (sodium, potassium, creatinine and urea) was performed using a Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, Indiana, USA).

Histopathology of the colon

Tissue samples of a colon were collected from all experimental groups at the end of the experiment and prepared for histopathological examination (see Histopathology of the colon section in Supplementary Digital Content 1, http://links.lww.com/HJH/B639). The microscopic evaluation was performed in a blinded fashion, using a standard light microscope Axiolab A5 with Axiocam 208 color and ZEN 3.0 software (Zeiss, Jena, Germany).

Statistical analyses

Data were analyzed using Dell Statistica, version 13 (Dell Inc, Tulsa, Oklahoma, USA). Hemodynamic data were evaluated by multivariate ANOVA, followed by Tukey’s post hoc test. In metabolic experiments, a t-test was performed. The Kolmogorov–Smirnov test was used to test the normality of the distribution. In data without normality of distribution, the Mann–Whitney U-test and Wilcoxon test were used. A value of two-sided P less than 0.05 was considered significant.

RESULTS

Wistar–Kyoto, rats vs. spontaneously hypertensive rats [WKY(wky)+WKY(shr) vs. SHR(wky)+SHR(shr)]

General metabolic and plasma biochemistry parameters

SHR had significantly lower body mass at the beginning and the end of the experiment compared to WKY. There were no significant differences in metabolic and plasma biochemical parameters, including food and water intake, urine output or sodium, potassium, or creatinine in the plasma (see Table S2, Supplementary Digital Content 1, http://links.lww.com/HJH/B639).
Hypertension affects gut microbiota

TABLE 1. Comparison of metabolic parameters and plasma biochemistry between all experimental groups

| Metabolic parameters                  | WKY(wky) (n = 7) | WKY(shr) (n = 7) | SHR(shr) (n = 7) | SHR(wky) (n = 7) |
|---------------------------------------|------------------|------------------|-----------------|-----------------|
| Body mass start (g)                   | 235.50 ± 7.35    | 246.89 ± 8.50    | 212.81 ± 11.11  | 207.34 ± 6.21   |
| Body mass end (g)                     | 347.75 ± 13.03   | 371.39 ± 12.09   | 335.94 ± 7.12   | 328.00 ± 12.73  |
| Weight gain (g)                       | 112.26 ± 10.23   | 124.50 ± 15.99   | 123.13 ± 10.72  | 121.26 ± 11.87  |
| Food intake (g/24h)                   | 24.08 ± 1.53     | 23.13 ± 3.65     | 24.74 ± 1.21    | 22.97 ± 0.72    |
| Water intake (ml/24h)                 | 33.94 ± 2.04     | 32.17 ± 3.65     | 37.54 ± 3.09    | 37.61 ± 2.31    |
| Urine output (ml/24h)                 | 8.00 ± 0.52      | 10.00 ± 1.51     | 9.67 ± 2.03     | 13.14 ± 1.56    |
| Stool output (g/24h)                  | 13.83 ± 1.05     | 13.70 ± 0.93     | 14.79 ± 0.79    | 13.38 ± 1.08    |
| Sodium (mmol/l)                       | 134.29 ± 1.66    | 136.14 ± 2.96    | 139.14 ± 1.52   | 138.14 ± 2.15   |
| Potassium (mmol/l)                    | 5.56 ± 0.45      | 5.02 ± 0.12      | 5.10 ± 0.06     | 5.49 ± 0.07     |
| Creatinine (mg/dl)                    | 0.61 ± 0.05      | 0.50 ± 0.05      | 0.57 ± 0.05     | 0.54 ± 0.04     |

Values are means ± SE. SHR, spontaneously hypertensive rats; SHR(shr), SHR rats receiving fecal transplantation from SHR rats; SHR(wky), SHR rats receiving fecal transplantation from WKY rats; WKY, Wistar–Kyoto rats; WKY(shr), WKY rats receiving fecal transplantation from SHR rats; WKY(wky), WKY rats receiving fecal transplantation from WKY rats.

*p less than 0.05 for comparison between the groups.

### Hemodynamic parameters

SHR showed significantly higher SBP and DBP at the start and end of the experiment. In SHR, there was a significant, gradual increase in SBP (F6,54 = 14.68, P < 0.05) throughout the entire experiment, whereas in WKY, there was only a transient increase in SBP following the metabolic measurements. SHR and WKY showed a significant increase in DBP (F6,54 = 47.55, P < 0.05 and F6,72 = 20.43, P < 0.05, respectively) (see Fig. S1, Supplementary Digital Content 1, http://links.lww.com/HJH/B639). There was a gradual decrease in HR in SHR (F6,54 = 6.50 P < 0.05) and WKY (F6,72 = 2.77 P < 0.05). Throughout the whole experiment, SHR had significantly higher SBP (F1,21 = 605.82, P < 0.05) and DBP (F1,21 = 276.79, P < 0.05) than WKY.

### Gut microbiota composition

There were significant differences in gut microbiota between WKY and SHR at the start and at the end of the experiment (see Fig. S2, Supplementary Digital Content 1, http://links.lww.com/HJH/B639). A comparison of alpha diversity (within-sample microbial diversity) showed that SHR had a higher average number of species and higher phylogenetic diversity than the normotensive rats (see Fig. S3, Supplementary Digital Content 1, http://links.lww.com/HJH/B639).

### Concentration of gut microbiota metabolites in stools

SHR showed a significantly higher concentration of butyric and valeric acids in stools than WKY at the start of the experiment. However, there was a substantial intra-group variation. At the end of the experiment, there was no significant difference between the concentration of stool metabolites between SHR and WKY (see Table S3, Supplementary Digital Content 1, http://links.lww.com/HJH/B639).

### Wistar—Kyoto, intra-Wistar–Kyoto group comparison: WKY(wky) series vs. WKY(shr) series

### General metabolic and plasma biochemistry parameters

There were no significant differences in any metabolic or plasma biochemical parameters between rats receiving fecal microbiota transplant (FMT) from WKY [WKY(wky) series] or SHR [WKY(shr) series] (Table 1).

### Hemodynamic parameters

There were no significant differences in SBP, DBP or HR between the WKY(wky) and WKY(shr) series at the start or end of the experiment (Fig. 2). In both series, there was a transient increase in HR associated with the time of FMT and the metabolic experiments.

There were no significant differences in SBP, DBP or HR between the experimental series at any point during the experiment. Additionally, no differences were found between the series in diurnal variation in BP or HR (see Fig. S4, Supplementary Digital Content 1, http://links.lww.com/HJH/B639).

### Changes in gut microbial community

**Between series WKY(wky) vs. WKY(shr) analysis**

No significant differences in gut bacteria were observed between WKY(wky) and WKY(shr) at either the start or end of the experiment in gut bacteria based on an unweighted UniFrac analysis. However, using a weighted UniFrac analysis, differences in gut microbiota abundance were found between the two series. A comparison of alpha diversity between WKY(wky) and WKY(shr) did not reveal significant differences in average species richness or phylogenetic diversity (see Fig. S5, Supplementary Digital Content 1, http://links.lww.com/HJH/B639).

**Change in time (the start vs. the end of the experiment) analysis**

In WKY(wky), there was no significant difference in gut bacteria composition or abundance when comparing stools harvested at the start and the end of the experiment (Fig. 3). In WKY(shr), differences in gut bacteria composition between the start and the end of the experiment were found; however, the abundance of bacteria was unchanged (Fig. 3).

### Stool concentration of gut microbiota metabolites

**Between series WKY(wky) vs. WKY(shr) analysis**

There were no significant differences in the concentration of gut microbiota metabolites between the WKY(wky)
WKY(shr) series at the start or end of the experiment (Table 2).

**Change in time (the start vs. the end of the experiment) analysis**

WKY(wky) group showed a significant decrease in acetic acid throughout the experiment (Table 2).

**Spontaneously hypertensive rats, intra-group comparison: SHR(shr) series vs. SHR(wky) series**

**General metabolic and plasma biochemical parameters**

There were no significant differences in general metabolic or plasma biochemical parameters between rats receiving FMT from SHR [SHR(shr) series] or rats receiving FMT from WKY [SHR(wky) series] (Table 1).

**Hemodynamic parameters**

There was no significant difference in SBP, DBP or HR between the SHR(shr) or SHR(wky) series (SHR transplanted with stools from SHR and WKY, respectively) at either the start or end of the experiment (Fig. 2). In both series, there was a significant gradual increase in SBP \( F_{16.48} = 8.63, P < 0.05 \) for SHR(shr); and \( F_{16.80} = 22.27, P < 0.05 \) for SHR(wky) group and DBP \( F_{16.48} = 20.64, P < 0.05 \) for SHR(shr), and \( F_{16.80} = 21.24, P < 0.05 \) for SHR(wky) group. There was a significant decrease in HR in the SHR(shr) \( (F_{16.48} = 19.02, P < 0.05) \) and SHR(wky) \( (F_{16.80} = 3.41, P < 0.05) \) series.

There was no significant difference between SHR(shr) and SHR(wky) in SBP, DBP and HR, throughout the experiment. Additionally, no differences between the groups were found in diurnal variation in BP or HR (see Fig. S6, Supplementary Digital Content 1, http://links.lww.com/HJH/B639).

**Changes in gut microbial community**

**Between series analysis SHR(shr) vs. SHR(wky)**

There was no significant difference in the composition of gut-microbiota between SHR(shr) and SHR(wky) at the start and the end of the experiment based on an unweighted and weighted UniFrac analysis. Comparison of alpha diversity between experimental series did not reveal differences in the average species richness or the phylogenetic diversity (see Fig. S7, Supplementary Digital Content 1, http://links.lww.com/HJH/B639).

**Change in time analysis (the start vs. the end of the experiment).**

In SHR(shr), there was no significant difference in bacteria composition between stools harvested at the start or the end of the experiment. However, the weighted UniFrac analysis...
found significant differences in the abundance of gut microbiota. Conversely, in SHR(wky), there was no significant difference in bacterial composition between stools harvested at either the start or end of the experiment (Fig. 3).

Phylogenetic changes between all experimental groups on a family level are presented in the Supplementary Material (see Fig. S8, Supplementary Digital Content 1, http://links.lww.com/HJH/B639).
The interaction between the host and gut bacteria is bidirectional. On the one hand, gut bacteria may affect the host’s homeostasis via blood-borne bacterial products [22] and/or afferent gut nervous signaling [23]. On the other hand, the host can affect the gut bacteria by dietary habits, ingestion of antibacterial food preservatives, medicinal products, or other substances that may alter the gut environment [4, 24].

Previous research suggests that hypertension is associated with alterations in gut bacterial composition [25] and that altered gut microbiota may contribute to hypertension [11, 26–29].

To evaluate if bacteria may affect rats’ hemodynamic and/or metabolic parameters, an experiment was designed involving rats subjected to FMT. Specifically, normotensive WKY rats were transplanted with stool masses from the WKY rats or hypertensive rats (SHR). Analogously, SHRs were implanted with stool masses from the SHRs or the WKY rats.

We have found significant differences in gut bacteria composition between normotensive and hypertensive animals through these studies, which is in line with previously published studies [14, 25].

In contrast to the previous hypotheses suggesting a hypertensive potential of altered bacterial composition, our findings provide evidence that alterations in gut bacteria composition are secondary to a hypertensive genotype and phenotype rather than the reciprocal. Firstly, we have found no significant effects of FMT on BP. To the best of our knowledge, our study is the first to evaluate the effect of FMT on BP in SHR and WKY using telemetry recordings. Some previous research has shown an increase in BP in normotensives rat after FMT from SHR and decreased BP after FMT from normotensive to hypertensive rats [30, 31]. However, all of those studies measured BP using repeated tail-cuff plethysmography. This method only permits short recordings (on the order of minutes) and requires physical restraint and warming of the animal. Such treatment produces stress-related hemodynamic effects [32]. Therefore, inter-strain differences in the reactivity of BP to stress may significantly affect the results. In fact, it was shown that this method is not appropriate for evaluating neither physiologically induced nor drug-induced hemodynamic effects, as WKY have a more pronounced response to stress than SHR [33]. In contrast, telemetry measurements offer a high-fidelity, uninterrupted recording of BP for long periods of time, in freely moving animals, and without the limitations of physical restraint [32].

Differences between the outcomes of the present study and studies by others [29, 30] may also result from other factors, such as age or strain. Even rat breeding techniques can be a source of variability, as it has been found that within the SHRs, there is a genetic variability associated with specific breeders [34, 35]. In our study, we found no effect of FMT on BP in WKY and SHR, which were subjected to cross-transplantation of fecal matter with stools harvested from either WKY or SHR. In contrast to our findings, Adnan et al. [29] have observed an increase in SBP in normotensive rats receiving FMT from stroke-prone SHRs but without reciprocal hypotensive effect in hypertensive

**DISCUSSION**

Our findings provide evidence that in an animal model of genetically induced hypertension, the host affects gut bacteria’s composition more than gut bacteria’s influence on the host’s hemodynamic and metabolic features. We postulate that the differences in the composition of gut bacteria between the normotensive and hypertensive animals result from structural and functional differences between the normotensive and hypertensive intestines, which constitute the bacterial habitat.

| Experimental group | Start of the experiment | End of the experiment |
|--------------------|-------------------------|-----------------------|
| Microbiota-derived SCFAs |                      |                       |
| WKY(wky) (n = 7) | 3764.92 ± 755.29 | 3850.83 ± 873.84 |
| WKY(shr) (n = 7) | 2617.07 ± 1485.65 | 4049.86 ± 1098.28 |
| SHR(shr) (n = 7) | 6391.23 ± 1598.06 | 4070.04 ± 699.06 |
| SHR(wky) (n = 7) | 4754.66 ± 732.56 | 4472.71 ± 658.91 |
| Valeric acid (µmol/L) |                      |                       |
| WKY(wky) (n = 7) | 362.89 ± 45.26 | 355.89 ± 46.70 |
| WKY(shr) (n = 7) | 209.02 ± 91.56 | 279.70 ± 68.05 |
| SHR(shr) (n = 7) | 511.26 ± 102.17 | 220.11 ± 16.51* |
| SHR(wky) (n = 7) | 487.31 ± 41.69 | 362.84 ± 44.36 |
| Acetic acid (µmol/L) |                      |                       |
| WKY(wky) (n = 7) | 2664.40 ± 2191.18 | 17388.49 ± 1968.79* |
| WKY(shr) (n = 7) | 22825.35 ± 3430.11 | 14538.71 ± 935.13 |
| SHR(shr) (n = 7) | 3109.47 ± 1420.63 | 14703.78 ± 1302.69* |
| SHR(wky) (n = 7) | 27741.28 ± 2659.91 | 16574.02 ± 807.71* |

Values are means ± s.e. SCFAs, short-chain fatty acids; SHR, spontaneously hypertensive rats; SHR(shr), SHR rats receiving fecal transplantation from SHR rats; SHR(wky), SHR rats receiving fecal transplantation from WKY rats; WKY, Wistar-Kyoto rats; WKY(wky), WKY rats receiving fecal transplantation from WKY rats; SCFAs concentration in the supernatant of stools diluted with saline (1:2), to estimate concentration in stools multiply by a factor of 3.

*P least than 0.05 – the start vs. the end of the experiment (within a group).

**Stool concentration of gut microbiota metabolites**

**Between series SHR(shr) vs. SHR(wky) analysis**

There were no significant differences in gut microbiota metabolites between SHR(shr) or SHR(wky) at either the start or end of the experiment (Table 2).

**Change in time (the start vs. the end of the experiment) analysis.**

SHR(wky) showed significant decreases in acetic acid, whereas SHR(shr) showed a significant decrease in both acetic acid and valeric acid concentrations in stools (Table 2).

**Comparison of colon histopathology between all experimental groups**

The SHR(shr) and SHR(wky) groups showed a significantly lower height of the colonic mucosa and a greater number of infiltrating lymphocytes in the epithelium than the WKY(wky) and WKY(shr) groups. Moreover, SHR(wky) and SHR(shr) showed a decrease in the number of goblet cells (Fig. 4). Nevertheless, histopathological images showed no significant morphological changes in the colon that would indicate pathological processes, active inflammation or other disorders.
Regarding the effect of age, in our study, to evaluate the influence of FMT on the development of hypertension, we performed FMT in relatively young 9-week-old recipients. However, Toral et al. [30] tested the effect of FMT in 21-week-old rats that had developed hypertension and likely pronounced hypertension-dependent tissue damage.

By demonstrating no effect of FMT on the hemodynamic parameters, our findings are in line with other studies using telemetry measurements, albeit conducted on a different hypertension model. Specifically, Mell et al., showed a lack of hypertensive effect of FMT from Dahl salt-sensitive rats (S) to Dahl salt-resistant (R) and no attenuation of hypertension after FMT from R to S [27].

FIGURE 4 (a) Histopathology of the colonic mucosa (10×) and (b) morphometry of the colon in Wistar-Kyoto rats transplanted with stools from Wistar-Kyoto rats: WKY(wky) (n = 5) or with stools from SHR: WKY(shr) (n = 5) and in SHR transplanted with stools from SHR: SHR(shr) (n = 5) or with stools from WKY: SHR(wky) (n = 6). (b) The height of the colonic mucosa (µm), the height of the colonic epithelium (µm), the number of infiltrating lymphocytes in the colonic epithelium (per 100 enterocytes), the number of goblet cells in the colonic epithelium (per 100 enterocytes). *P less than 0.05 – vs. WKY(wky); #P less than 0.05 – vs. WKY(shr), One-way ANOVA followed by post hoc Tukey’s test. SHR(shr), spontaneously hypertensive rats (SHR) rats receiving fecal transplantation from SHR rats; SHR(wky), SHR rats receiving fecal transplantation from Wistar-Kyoto (WKY) rats; WKY(shr), WKY rats receiving fecal transplantation from SHR rats; WKY(wky), WKY rats receiving fecal transplantation from WKY rats.
Secondly, our study suggests that the composition of bacteria in the gut is strongly affected by the host’s intestinal phenotype. In general, the composition of gut bacteria is shaped by the host demographic characteristics and dietary habits [4–6]. Here, WKY and SHR had the same diet, were maintained under the same conditions and underwent the same experimental interventions. However, they still showed significant differences in the composition of gut bacteria. This was associated with significant differences between SHR and WKY in the colon’s morphology, which is one of the major habitats of gut bacteria. Specifically, hypertensive rats showed a significantly lower height of the colonic mucosa, a decreased number of goblet cells and greater infiltration of lymphocytes in the epithelium than did the WKYs.

Hypertension-associated alterations in the intestines have also been reported in our previous [12] and several other studies [13,14,36].

Therefore, we postulate that differences in the gut bacteria composition between WKY and SHR result from the differences in bacterial habitat, that is, differences between the normotensive and hypertensive intestines.

Notably, for either the SHR and WKY, we did not find a significant difference in the intestinal structure between rats transplanted with stools from WKY and SHR. This suggests no significant effect of FMT on the intestines of rats. Due to a notable inter-individual variation, we did not observe a significant difference in stool concentration of bacterial metabolites between rats transplanted with hypertensive and normotensive stools. However, SHR tended to have a higher concentration of butyric and valeric acid in stools. In this regard, previous findings in SHR show decreased colonic absorption of butyrate [37]. Such observations, at least in part, may explain our findings. Furthermore, it is worth noting that the levels of bacterial metabolites may or may not reflect gut bacteria’s composition; as, although specific bacterial genera produce various metabolites, the same metabolite may be produced by various bacterial genera.

Finally, we have found no effect of intrastrain FMT on metabolic parameters, such as body mass or food and water intake.

There are several methods of fecal transplantation, including administration of fecal content through the upper gastrointestinal tract (oral administration), the middle gastrointestinal tract (endoscopy, nasogastric tube), or the lower gastrointestinal tract (sigmoidoscopy, colonoscopy, or enema) [38,39]. For studies in rodents, oral administration is the route most commonly used because of its relative simplicity [11,40]. For our study, we decided to perform a repeated intracolonic administration of the fecal content preceded by antibiotics treatment. We have chosen this procedure as it is the most radical method for changing microbiota composition, and it closely resembles the FMT protocols for humans [41,42]. Thirty-six days after the FMT, we found no difference in the microbiota composition between rats transplanted with stool masses from SHR and WKY, which provides evidence for the lack of long-term effects of FMT on gut microbiota composition.

Notably, clinical studies have shown no prolonged effect of FMT on gut microbiota composition. Although individual microbiota strains from the donor can be detected in stool samples of FMT recipient for up to 3 months following the FMT, no persistent changes in gut microbiota composition have been shown [43].

It is also worth noting that there are significant differences in gut bacteria composition and bacterial metabolite concentration between specific gastrointestinal tract segments [44]. We chose to focus on the colon, which is the most evaluated part of the gastrointestinal tract concerning the interaction between microbiota and the circulatory system [12,37,45,46] and a major site of gut bacteria metabolism in humans. However, in rats, the cecum seems to play a critical role in bacterial fermentation. Regrettably, the noninvasive access to the cecum in rats is virtually impossible, making FMT studies targeting the cecum in freely moving rats not feasible.

In conclusion, we postulate that differences in gut bacteria composition between WKY and SHR result from differences in bacterial habitat, that is, differences between normotensive and hypertensive intestines. This is because microbiota adjusts to the habitat provided by the host. In this regard, in our experimental settings in WKY and SHR rats, the host had a more significant impact on its gut bacteria than the bacteria had on the host. Further studies evaluating hemodynamic and neurohormonal responses to FMT, including assessing sympathetic and parasympathetic activity, are needed to reveal the impact of the hypertension-induced changes in the intestines on the crosstalk between microbiota and the host.

**Perspectives**

The present study highlights significant differences between normotensive and hypertensive animals in the gastrointestinal system. Further studies on ‘a hypertensive gut’ are needed as hypertension-specific changes in the intestines may affect gut bacteria composition and contribute to hypertension and cardiovascular disease.

**ACKNOWLEDGEMENTS**

Funding: this work was supported by the National Science Centre, Poland grants: 2016/21/B/NZ5/02544 and 2018/31/B/NZ5/00038.

**Conflicts of interest**

M.U. is currently receiving grants (2016/21/B/NZ5/02544 and 2018/31/B/NZ5/00038) from National Science Centre, Poland. For the remaining authors, none were declared.

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