Increased Systolic and Diastolic Blood Pressure Is Associated With Altered Gut Microbiota Composition and Butyrate Production in Early Pregnancy

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Abstract—The risk of developing pregnancy-induced hypertension and preeclampsia is higher in obese pregnant women. In obesity, the composition of the gut microbiota is altered. Obesity is also associated with low-grade inflammation. Metabolites from the gut microbiota may contribute to both hypertension and inflammation. The aim of this study is to investigate whether the composition of the gut microbiota in overweight and obese pregnant women is associated with blood pressure and levels of plasminogen activator inhibitor-1. The composition of the gut microbiota was determined with 16S ribosomal RNA sequencing in 205 women at 16 weeks gestation from the SPRING study (the Study of Probiotics in Gestational Diabetes). Expression of butyrate-producing genes in the gut microbiota was assessed by real-time polymerase chain reaction. Plasminogen activator inhibitor-1 levels were measured in fasting serum of a subset of 70 women. Blood pressure was slightly but significantly higher in obese compared with overweight women. The abundance of the butyrate-producing genus *Odoribacter* was inversely correlated with systolic blood pressure. Butyrate production capacity was decreased, but plasminogen activator inhibitor-1 concentrations increased in obese pregnant women. Plasminogen activator inhibitor-1 levels were inversely correlated with expression of butyrate kinase and *Odoribacter* abundance. This study shows that in overweight and obese pregnant women at 16 weeks gestation, the abundance of butyrate-producing bacteria and butyrate production in the gut microbiota is significantly negatively associated with blood pressure and with plasminogen activator inhibitor-1 levels. Increasing butyrate-producing capacity may contribute to maintenance of normal blood pressure in obese pregnant women. **(*Hypertension*. 2016;68:974-981. DOI: 10.1161/HYPERTENSIONAHA.116.07910.)** ● **Online Data Supplement**

Key Words: blood pressure ■ butyrate ■ dysbiosis ■ hypertension ■ pregnancy

Hypertension is clearly associated with obesity,[1] predisposing obese individuals to adverse cardiovascular outcomes. In obesity, the gut microbiome is altered, a phenomenon known as dysbiosis, which could contribute to hypertension and its complications, potentially by increasing low-grade inflammation.[2] Gut microbiome dysbiosis is also associated with cardiovascular disease.[3] In a rat model of spontaneous hypertension, the gut microbiome is less diverse, is less rich, and shows a higher *Firmicutes:Bacteroidetes* ratio.[2] A higher *Firmicutes:Bacteroidetes* ratio has also been reported for obese humans[4] and in people with systolic hypertension.[2] Obese school girls have higher blood pressure and a lower abundance of *Bacteroidetes* in their gut microbiota than normal-weight girls.[5]

The gut microbiota can potentially influence host blood pressure through multiple mechanisms. Bacteria belonging to the *Streptococcus, Escherichia, Lactobacillus*, and *Bifidobacterium* genera can synthesize neurotransmitters within the autonomic nervous system.[6] Alterations in the prevalence of these bacteria may alter vascular tone and contribute to the development of hypertension.[7] The gut microbiota influences host inflammatory response,[8] altering endothelial function, which can impact on host blood pressure. Short-chain fatty acid (SCFA) production by the gut microbiota is associated with hypertension,[9] as a result of the influence of SCFA on vascular tone.

The gut microbiota of hypertensive rats has lower abundance of *Bifidobacteria* and fewer SCFA-producing bacteria.[2] Treatment with the antibiotic minocycline increased the numbers of SCFA-producing bacteria while reducing mean arterial blood pressure.[2] Supplementation with probiotics reportedly reduces blood pressure, indicating the importance of the gut microbiota in the regulation of blood pressure outside pregnancy.[10]

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The SCFA butyrate is produced from dietary fiber by bacteria in the gastrointestinal lumen. There are 2 main enzymes catalyzing butyrate production: butyrate kinase (*Buk*) and butyryl-CoA:acetate CoA-transferase *(But),* which are often used as biomarkers for the detection of butyrate-producing bacteria.[11] *Buk* catalyzes the formation of butyrate from butyryl-CoA, releasing ATP in the process.[12,13] *But* catalyzes the reaction of butyryl-CoA with exogenous acetate to form butyrate and acetyl-CoA.[13,14] Some butyrate-producing bacteria only express *But* or *Buk*, whereas others express both.[12] The main butyrate producers in the human gut belong to the *Firmicutes* phylum (*Coprococci, Eubacterium, Roseburia*, and *Faecalibacterium* genera),[14] but members of other phyla, especially *Bacteroidetes* (*Odoribacter* and *Alistipes* genera), often contribute to the overall butyrogenic pool. SCFA-producing bacteria may affect blood pressure by direct effects of SCFA on vasodilation or through plasminogen activator inhibitor-1 (PAI-1). n-Butyrate increases PAI-1 mRNA in cultured hepatocytes,[15] and SCFA enemas stimulate rectal microcirculation and PAI-1 after aortic graft surgery.[16] It is unclear whether there is a relationship between butyrate production and hypertension in pregnancy.

In obesity, adipocytes from visceral fat secrete inflammatory markers and angiotensinogen. Angiotensinogen activates the renin–angiotensin–aldosterone system,[1] which may result in hypertension.[1] Hypertension causes an injury to the vascular endothelium, eliciting an inflammatory response causing a rise in C-reactive protein, which can induce PAI-1.[17] Circulating PAI-1 is increased in established hypertension and in normotensive children of hypertensive parents.[17] PAI-1 is expressed in adipose tissue, and its levels are increased in obesity.[18] In pregnancies affected by hypertension, including preeclampsia and HELLP syndrome (hemolysis elevated liver enzymes and low platelet), placental PAI-1 expression[19] and circulating PAI-1 levels[20–24] are increased.

Aim

In this study, the relationships between obesity, the gut microbiome, blood pressure, and PAI-1 levels in overweight and obese pregnant women are examined at 16 weeks gestation.

Materials and Methods

Study Population and Sample Collection

For this study, samples obtained from 205 participants of the SPRING study (the Study of Probiotics in Gestational Diabetes) were obtained. The SPRING study is a registered randomized controlled trial aiming to prevent gestational diabetes mellitus by probiotics supplementation in overweight and obese pregnant women (ANZCTR 12611001208998).[25] At baseline (<16 weeks gestation), detailed demographic and medical history data and a fasting serum sample were collected before randomization. Participants self-collected a fecal sample, which was refrigerated until storage at −80 °C within 24 hours of collection. Pregnancy-induced hypertension encompassing gestational hypertension, preeclampsia, and HELLP syndrome,[26] developing during pregnancy was noted.

Fecal DNA Extraction

0.25 g of stool sample was thawed at 4 °C overnight. For DNA extraction, the RBB+C (repeated bead beating and column) protocol was followed[27] with mechanical sample lysis performed for 3 minutes with a mix of 0.1 and 0.5 mm sterile zirconia beads in the TissueLyser II (Qiagen). The Qiagen AllPrep DNA extraction kit was used to isolate fecal DNA. DNA quantity and quality was analyzed with a Nanodrop ND 1000 spectrophotometer.

Gut Microbiome Sequencing

The V6–V8 regions of the bacterial 16S rRNA gene were amplified from the isolated DNA using the 926F and 1392R primer pair. The sequence for 926F is (5′ - TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAACTYAAAKGAATTGRCGG - 3′) and for 1392R is (5′ - GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGGGCGGTGWGTRC - 3′). This primer pair yields a product of 500 bps, which was cleaned with AMPure XP beads. DNA isolated from *Escherichia coli* Jm109 was used as positive control and sterile water as the negative control. The Nextera XT Index kit was used to add barcodes, synthesize, quantify, normalize, and pool libraries following the manufacturer's recommendations (Illumina). An Illumina MiSeq system was used for sequencing at the Australian Center for Ecogenomics in Brisbane, Australia. Sequencing data were assembled, demultiplexed, and quality filtered using the QIIME software (Quantitative Insights Into Microbial Ecology v 1.9.1; http://www.qiime.org).[28] Sequences were assigned to operational taxonomic units with a pairwise identity threshold of 97%, and taxonomic classification was determined using the Greengenes reference database. After excluding operational taxonomic units with an overall abundance of <0.0001, a median of 25 400 reads per sample were obtained. Data were rarefied to 3000 sequences per sample to standardize sequence reads across samples. Operational taxonomic unit abundance was analyzed on taxonomic level including Phylum, Class, Order, Family, and Genus.

Butyrate Production Capacity

The 2 main genes for bacterial butyrate production are *But* and *Buk*. *But* encodes butyryl-CoA:acetate-CoA transferase and *Buk* encodes butyrate kinase. Degenerate real-time polymerase chain reaction was performed to assess expression of *But* and *Buk* in each fecal DNA sample. The primer sequences for these assays were *Buk* (forward: 5′ - TGCTGTWGTTGGWAGAGGYGGA - 3′ and reverse: 5′ GCAACIGCYTTTTGATTTAATGCATGG) and *But* (forward: 5′ GCIGAICATTTCACITGGAAYWSITGGCAYATG 3′ and reverse: 5′ CCTGCCTTTGCAATRTCIACRAANGC 3′). Gene amplification and detection was performed in 96-well plates with SYBR Green polymerase chain reaction 2X master mix (Bio-Rad Laboratories). Each reaction of 15 ng DNA was run in duplicate with a final volume of 20 µL and sterile deionized water as nontemplate control. The amplification cycle used for *Buk* was 1 cycle of 95 °C for 2 minutes and 40 cycles of 95 °C, 54 °C, 72 °C for 45 seconds, and for *But* was 1 cycle of 95 °C for 3 minutes and 40 cycles of 94 °C, 53 °C, 72 °C for 30 seconds using the iQ5 real-time polymerase chain reaction machine (Bio-Rad Laboratories, Hercules, CA). *Clostridium difficile* and *Faecalibacterium prausnitzii* A2-165 were used to generate standard curves for *Buk* and B*ut,* respectively. Levels of *Buk* and *But* gene expression were normalized to the level of total bacteria content assessed using total bacterial gene primer sets (forward: 5′ GCAGGCCTAACACATGCAAGTC 3′ and reverse: 5′ CTGCTGCCTCCCGTAGGAGT 3′). Relative gene expression was calculated using the ΔΔCt method. Overall butyrate production was calculated by determining the centroid values for the expression of *Buk* and *But* separately and adding these to provide an indication of the total butyrate-producing gene load for each woman. From each subject, dietary fiber intake was obtained from food frequency records, was stratified into tertiles, and correlated to *Buk* and *But* expression.

Circulating PAI-1 Levels

PAI-1 levels were determined in serum samples of 28 overweight and 42 obese women at 16 weeks gestation by multiplex ELISA using the BioPlex Pro human diabetes immunoassay (Bio-Rad). The proportion of overweight and obese women in the subsample was proportional to that of the 205 women. Samples were thawed overnight at 4 °C, and 50 µL was assayed in duplicate according to the manufacturer's

recommendations before analysis with the BioPlex 200 using the BioPlex Manager 6.1 software (Bio-Rad).

Statistical Analysis

The data were not normally distributed and are therefore presented as median with interquartile range in all instances. Comparisons between groups were performed with the nonparametric Mann–Whitney *U* test. The proportions of women developing pregnancy-induced hypertension were compared with a Fisher exact test. Correlation analyses were performed with Spearman rank correlation coefficient tests for gene expression and hormone analyses using GraphPad Prism v6, with a *P* value <0.05 considered to be statistically significant.

For associations between clinical characteristics and gut microbial taxa abundance, bootstrapped Spearman rank correlation coefficient tests were performed using 1000 permutations. Correction for multiple testing was performed using false discovery rate with the Benjamini–Hochberg procedure within the QIIME software. False discovery rate values <0.05 were considered statistically significant.

Results

The characteristics of the cohort at 16 weeks gestation are described in Table 1. Body mass index (BMI) is positively correlated with both systolic and diastolic blood pressure (Table 2). When comparing overweight to obese women, obese women have slightly but significantly higher systolic and diastolic blood pressures (Figure 1A). The rate of pregnancy-induced hypertension trended to be twice as high in obese women (Table 1). When analyzing the associations between blood pressure and the proportions of *Firmicutes* and *Bacteroidetes* or the *Firmicutes:Bacteroidetes* ratio, no differences were observed (Table 1). However, systolic and diastolic blood pressures were negatively correlated to specific families and genera in the gut microbiota including the *Odoribacteraceae* and *Clostridiaceae* families (Table 3). In addition, the *Christensenellaceae* family and the *Blautia* genus were also negatively associated with systolic blood pressure. The abundance of the genus *Odoribacter* was negatively correlated with systolic blood pressure (Figure 1B). To exclude that this effect was driven by BMI, associations between gut microbiota composition and systolic blood pressure were analyzed in overweight and obese women, respectively. The same families and genera were correlated with systolic blood pressure in overweight and obese women when analyzed separately (Table S1 in the online-only Data Supplement; Figure 1C and 1D). However, the correlation coefficients were stronger in overweight women than in obese women. Furthermore, abundance of *Odoribacter* in the gut microbiota of overweight and obese pregnant women was not independently correlated with BMI (Table S2).

Because the *Odoribacteraceae* and *Clostridiaceae* families are known butyrate producers, we investigated butyrate production capacity by assessing gene expression of 2 known

Table 1. Participant Characteristics

Characteristic	Overweight	Obese	*P* Value
N	86	119	
BMI	27.5 (26.4–28.4)	34.9 (32.1–38.5)	<0.0001
Age	36 (33–38)	36 (32–40)	0.51
SBP, mm Hg	106 (100–110)	110 (106–118)	<0.0001
DBP, mm Hg	65 (60–70)	70 (65–75)	<0.0001
PIH, n(%)*	8 (9.3)	22 (18.4)	0.066
PAI-1, ng/mL†	6.76 (2.06–9.21)	10.12 (6.41–14.81)	0.0054
Fiber intake	137 (120–150)	136 (117–154)	0.78
% *Firmicutes*	67.1 (57.6–75.2)	67.8 (57.1–78.6)	0.30
% *Bacteroidetes*	25.0 (16.9–34.5)	21.7 (12.2–30.2)	0.077
F:B ratio	2.7 (1.7–4.4)	2.7 (1.9–6.5)	0.11
Butyrate production capacity centroid value	0.16 (0.04–0.36)	0.09 (0.04–0.22)	0.06
But expression, AU	0.97 (0.38–2.97)	0.79 (0.24–1.71)	0.058
Buk expression, AU	1.00 (0.31–4.99)	0.81 (0.09–3.53)	0.12

Data are presented as median (interquartile range). AU indicates arbitrary units; BMI, body mass index; *Buk,* butyrate kinase; *But*, butyryl-CoA:acetate-CoA transferase; DBP, diastolic blood pressure; F:B ratio, *Firmicutes:Bacteroidetes* ratio; HELLP, hemolysis elevated liver enzymes and low platelet; PIH, pregnancy-induced hypertension includes diagnosis of gestational hypertension only, preeclampsia, or HELLP syndrome after 20 wk gestation[26]; and SBP, systolic blood pressure.

*As assessed by χ^2 analysis.

†n=28 overweight, n=42 obese.

Table 2. Correlation Coefficients

Parameter	Spearman ρ	*P* Value	Spearman ρ	*P* Value
	Systolic Blood Pressure		Diastolic Blood Pressure	
BMI	0.34	<0.0001	0.36	<0.0001
Buk gene expression	−0.13	0.055	−0.16	0.02
But gene expression	−0.01	NS	−0.11	NS
Butyrate-producing capacity	−0.09	NS	−0.15	0.03
PAI-1 levels	0.24	0.05	0.25	0.03
	Buk gene expression		*But* gene expression	
BMI	−0.15	0.04	−0.13	0.06
PAI-1 levels	−0.38	0.0012	0.02	NS
	PAI-1 levels			
BMI	0.37	0.0016		
Butyrate-producing capacity	−0.04	NS		
Odoribactereae abundance	−0.25	0.04		
Odoribacter abundance	−0.28	0.02		
Rikenellaceae abundance	−0.39	0.0001		

BMI indicates body mass index; *Buk,* butyrate kinase; *But*, butyryl-CoA:acetate-CoA transferase; NS, nonsignificant; and PAI, plasminogen activator inhibitor.

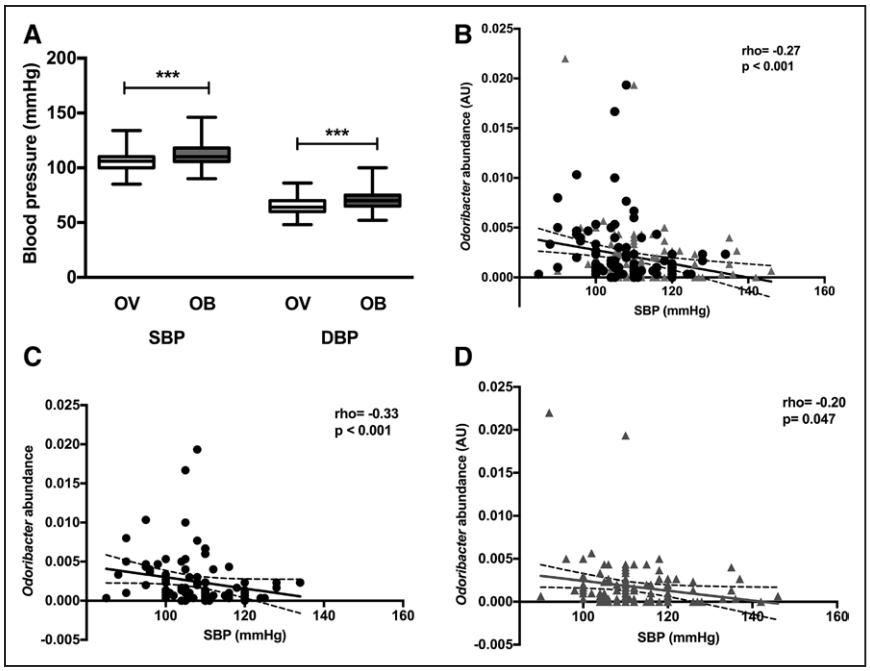

Figure 1. Blood pressure and abundance of genus Odoribacter in overweight and obese pregnant women. **A**, Blood pressure in overweight (OV) and obese (OB) pregnant women at 16 wk gestation. Correlation between abundance of Odoribacter and systolic blood pressure (SBP) in all study participants (**B**), in overweight participants (**C**), and obese participants (**D**). Data are represented as median with interquartile range, error bars representing minimum and maximum. White bars/black circles, overweight women; gray bars/grey triangles, obese women; ***P<0.001.

bacterial butyrate-producing genes: *Buk* (butyrate kinase) and *But* (butyryl-CoA:acetate-CoA transferase). Overall butyrate-producing capacity was negatively correlated with BMI (ρ=–0.19; P=0.005), and obese women showed a trend toward lower overall butyrate production (Figure 2A). BMI was negatively correlated with *Buk* expression (ρ=–0.15; P=0.04; Figure 2B) and nominally negatively correlated with *But* expression (ρ=–0.13; P=0.06). *Buk* but not *But* expression was negatively correlated with both systolic and diastolic blood pressure (Figure 2C and Table 2). *Buk* and *But* expression are not correlated with the abundance of *Firmicutes* or *Bacteroidetes* nor with the F:B ratio (data not shown). Because dietary fiber intake may affect butyrate production by increasing the availability of substrate, the relationship between fiber intake and expression of *Buk* and *But* was investigated, but no correlation was found.

Because decreased butyrate production was associated with higher blood pressure, which itself is linked to circulating PAI-1 levels, we investigated PAI-1 in a subset of 28 overweight and 42 obese women. Obese women had significantly higher circulating PAI-1 levels than overweight women (Figure 2D and Table 1). Furthermore, there was a positive correlation between BMI and PAI-1 levels (Table 2). PAI-1 levels were also positively correlated to systolic and diastolic blood pressure (Table 2). Although there was no correlation between PAI-1 levels and *But* expression, there was a negative correlation with *Buk* expression (Figure 2E and Table 2). PAI-1 levels were also negatively correlated with abundance of the butyrate-producing family *Odoribactereae*, the genus of *Odoribacter* (Figure 2F and Table 2), and the family *Rikenellaceae* (Table 2).

Discussion

This study shows that systolic blood pressure is negatively correlated with the abundance of the butyrate producers *Odoribacter* and *Clostridiaceae* and expression of the *Buk* gene in the gut microbiota in overweight and obese pregnant women at 16 weeks gestation. *Odoribacter* abundance and *Buk* expression are negatively correlated with the inflammatory marker PAI-1, which itself is positively correlated with blood pressure (Figure 3).

The results of this study suggest that the expression of the *Buk* gene by *Odoribacteraceae* and *Clostridiaceae* in the gut microbiota in pregnant women at 16 weeks gestation is associated with decreased blood pressure. This relationship is independent of BMI because the correlation between *Odoribacter* abundance and blood pressure is observed in overweight and obese women separately with stronger correlation coefficients in overweight pregnant women. This suggests that BMI may actually attenuate the effect of *Odoribacter* abundance on blood pressure especially because BMI itself is not correlated with *Odoribacter* abundance. This study cannot demonstrate causality, but there are potential mechanisms by which butyrate-producing bacteria could exert influence on host blood pressure. These include via SCFA production and more tentatively through influencing inflammation and PAI-1.

SCFAs can dilate isolated resistance arteries from the human colon in vitro.[29] SCFAs reduce systemic blood pressure via activation of GPR41.[30] The butyrate kinase gene (*Buk*) is selectively expressed among human butyrate-producing bacteria belonging to Clostridial clusters XI and I.[12] The butyryl-CoA:acetate CoA-transferase (*But)* is expressed by bacteria belonging mainly to Clostridial clusters XVIa and IV.[12] It is unclear which butyrate-producing pathway is mostly used in the gut microbiota; however, one study suggested that butyrate kinase is the pathway used in most intestinal ecosystems.[12] Members of the *Odoribacteraceae*, *Clostridiaceae*, and *Rikenellaceae* families primarily express the *Buk* gene.[12] Oral supplementation with butyrate lowers the concentrations of interleukin-1β and tumor necrosis factor-α in response to high-fat diet in mice.[31] Lower production capacity of butyrate may therefore contribute to

Table 3. Correlations of Systolic and Diastolic Blood Pressure With the Gut Microbiome

Phylum	Class	Order	Family	Genus	Spearman Correlation Coefficient ρ	*P* Value	FDR-Adjusted *P* Value
Systolic blood pressure (n=209)							
Bacteroidetes	*Bacteroidia*	*Bacteroidales*	*Odoribacteraceae*	…	−0.25	<0.001	<0.001
Bacteroidetes	*Bacteroidia*	*Bacteroidales*	*Odoribacteraceae*	*Odoribacter*	−0.27	<0.001	<0.001
Bacteroidetes	*Bacteroidia*	*Bacteroidales*	*Rikenellaceae*	…	−0.19	0.007	0.088
Firmicutes	*Clostridia*	*Clostridiales*	*Christensenellaceae*	…	−0.20	0.008	0.088
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	−0.25	<0.001	<0.001
Diastolic blood pressure (n=209)							
Firmicutes	*Clostridia*	*Clostridiales*	*Clostridiaceae*	…	−0.25	<0.001	<0.001
Bacteroidetes	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	−0.26	<0.001	<0.001

FDR indicates false discovery rate.

higher blood pressure in obese pregnant women. The use of sodium butyrate during pregnancy in a rat model increased the expression of antioxidant and nutrient-related genes.[32] In pigs, sodium butyrate supplementation improved embryo development in vitro and enhanced gene expression involved in early development.[33,34] Lastly, a recent study in rats suggested that hypertension was associated with reductions in the proportions of butyrate-producing bacteria but increases in bacteria that produce lactate.[35]

Here, *Odoribacter* abundance is inversely correlated with both blood pressure and PAI-1. *Odoribacter* is an anaerobic nonmotile Gram-negative rod, which makes succinate, propionate, acetate, butyrate, isobutyrate, and isovalerate by fermenting carbohydrates in vitro.[36,37] In the human gastrointestinal tract, there are only 3 species belonging to this genus, with *Odoribacter splanchnicus* being the prominent one.[37] Fecal butyrate and 3-phenylpropionate concentrations correlate positively with *O. splanchnicus* abundance in humans.[38] Moreover, lower *Odoribacter* abundance has been detected in subjects with Crohn's disease,[39] which is characterized by low butyrate-producing capacity. The associations between *Odoribacter* and blood pressure (data presented here) and metabolism[38] indicate that this species could be a candidate to use as a probiotic.

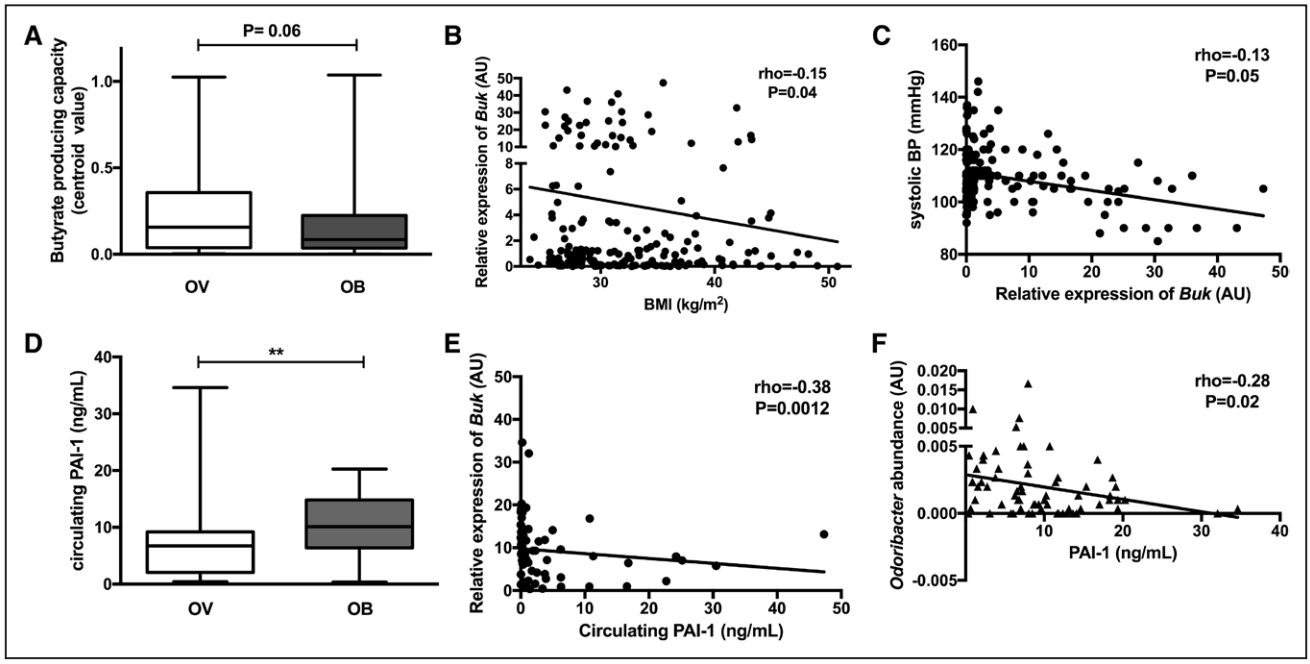

Figure 2. Blood pressure, butyrate production, and plasminogen activator inhibitor-1 (PAI-1) concentrations in overweight and obese pregnant women. **A**, Overall butyrate-producing capacity in the gut microbiome of overweight (OV) and obese (OB) pregnant women at 16 wk gestation. **B**, Correlation between relative expression levels of the *Buk* (butyrate kinase) gene and body mass index. **C**, Correlation between relative expression levels of the Buk (butyrate kinase) gene and systolic blood pressure. **D**, Circulating PAI-1 concentrations in overweight (OV) and obese (OB) pregnant women at 16 wk gestation. Correlation between PAI-1 concentrations and the relative expression levels of the *Buk* (butyrate kinase) gene (**E**) and Odoribacter abundance (**F**). White bars, overweight women; gray bars, obese women; $^{**}P<0.01$.

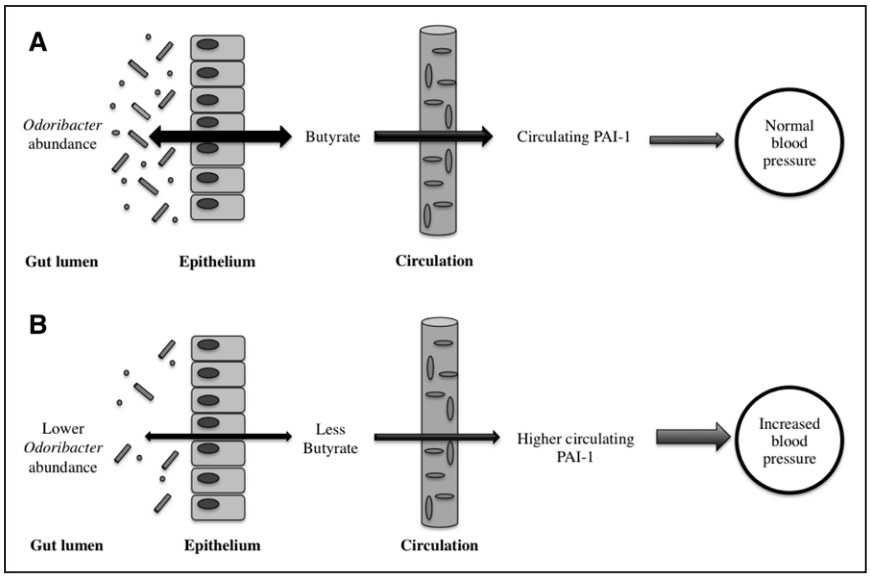

Figure 3. Relationship between the gut microbiome and blood pressure. Effects of Odoribacter abundance on butyrate production, plasminogen activator inhibitor-1 (PAI-1) levels, and blood pressure in overweight (**A**) and obese (**B**) pregnant women.

Low-grade inflammation, such as in obesity, can be the result of reduced microbial diversity. The gut microbiome may reduce the synthesis of inflammatory markers by metabolizing the plant polyphenols into urolithins, which can reduce PAI-1 secretion from enterocytes and aortic endothelial cells.[40,41] In our study, PAI-1 levels were higher in obese women. PAI-1 levels are increased in diabetes,[42] and its gene expression levels are positively correlated with BMI.[43] In men with mild hypertension, BMI and PAI-1 activity are positively correlated, and PAI-1 levels decrease with weight loss.[43] Furthermore, pharmacological treatment of newly diagnosed hypertension reduces PAI-1 levels.[44,45] In early pregnancy, increased PAI-1 levels are associated with higher risk of later hypertensive disorders in pregnancy.[46] PAI-1 levels are also increased in severe preeclampsia,[23] and polymorphisms in the PAI-1 gene (*SERPINE2)* have been associated with increased risk of preeclampsia in a meta-analysis.[47] It is not clear whether PAI-1 regulates blood pressure or vice versa; however, one study suggests that inhibition of PAI-1 can reduce hypertension caused by inhibition of nitric oxide synthase.[48] Therefore, manipulation of gut microbiome metabolites reducing PAI-1 levels may lower blood pressure both in pregnancy and outside pregnancy.

In this study, blood pressure is inversely correlated with the abundance of other bacteria from the order of *Clostridiales*, including the *Christensellaceae*, *Blautia*, and *Clostridiidiaceae* families; the last 2 families are known to be important butyrate producers. In addition, members of the *Bacteroidales* order, *Rikenellaceae* and *Bacteroides*, which can harbor butyrate-producing members, were also negatively correlated with blood pressure. A negative correlation between *Bacteroides* and blood pressure, body weight, and fat mass has been reported.[49,50] Therefore, supplementation with bacteria belonging to this genus may improve multiple clinical parameters.

The gut microbiota can regulate ≈10% of the host's transcriptome, particularly genes involved in immune response, proliferation, and metabolism.[51] Preeclampsia incidence can be reduced by consumption of probiotics in dairy products.[52] It has been hypothesized that probiotics reduce blood pressure by fermenting food to release bioactive peptides including angiontensin-converting enzyme inhibitory peptides[53] and reduce the production of proinflammatory cytokines. Randomized controlled clinical trials of adequate size should be performed to definitively establish the effects of probiotics on lowering blood pressure.

Perspectives

This study shows that the abundance of butyrate-producing bacteria in the gut microbiome and the number of copies of the butyrate-producing *Buk* enzyme are inversely correlated to systolic and diastolic blood pressure and the inflammatory marker PAI-1 in overweight and obese women at 16 weeks gestation. Whether this relationship is true for normal-weight pregnant women remains to be investigated. Future studies aimed at manipulation of the gut microbiome by probiotics, prebiotics, or dietary intervention may shed light on whether this is a novel way to aid pregnant women in maintaining healthy blood pressure and reducing low-grade inflammation thereby improving outcomes for mother and baby.

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Disclosures

None.

References

1. Kotsis V, Nilsson P, Grassi G, Mancia G, Redon J, Luft F, Schmieder R, Engeli S, Stabouli S, Antza C, Pall D, Schlaich M, Jordan J; WG on Obesity, Diabetes, the High Risk Patient, European Society of Hypertension. New developments in the pathogenesis of obesity-induced hypertension. *J Hypertens*. 2015;33:1499–1508. doi: 10.1097/HJH.0000000000000645.
2. Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, Carvajal JM, Zadeh M, Gong M, Qi Y, Zubcevic J, Sahay B, Pepine CJ, Raizada MK, Mohamadzadeh M. Gut dysbiosis is linked to hypertension. *Hypertension*. 2015;65:1331–1340. doi: 10.1161/HYPERTENSIONAHA.115.05315.
3. Howitt MR, Garrett WS. A complex microworld in the gut: gut microbiota and cardiovascular disease connectivity. *Nat Med*. 2012;18:1188–1189. doi: 10.1038/nm.2895.
4. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature*. 2006;444:1022–1023. doi: 10.1038/4441022a.
5. Xu P, Li M, Zhang J, Zhang T. Correlation of intestinal microbiota with overweight and obesity in Kazakh school children. *BMC Microbiol*. 2012;12:283. doi: 10.1186/1471-2180-12-283.
6. Lyte M. Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. *Bioessays*. 2011;33:574–581. doi: 10.1002/bies.201100024.
7. El Aidy S, Dinan TG, Cryan JF. Immune modulation of the brain-gut-microbe axis. *Front Microbiol*. 2014;5:146. doi: 10.3389/fmicb.2014.00146.
8. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*. 2007;56:1761–1772. doi: 10.2337/db06-1491.
9. Mell B, Jala VR, Mathew AV, Byun J, Waghulde H, Zhang Y, Haribabu B, Vijay-Kumar M, Pennathur S, Joe B. Evidence for a link between gut microbiota and hypertension in the Dahl rat. *Physiol Genomics*. 2015;47:187–197. doi: 10.1152/physiolgenomics.00136.2014.
10. Khalesi S, Sun J, Buys N, Jayasinghe R. Effect of probiotics on blood pressure: a systematic review and meta-analysis of randomized, controlled trials. *Hypertension*. 2014;64:897–903. doi: 10.1161/HYPERTENSIONAHA.114.03469.
11. Vital M, Howe AC, Tiedje JM. Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. *MBio*. 2014;5:e00889. doi: 10.1128/mBio.00889-14.
12. Louis P, Duncan SH, McCrae SI, Millar J, Jackson MS, Flint HJ. Restricted distribution of the butyrate kinase pathway among butyrate-producing bacteria from the human colon. *J Bacteriol*. 2004;186:2099–2106.
13. Pryde SE, Duncan SH, Hold GL, Stewart CS, Flint HJ. The microbiology of butyrate formation in the human colon. *FEMS Microbiol Lett*. 2002;217:133–139.
14. Duncan SH, Barcenilla A, Stewart CS, Pryde SE, Flint HJ. Acetate utilization and butyryl coenzyme A (CoA):acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. *Appl Environ Microbiol*. 2002;68:5186–5190.
15. Smith TJ, Piscatelli JJ, Andersen V, Wang HS, Lance P. n-Butyrate induces plasminogen activator inhibitor type 1 messenger RNA in cultured Hep G2 cells. *Hepatology*. 1996;23:866–871. doi: 10.1002/hep.510230430.
16. Mortensen FV, Jørgensen B, Christiansen HM, Sloth-Nielsen J, Wolff B, Hessov I. Short-chain fatty acid enemas stimulate plasminogen activator inhibitor-1 after abdominal aortic graft surgery: a double-blinded, placebo-controlled study. *Thromb Res*. 2000;98:361–366.
17. Martin C, Cameron J, McGrath B. Mechanical and circulating biomarkers in isolated clinic hypertension. *Clin Exp Pharmacol Physiol*. 2008;35:402–408. doi: 10.1111/j.1440-1681.2008.04886.x.
18. Mertens I, Ballaux D, Funahashi T, Matsuzawa Y, Van der Planken M, Verrijken A, Ruige JB, Van Gaal LF. Inverse relationship between plasminogen activator inhibitor-I activity and adiponectin in overweight and obese women. Interrelationship with visceral adipose tissue, insulin resistance, HDL-chol and inflammation. *Thromb Haemost*. 2005;94:1190–1195.
19. Chen YS, Shen L, Mai RQ, Wang Y. Levels of microRNA-181b and plasminogen activator inhibitor-1 are associated with hypertensive disorders complicating pregnancy. *Exp Ther Med*. 2014;8:1523–1527. doi: 10.3892/etm.2014.1946.
20. Sartori MT, Serena A, Saggiorato G, Campei S, Faggian D, Pagnan A, Paternoster DM. Variations in fibrinolytic parameters and inhibin-A in pregnancy: related hypertensive disorders. *J Thromb Haemost*. 2008;6:352–358. doi: 10.1111/j.1538-7836.2007.02840.x.
21. Reith A, Booth NA, Moore NR, Cruickshank DJ, Bennett B. Plasminogen activator inhibitors (PAI-1 and PAI-2) in normal pregnancies, pre-eclampsia and hydatidiform mole. *Br J Obstet Gynaecol*. 1993;100:370–374.
22. Estellés A, Gilabert J, Aznar J, Loskutoff DJ, Schleef RR. Changes in the plasma levels of type 1 and type 2 plasminogen activator inhibitors in normal pregnancy and in patients with severe preeclampsia. *Blood*. 1989;74:1332–1338.
23. Pinheiro MB, Carvalho MG, Martins-Filho OA, Freitas LG, Godoi LC, Alpoim PN, Gomes KB, Dusse LM. Severe preeclampsia: are hemostatic and inflammatory parameters associated? *Clin Chim Acta*. 2014;427:65–70. doi: 10.1016/j.cca.2013.09.050.
24. Guven S, Sonmez M, Karahan SC. The role of fibrinolytic and antifibrinolytic activities in the pathophysiology of HELLP syndrome. *Hypertens Pregnancy*. 2011;30:275–286. doi: 10.3109/10641950902968718.
25. Nitert MD, Barrett HL, Foxcroft K, Tremellen A, Wilkinson S, Lingwood B, Tobin JM, McSweeney C, O'Rourke P, McIntyre HD, Callaway LK. SPRING: an RCT study of probiotics in the prevention of gestational diabetes mellitus in overweight and obese women. *BMC Pregnancy Childbirth*. 2013;13:50. doi: 10.1186/1471-2393-13-50.
26. Lowe SA, Bowyer L, Lust K, McMahon LP, Morton MR, North RA, Paech MJ, Said JM; Society of Obstetric Medicine of Australia and New Zealand. The SOMANZ guidelines for the management of hypertensive disorders of pregnancy 2014. *Aust N Z J Obstet Gynaecol*. 2015;55:11–16. doi: 10.1111/ajo.12253.
27. Yu Z, Morrison M. Improved extraction of PCR-quality community DNA from digesta and fecal samples. *Biotechniques*. 2004;36:808–812.
28. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. 2010;7:335–336. doi: 10.1038/nmeth.f.303.
29. Mortensen PB, Holtug K, Bonnén H, Clausen MR. The degradation of amino acids, proteins, and blood to short-chain fatty acids in colon is prevented by lactulose. *Gastroenterology*. 1990;98:353–360.
30. Pluznick J. A novel scfa receptor, the microbiota, and blood pressure regulation. *Gut Microbes*. 2014;5:202–207.
31. Li HP, Chen X, Li MQ. Butyrate alleviates metabolic impairments and protects pancreatic β cell function in pregnant mice with obesity. *Int J Clin Exp Pathol*. 2013;6:1574–1584.
32. Lin Y, Fang ZF, Che LQ, Xu SY, Wu D, Wu CM, Wu XQ. Use of sodium butyrate as an alternative to dietary fiber: effects on the embryonic development and anti-oxidative capacity of rats. *PLoS One*. 2014;9:e97838. doi: 10.1371/journal.pone.0097838.
33. Goicoa S, Alvarez S, Ricordi C, Inverardi L, Domínguez-Bendala J. Sodium butyrate activates genes of early pancreatic development in embryonic stem cells. *Cloning Stem Cells*. 2006;8:140–149. doi: 10.1089/clo.2006.8.140.
34. Das ZC, Gupta MK, Uhm SJ, Lee HT. Increasing histone acetylation of cloned embryos, but not donor cells, by sodium butyrate improves their *in vitro* development in pigs. *Cell Reprogram*. 2010;12:95–104. doi: 10.1089/cell.2009.0068.
35. Durgan DJ, Ganesh BP, Cope JL, Ajami NJ, Phillips SC, Petrosino JF, Hollister EB, Bryan RM Jr. Role of the gut microbiome in obstructive sleep apnea-induced hypertension. *Hypertension*. 2016;67:469–474. doi: 10.1161/HYPERTENSIONAHA.115.06672.
36. Nagai F, Morotomi M, Watanabe Y, Sakon H, Tanaka R. Alistipes indistinctus sp. nov. and Odoribacter laneus sp. nov., common members of the human intestinal microbiota isolated from faeces. *Int J Syst Evol Microbiol*. 2010;60(pt 6):1296–1302. doi: 10.1099/ijs.0.014571-0.
37. Göker M, Gronow S, Zeytun A, et al. Complete genome sequence of Odoribacter splanchnicus type strain (1651/6). *Stand Genomic Sci*. 2011;4:200–209. doi: 10.4056/sigs.1714269.
38. Mosele JI, Gosalbes MJ, Macià A, Rubió L, Vázquez-Castellanos JF, Jiménez Hernández N, Moya A, Latorre A, Motilva MJ. Effect of daily intake of pomegranate juice on fecal microbiota and feces metabolites from healthy volunteers. *Mol Nutr Food Res*. 2015;59:1942–1953. doi: 10.1002/mnfr.201500227.
39. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, Reyes JA, Shah SA, LeLeiko N, Snapper SB, Bousvaros A, Korzenik J, Sands BE, Xavier RJ, Huttenhower C. Dysfunction of the intestinal microbiome

in inflammatory bowel disease and treatment. *Genome Biol*. 2012;13:R79. doi: 10.1186/gb-2012-13-9-r79.
40. Giménez-Bastida JA, Larrosa M, González-Sarrías A, Tomás-Barberán F, Espín JC, García-Conesa MT. Intestinal ellagitannin metabolites ameliorate cytokine-induced inflammation and associated molecular markers in human colon fibroblasts. *J Agric Food Chem*. 2012;60:8866–8876. doi: 10.1021/jf300290f.
41. Giménez-Bastida JA, González-Sarrías A, Larrosa M, Tomás-Barberán F, Espín JC, García-Conesa MT. Ellagitannin metabolites, urolithin A glucuronide and its aglycone urolithin A, ameliorate TNF-α-induced inflammation and associated molecular markers in human aortic endothelial cells. *Mol Nutr Food Res*. 2012;56:784–796. doi: 10.1002/mnfr.201100677.
42. Lyon CJ, Hsueh WA. Effect of plasminogen activator inhibitor-1 in diabetes mellitus and cardiovascular disease. *Am J Med*. 2003;115(suppl 8A):62S–68S.
43. Correia ML, Haynes WG. A role for plasminogen activator inhibitor-1 in obesity: from pie to PAI? *Arterioscler Thromb Vasc Biol*. 2006;26:2183–2185. doi: 10.1161/01.ATV.0000244018.24120.70.
44. Ali Nahit Sendur M, Sain Guven G, Yorgun H, Hakan Ates A, Canpolat U, Sunman H, Karahan S, Kaya B. Effect of antihypertensive therapy on endothelial markers in newly diagnosed stage 1 hypertension: a randomized single-centre study. *Anatol J Cardiol*. 2014;14:363–369.
45. Gavriilaki E, Gkaliagkousi E, Nikolaidou B, Triantafyllou G, Chatzopoulou F, Douma S. Increased thrombotic and impaired fibrinolytic response to acute exercise in patients with essential hypertension: the effect of treatment with an angiotensin II receptor blocker. *J Hum Hypertens*. 2014;28:606–609. doi: 10.1038/jhh.2014.18.
46. Prochazka M, Procházková J, Lubušký M, Pilka R, Úlehlová J, Michalec I, Polák P, Kacerovský M, Slavik L. Markers of endothelial activation in preeclampsia. *Clin Lab*. 2015;61:39–46.
47. Morgan JA, Bombell S, McGuire W. Association of plasminogen activator inhibitor-type 1 (-675 4G/5G) polymorphism with pre-eclampsia: systematic review. *PLoS One*. 2013;8:e56907. doi: 10.1371/journal.pone.0056907.
48. Boe AE, Eren M, Murphy SB, Kamide CE, Ichimura A, Terry D, McAnally D, Smith LH, Miyata T, Vaughan DE. Plasminogen activator inhibitor-1 antagonist TM5441 attenuates Nω-nitro-L-arginine methyl ester-induced hypertension and vascular senescence. *Circulation*. 2013;128:2318–2324. doi: 10.1161/CIRCULATIONAHA.113.003192.
49. Queipo-Ortuño MI, Boto-Ordóñez M, Murri M, Gomez-Zumaquero JM, Clemente-Postigo M, Estruch R, Cardona Diaz F, Andrés-Lacueva C, Tinahones FJ. Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. *Am J Clin Nutr*. 2012;95:1323–1334. doi: 10.3945/ajcn.111.027847.
50. Munukka E, Wiklund P, Pekkala S, Völgyi E, Xu L, Cheng S, Lyytikäinen A, Marjomäki V, Alen M, Vaahtovuo J, Keinänen-Kiukaanniemi S, Cheng S. Women with and without metabolic disorder differ in their gut microbiota composition. *Obesity (Silver Spring)*. 2012;20:1082–1087. doi: 10.1038/oby.2012.8.
51. Jose PA, Raj D. Gut microbiota in hypertension. *Curr Opin Nephrol Hypertens*. 2015;24:403–409. doi: 10.1097/MNH.0000000000000149.
52. Brantsaeter AL, Myhre R, Haugen M, Myking S, Sengpiel V, Magnus P, Jacobsson B, Meltzer HM. Intake of probiotic food and risk of preeclampsia in primiparous women: the Norwegian Mother and Child Cohort Study. *Am J Epidemiol*. 2011;174:807–815. doi: 10.1093/aje/kwr168.
53. Lye HS, Kuan CY, Ewe JA, Fung WY, Liong MT. The improvement of hypertension by probiotics: effects on cholesterol, diabetes, renin, and phytoestrogens. *Int J Mol Sci*. 2009;10:3755–3775. doi: 10.3390/ijms10093755.

Novelty and Significance

What Is New?

- This study shows that the abundance of *Odoribacter* and the expression of the butyrate-producing gene butyrate kinase in the gut microbiome of overweight and obese pregnant women in early second trimester are negatively correlated with PAI-1 concentrations in the circulation and with systolic and diastolic blood pressure.

What Is Relevant?

- The relationship between the composition of the gut microbiome and blood pressure in pregnancy suggests that manipulation of the gut microbiome by altered dietary intake, probiotics, and prebiotics may support the maintenance of normal blood pressure in pregnancy.

Summary

It is not clear whether the composition of the gut microbiome in early pregnancy is related to blood pressure in overweight and obese pregnant women. In this study, this relationship was explored. The results show that there is a relationship between the composition of the gut microbiome and blood pressure. Higher abundance of the butyrate-producing *Odoribacter* genus is associated with lower systolic and diastolic blood pressure. Higher expression of the butyrate kinase in the gut microbiome is associated with lower blood pressure. Higher *Odoribacter* abundance and butyrate expression are associated with lower serum plasminogen activator inhibitor-1 concentrations.