High-Fiber Diet and Acetate Supplementation Change the Gut Microbiota and Prevent the Development of Hypertension and Heart Failure in Hypertensive Mice

BACKGROUND: Dietary intake of fruit and vegetables is associated with lower incidence of hypertension, but the mechanisms involved have not been elucidated. Here, we evaluated the effect of a high-fiber diet and supplementation with the short-chain fatty acid acetate on the gut microbiota and the prevention of cardiovascular disease.

METHODS: Gut microbiome, cardiorenal structure/function, and blood pressure were examined in sham and mineralocorticoid excess–treated mice with a control diet, high-fiber diet, or acetate supplementation. We also determined the renal and cardiac transcriptome of mice treated with the different diets.

RESULTS: We found that high consumption of fiber modified the gut microbiota populations and increased the abundance of acetate-producing bacteria independently of mineralocorticoid excess. Both fiber and acetate decreased gut dysbiosis, measured by the ratio of Firmicutes to Bacteroidetes, and increased the prevalence of *Bacteroides acidifaciens*. Compared with mineralocorticoid-excess mice fed a control diet, both high-fiber diet and acetate supplementation significantly reduced systolic and diastolic blood pressures, cardiac fibrosis, and left ventricular hypertrophy. Acetate had similar effects and markedly reduced renal fibrosis. Transcriptome analyses showed that the protective effects of high fiber and acetate were accompanied by the downregulation of cardiac and renal *Egr1*, a master cardiovascular regulator involved in cardiac hypertrophy, cardiorenal fibrosis, and inflammation. We also observed the upregulation of a network of genes involved in circadian rhythm in both tissues and downregulation of the renin-angiotensin system in the kidney and mitogen-activated protein kinase signaling in the heart.

CONCLUSIONS: A diet high in fiber led to changes in the gut microbiota that played a protective role in the development of cardiovascular disease. The favorable effects of fiber may be explained by the generation and distribution of one of the main metabolites of the gut microbiota, the short-chain fatty acid acetate. Acetate effected several molecular changes associated with improved cardiovascular health and function.

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Sources of Funding, see page 975

Key Words: blood pressure ■ diet ■ fibrosis ■ gut microbiome ■ gut microbiota ■ heart failure ■ hypertension

Clinical Perspective

What Is New?

- We tested the hypothesis that a high-fiber diet modifies the gut microbiota and that acetate, a product of fiber fermentation, mediates part of the fiber benefit in lowering cardiovascular disease and, in particular, blood pressure.
- Fiber and acetate changed the gut microbiota composition, increasing the prevalence of acetate-producing bacteria, improving the levels of *Bacteroides acidifaciens,* and decreasing gut dysbiosis.
- Dietary intake of fiber and supplementation with acetate modulated renal and cardiac molecular pathways beneficial for cardiovascular function, lowered blood pressure, decreased cardiac hypertrophy and fibrosis, and improved heart function in experimental hypertension.

What Are the Clinical Implications?

- Our study highlights the important role played by dietary fiber and the gut microbiota in regulating cardiovascular homeostasis.
- Further clinical studies to examine the potential utility of dietary supplementation with short-chain fatty acids such as acetate in addition to a diet rich in fiber are warranted.

Despite major advances in the identification of key pathophysiological mechanisms and in treatment, hypertension remains one of the most important causes of acute and chronic cardiovascular diseases (CVDs), including stroke, myocardial infraction, and heart failure.[1] Although dietary sodium content is a clear contributor to hypertension, it is starting to emerge that other dietary components such as fiber might modulate cardiovascular risk factors. Epidemiological studies have shown that a high dietary intake of fruit and vegetables is accompanied by a lower incidence of cardiovascular mortality[2] and reduced blood pressure (BP).[3,4] This is supported by short-term intervention studies such as those following the DASH (Dietary Approaches to Stop Hypertension) diet.[5] Even combined with a diet high in fat (37% fat), a diet rich in fruit and vegetables was inversely associated with BP.[6] Similarly, 2 meta-analyses found that interventions aimed at increasing total dietary fiber intake significantly reduced systolic and diastolic BPs in hypertensive patients.[7,8]

The mechanism by which dietary fiber lowers BP is not known. Although some types of fiber are digestible, most pass through the small intestine undigested and are fermented in the distal colon, where they feed the community of commensal bacteria known as the gut microbiota. Consumption of a diet high in fiber increases gut microbiota populations that generate short-chain fatty acids (SCFAs) such as acetate.[9–11] Gut microbiota dysbiosis has been recently associated with high BP in animal and human hypertension.[12] In the present study, we aimed to investigate whether dietary fiber or acetate supplementation, through changes in the gut microbiota, prevents the development of hypertension and associated renal and cardiac fibrosis in the deoxycorticosterone acetate (DOCA)–salt model. Last, we also determined the transcriptome involved in the cross-talk between the gut, kidney, and heart.

METHODS

All experimental protocols were approved by the Alfred Medical Research and Education Precinct Animal Experimentation Ethics Committee under the guidelines of the National Medical and Health Research Council of Australia.

Animal Model and Treatment

Male C57Bl/6 mice were randomly selected to the various diet intervention groups: control, high fiber, or acetate. The diets used in this study were control diet (normal chow, 47.6% fiber) and high-fiber diet (72.7% fiber, SF11-025; Specialty Feeds, Perth, Australia), refreshed 3 times per week (specific components described in Table I in the online-only Data Supplement). SCFA supplementation was given as 200 mmol/L magnesium acetate (Merck Millipore, 1.05819.1000) in drinking water as we described earlier,[13] refreshed 3 times per week. Mice received the high-fiber diet or acetate supplementation for 3 weeks before sham or DOCA surgery and were housed only with other mice receiving the same diet to avoid cross-contamination of gut microbiota. After the surgery, mice were housed individually. The study included the following treatment groups: sham+control (uninephrectomy, placebo pellet, normal diet; n=13), DOCA+control (uninephrectomy, DOCA pellet, normal diet; n=11), DOCA+fiber (uninephrectomy, DOCA pellet, high-fiber diet; n=15), DOCA+acetate (uninephrectomy, DOCA pellet, magnesium acetate in drinking water; n=13), sham+fiber (uninephrectomy, placebo pellet, high-fiber diet; n=6), and sham+acetate (uninephrectomy, placebo pellet, magnesium acetate in drinking water; n=6). After 3 weeks of initial diet intervention or SCFA supplementation, 6-week-old mice underwent a left unilateral uninephrectomy and were implanted with a slow-release 21-day DOCA pellet (Innovative Research of America) or a placebo pellet in the right flank while anesthetized with isoflurane. After 21 days, an additional pellet was implanted in the left flank for the remainder of the experiment for a total of 6 weeks. All animals were provided 1% saline (with or without magnesium acetate) and had access to food and water ad libitum. Animals were monitored and weighed regularly over the protocol. Body weight at the end point is shown in Figure IA and IB in the online-only Data Supplement.

Gut Microbiome

Stool samples were collected sterilely from the colon during euthanasia and stored at −80°C. DNA was extracted from 4 samples per group with the PowerSoil DNA isolation

kit (MoBio), and the V3-V4 region of the bacterial 16S rRNA was sequenced with the 341F and 806R primers in a Illumina MiSeq sequencer (300-bp paired-end reads) as a service by the Australian Genome Research Facility. Raw data were filtered, and primers were trimmed. Reads were assembled with PEAR (version 0.9.5).[14] Sequences were analyzed with Quantitative Insights Into Microbial Ecology (1.9.1),[15] as we previously described.[10,11] To determine whether fiber would increase SCFAs, major genera were classified according to their primary fermentation product as acetate or butyrate according to previously published work.[16]

Functional Measurements

The day before the completion of the study, mice were anesthetized with isoflurane, and echocardiography was performed to image the left ventricle with a PHILIPS IE33 ultrasound machine (Royal Philips Electronics, Amsterdam, the Netherlands) with a 15-MHz linear transducer. Images were analyzed blindly. Immediately before the completion of the study, mice were anesthetized, and a 1.4F microtipped transducer catheter (Millar, Houston, TX) was inserted into the carotid artery to measure arterial BP. Urine glucose concentration was measured with a Beckman Coulter Glucose Kit in a Beckman Coulter DXC800 Analyzer as a service by Monash Pathology (Melbourne, Australia). Hemoglobin A_{1c} was measured with 2 μL whole blood in a Cobas b 101 system.

Morphological Analyses

After cardiac catheterization, a blood sample was taken, and the heart, kidney, and lung were rapidly removed. The tissues were weighed before being snap-frozen in liquid nitrogen, stored at −80°C, or fixed in 10% formalin in PBS.

Histological Analyses

Paraffin sections were cut into 4-μm sections and stained with Masson trichrome to analyze the collagen present in heart and kidney samples. Perivascular and interstitial fibrosis levels were quantified in the heart and interstitial and glomerular fibrosis levels were quantified in the kidney in 10 random fields of view per section with an Olympus BH2 microscope (×400 magnification) and ImagePro Plus software (Adept Electronic Solutions Pty Ltd, Moorabbin, Australia). Collagen levels were expressed as a percentage of the area of the region of interest.

Statistical Analyses

The GraphPad Prism (version 6) package was used for statistical analysis and graphing. Normal distribution of data was verified with the Shapiro-Wilk normality test. Two-factor ANOVA (with a Tukey adjustment for multiple comparisons, not for repeated measures) was used to compare the data between the disease models (sham and DOCA) and diets (control, fiber, and acetate). In mice that were not operated on, 1-way ANOVA with a Tukey adjustment for multiple comparison was used to compare between diet groups (control, fiber, and acetate). Values are presented as mean±SEM, and those with a value of $P<0.05$ were considered significant.

Renal and Cardiac Transcriptome

To determine the renal and cardiac genes and pathways involved without the confounding effect of phenotypes and changes in the prevalence of cell types, we examined the renal and cardiac transcriptome in mice fed a control diet, high-fiber diet, or acetate for 3 weeks (n=4 per group) without any surgeries. Tissue was collected as described above. RNA was extracted with the RNeasy (Qiagen) kit, and DNase was treated. RNA quality was assessed on a MultiNA bioanalyzer (Shimadzu). Total RNA (1 μg) underwent mRNA enrichment with the NEBNext Poly(A) mRNA Magnetic Isolation Module followed by library preparation with the NEBNext Ultra Directional RNA Library Prep Kit for Illumina. Libraries were validated with the MultiNA Bioanalyzer, and barcoded samples were pooled to equimolar concentrations before sequencing. Cluster generation was performed at a concentration of 13 pmol/L with HiSeq 2500 version 4 reagents. Single-end sequencing was undertaken at the Australian Genome Research Facility as a service, yielding 100-bp reads.

Bioinformatic Analyses

Quality trimming and adapter clipping were performed with Skewer version 0.2.2.[17] Read mapping was performed with STAR version 2.4.0g1,[18] and reads were annotated to the mouse genome from Ensembl release 69 (Mus_musculus.GRCm38.78.gtf). One control sample was excluded because of evidence of inflammation compared with the other samples in the same group. Galaxy[19] was used to analyze the data with EdgeR version 3.10.2[20] used to calculate differential expression between groups with default parameters and minimum mapQ of 20. Significance was set as a false discovery rate (FDR) <0.05. A principal component analysis was performed in the normalized data with Partek Flow software (version 5.0). Gene-set enrichment analysis was performed, interrogating the overlap of pathways in the Reactome and Kyoto Encyclopedia of Genes and Genomes databases.[21] The results were uploaded into Enrichment Map,[22] a plug-in of Cytoscape (version 3.4.0),[23] to build pathway networks.

RESULTS

Gut Microbiota Dysbiosis in Hypertension: Correction by Dietary Fiber and Acetate

We investigated whether dietary intake of fiber changed the gut microbiota composition in sham and DOCA-treated mice by sequencing the 16S bacterial gene. β-Diversity analysis, a measure of the distance between samples, showed in both unweighted and weighted UniFrac principal coordinate analyses (both $P=0.001$) that mice fed a high-fiber diet had a different gut microbiota composition from those fed a control diet (Figure 1A). This was supported by the different abundance in bacterial phyla (Figure 1B) and families (Figure 1C) between the diet groups. We also determined α diversity ($P=0.21$–1.00; Figure IIA in the online-only Data Supplement), and consistent with our previous findings,[10] a high-fiber diet or acetate did not

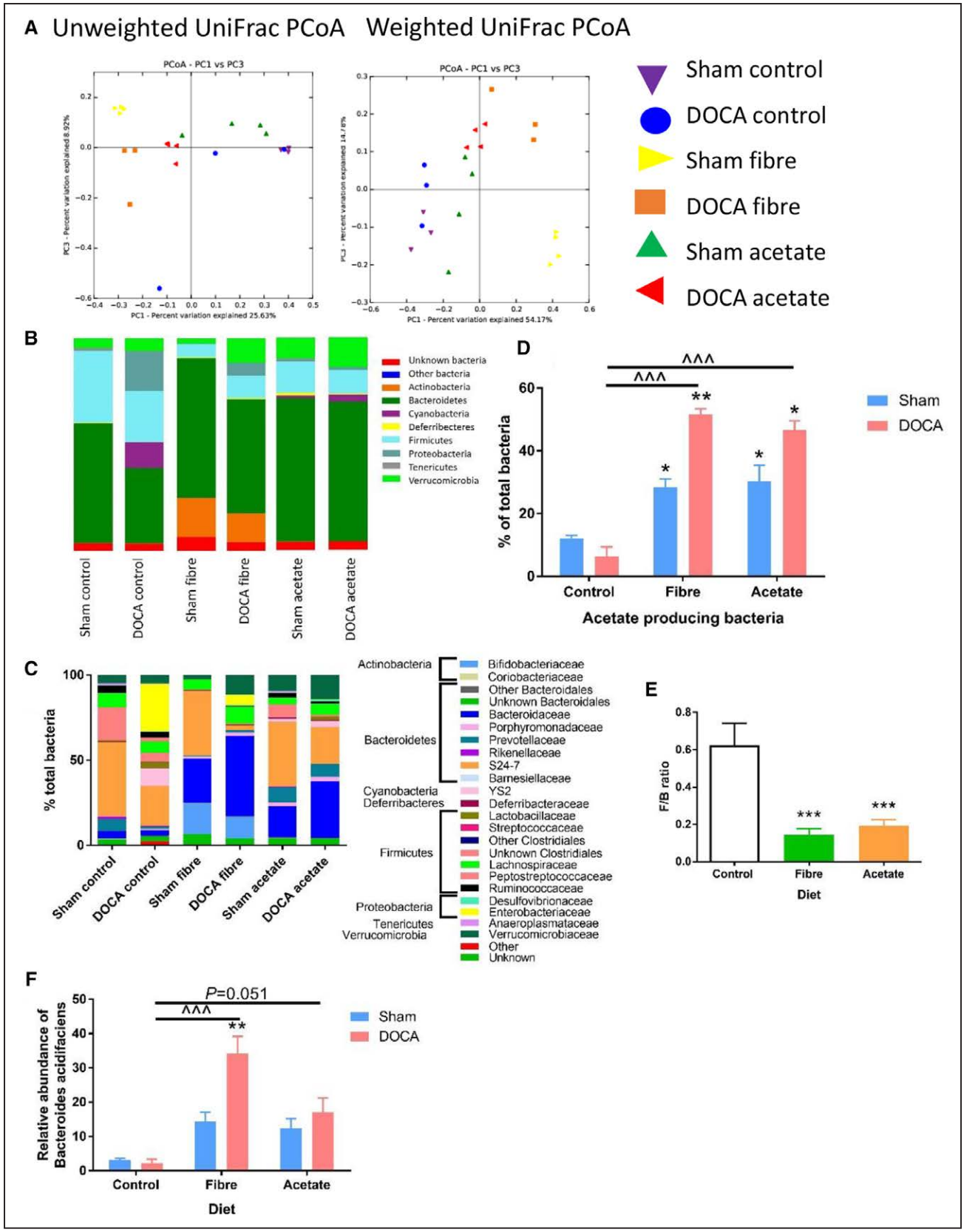

Figure 1. The effect of deoxycorticosterone acetate (DOCA)–salt, a diet rich in fiber and acetate supplementation, in the gut microbiome.

A, The composition of the gut microbiota between sham and DOCA mice fed a control or high-fiber diet is significantly different. Shown are unweighted (**left**; *P*=0.001) and weighted (**right**; *P*=0.001) UniFrac principal coordinate analyses (PCoA). (*Continued*)

increase the number of different types of bacteria in the gut.

We also found higher acetate-producing bacteria (Figure 1D), but not butyrate-producing bacteria (Figure IIB in the online-only Data Supplement), in mice fed a high-fiber diet. This is consistent with a previous study by us (J.K.T., C.R.M) that established that high-fiber diets increases levels of acetate in the blood and feces.[10] Thus, we decided to treat mice with acetate itself as the main SCFA metabolite from a high-fiber diet. It is interesting to note that we found that supplementation with acetate resulted in changes in the gut microbiota independently of fiber (Figure 1A–1C) but also increased the percentage of acetate-producing bacteria (Figure 1D).

We then calculated the ratio between Firmicutes and Bacteroidetes as a widely used marker of gut dysbiosis and found it to be significantly lower in mice fed a high-fiber diet or acetate compared with those fed a control diet, independently of mineralocorticoid excess (Figure 1E). Mice fed fiber and acetate had significantly higher levels of bacteria from the *Bacteroides* genus (FDR q=0.029), whereas the *Bifidobacterium* genus was higher only in mice fed fiber (q=0.05). The *Prevotella* genus was significantly lower in the fiber group compared with the other groups (q=0.029), and the YS2 genus was overrepresented in DOCA-treated mice fed a control diet (q=0.054). The only species that had high levels with fiber and acetate but low levels with the control diet was *Bacteroides acidifaciens* (q=0.037; Figure 1F).

High-Fiber Diet and Acetate Decreased BP in the DOCA Model

Mineralocorticoid excess, induced by DOCA administration for a 42-day period, led to a significant increase in systolic BP (sham-control versus DOCA-control, 87±4 versus 116±19 mm Hg [mean±SD]; $P<0.01$; Figure 2A), which was mirrored by similar increases in diastolic BP (63±2 versus 75±5 mm Hg; $P<0.01$; Figure 2B). Dietary supplementation with high fiber or acetate significantly reduced systolic BP (91±5 mm Hg [$P=0.002$] and 85±9 mm Hg [$P=0.0002$], respectively) and diastolic BP (58±5 mm Hg [$P=0.0001$] and 54±8 mm Hg [$P<0.0001$], respectively) in DOCA-treated mice. Similar results were found for changes in mean arterial pressure with high-fiber diet and acetate (Figure 2C). No significant change in BP was observed in sham mice treated with high-fiber diet or acetate alone (Figure 2A–2C).

High-Fiber Diet and Acetate Normalized Cardiac Hypertrophy

Consistent with changes in BP, DOCA-treated mice had increased ratios of heart and kidney to body weight (both $P<0.0001$; Figure 2D and 2E, respectively). Whereas a high-fiber diet reduced the heart/body weight ratio ($P<0.0001$; Figure 2D), it did not affect renal mass ($P=0.73$; Figure 2E). In contrast, acetate supplementation attenuated both cardiac ($P=0.0002$; Figure 2D) and renal ($P<0.0001$; Figure 2E) hypertrophy. No significant change in body/organ weight was observed in sham mice treated with high-fiber diet or acetate alone (Figure 2D and 2E). DOCA-treated mice did not have increased lung/body weight ratio (Figure 2F). There was no difference in heart rate between diets or surgical models (Figure IC in the online-only Data Supplement).

High-Fiber Diet and Acetate Improved Cardiac Function

Consistent with the changes in myocardial histology, significant increases in left ventricular wall thickness and systolic dimension were observed in DOCA-treated mice compared with control (Table). The increase in left ventricular wall thickness was significantly reduced by both high-fiber diet and acetate. Left ventricular chamber dilatation was reversed to normal levels by high-fiber treatment and to near-normal levels by exposure to acetate (Table).

High-Fiber Diet and Acetate Interventions Attenuated Cardiac and Renal Fibrosis

Administration of DOCA also led to a significant increase in glomerular (control 0.67±0.16% versus DOCA 7.48±2.09%; $P<0.0001$) and tubulointerstitial (control 0.46±0.25 versus DOCA 2.46±0.1.47%; $P=0.008$; Figure 3A–3C) fibrosis. Renal fibrosis was attenuated in mice provided with acetate supplementation, with glomerular fibrosis reduced to 2.16±0.26%

Figure 1 Continued. Percentage of total bacteria presented at the (**B**) phyla and (**C**) family level. **D**, Mice fed a high-fiber diet or acetate had a significantly higher percentage of acetate-producing bacteria ($P<0.0001$). **E**, Ratio of Firmicutes to Bacteroidetes (F/B), as a marker of gut dysbiosis, is lower in mice fed high fiber or acetate, independently of surgical state ($P<0.0001$). One-way ANOVA with a Tukey adjustment for multiple comparison was used. **F**, Mice fed a high-fiber diet or acetate had significantly higher levels of bacteria from the *Bacteroides acidifaciens* species than mice fed the control chow ($P<0.0001$). Three of the 24 samples were excluded because of low DNA and consequent low number of reads. Other samples had between 59478 and 129113 reads. **B** and **C** are shown as percent; values in **D** through **F** are mean±SEM. *$P<0.05$, **$P<0.01$ vs sham-control. ***$P<0.001$, ^^^$P<0.001$ vs DOCA-control.

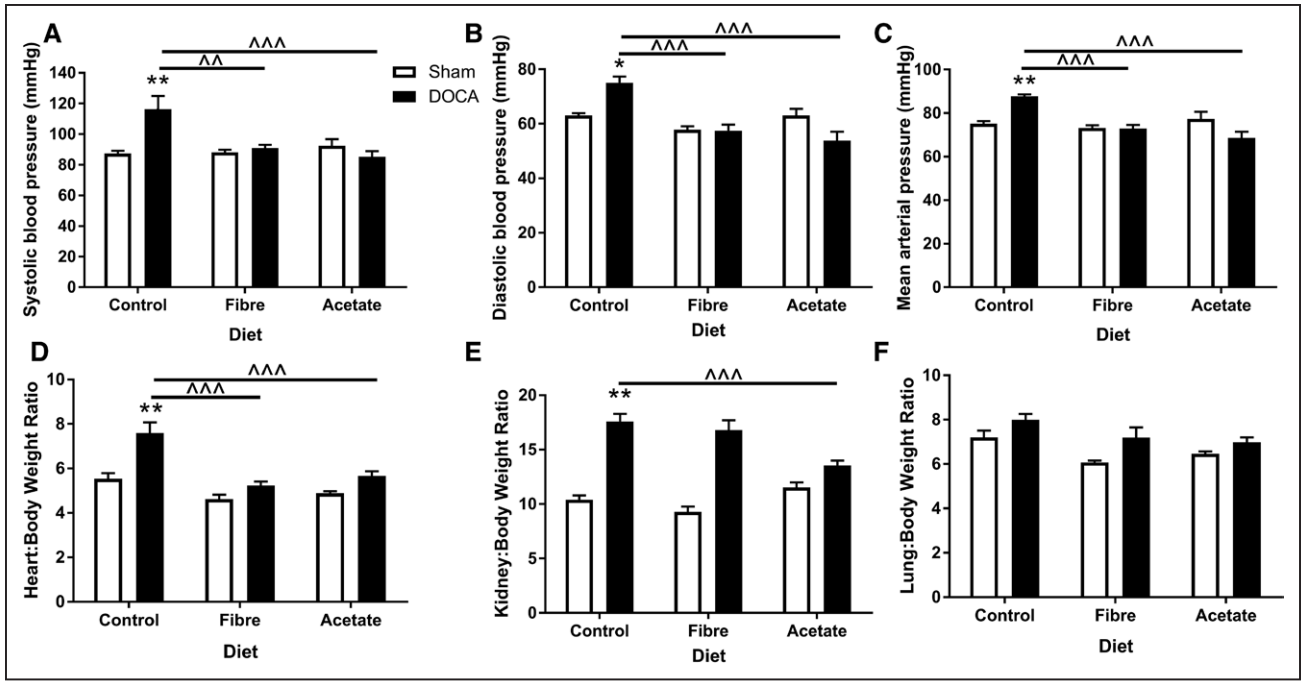

Figure 2. High-fiber diet and acetate modulate blood pressure and heart and kidney weight in deoxycorticosterone acetate (DOCA)–salt model.

Compared with controls, animals exposed to mineralocorticoid excess had a significant increase in (**A**) systolic blood pressure, (**B**) diastolic blood pressure, and (**C**) mean arterial pressure. All were significantly reduced by a high-fiber diet or acetate complementation. **D**, Compared with controls, DOCA-salt mice had significantly larger cardiac weight index, which was reduced by a high-fiber diet or acetate complementation. **E**, Kidney weight (relative to body weight) of DOCA-salt mice was also increased, and acetate significantly reduced it. **F**, There was no difference in lung weight (adjusted by body weight) between groups. Values are mean±SEM. **P*<0.05, ***P*<0.01, ^^^*P*<0.001 vs DOCA-control.

(*P*<0.0001; Figure 3A and 3B) and tubulointerstitial fibrosis to 0.31±0.11% (*P*=005; Figure 3A and 3C), whereas high-fiber diet did not have a significant effect (*P*=0.97 and *P*=0.94, respectively; Figure 3A–3C).

Consistent with the extent of renal fibrosis, compared with control mice, mice treated with DOCA for 6 weeks developed extensive perivascular and interstitial cardiac fibrosis (Figure 4). The perivascular collagen volume fraction increased from 2.2±0.8% in controls to 30.9±2.0% in DOCA-treated mice (*P*<0.0001). This was significantly reduced by both the high-fiber diet (19.4±2.2%; *P*<0.0001) and acetate supplementation (9.26±2.8%; *P*<0.0001; Figure 4A and 4B). The interstitial collagen volume percentage also increased in DOCA-treated mice, from control values of 0.5±0.2% to 5.1±2.0% (*P*<0.001). Again, both high-fiber diet (1.4±0.7%; *P*<0.0001) and acetate supplementation (0.47±0.15%; *P*<0.0001) attenuated this, reducing the interstitial collagen volume fraction significantly (Figure 4A and 4C).

Table. Echocardiographic Measurements

	Sham+Control	Sham+Fiber	Sham+Acetate	DOCA+Control	DOCA+Fiber	DOCA+Acetate
LVIDs, mm	3.3±0.3	2.6±0.4†	2.7±0.3*	3.8±0.5†	3.0±0.3§	2.9±0.3§
LVIDd, mm	4.2±0.3	3.7±0.3†	3.8±0.1*	4.5±0.6	4.0±0.2‡	3.8±0.2§
FS, %	25±3	29±6	29±7	18±6*	26±6‡	24±4
LVPWd, mm	0.73±0.07	0.60±0.07*	0.66±0.05	0.81±0.07*	0.63±0.07§	0.72±0.1
IVSd, mm	0.75±0.15	0.89±0.12	0.92±0.15	0.80±0.18	0.75±0.19	0.86±0.23

Values are mean±SD. FS indicates fractional shortening; IVSd, interventricular septal dimension; LVIDd, diastolic left ventricular internal dimension; LVIDs, systolic left ventricular internal dimension; and LVPWd, left ventricular posterior wall dimension.

**P*<0.05,

†*P*<0.01 versus sham+control.

‡*P*<0.01, §*P*<0.001 versus DOCA+control.

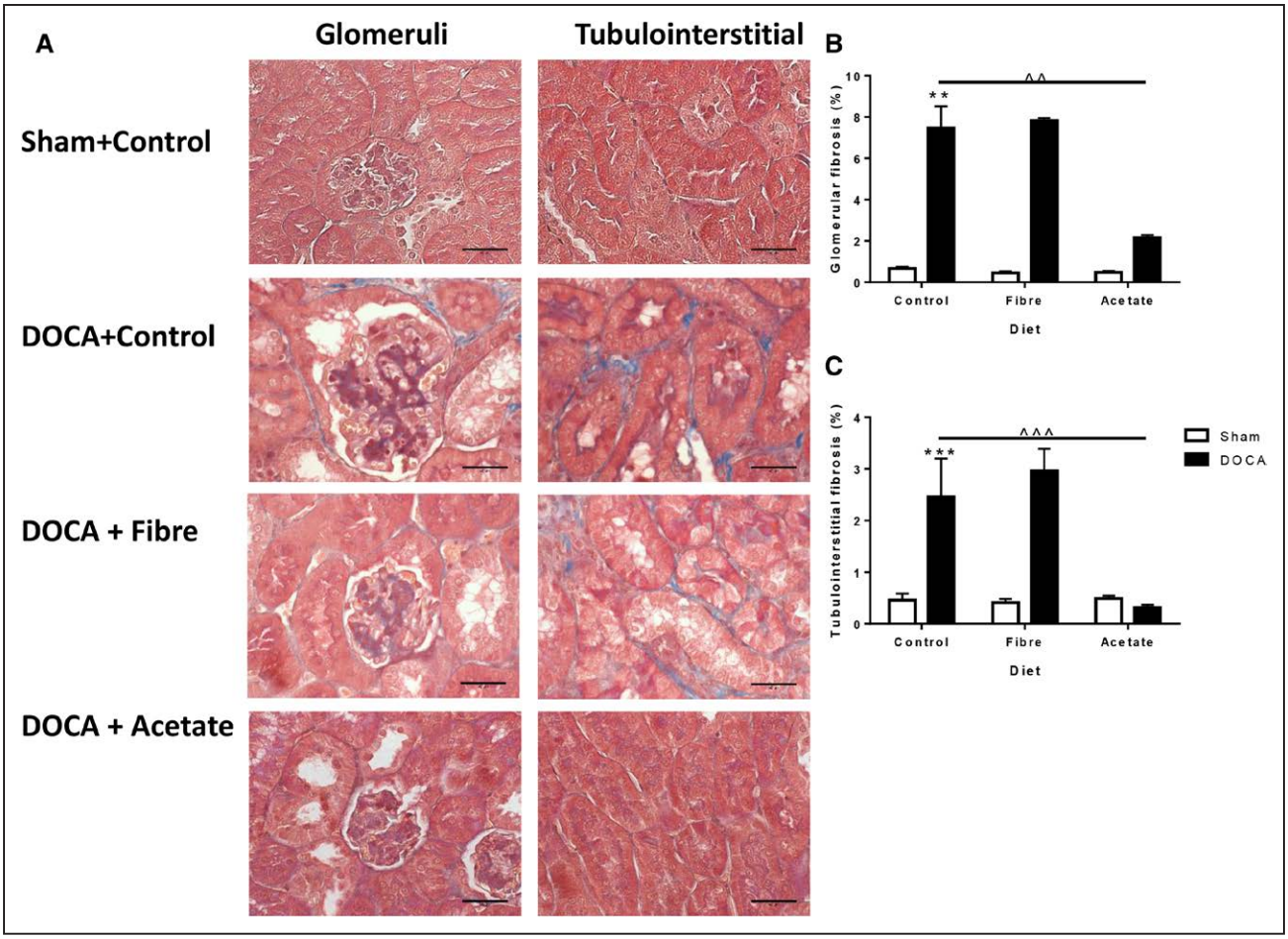

Figure 3. Acetate improves renal fibrosis in deoxycorticosterone acetate (DOCA)–salt model.
A, Representative histological images of Masson trichrome staining for each treatment (scale bar=40 μm). Compared with controls, DOCA-salt mice had a significant increase in renal (**B**) tubulointerstitial and (**C**) glomerular fibrosis, which was significantly reduced by acetate complementation. Values are mean±SEM. ***P*<0.01, ****P*<0.001 vs sham-control. ^^*P*<0.01, ^^^*P*<0.001 vs DOCA-control.

High-Fiber Diet and Acetate Supplementation Did Not Increase the Risk of Diabetes Mellitus

Mice fed a diet rich in fiber had body weight similar those fed the control diet, whereas supplementation with acetate led to reduced body weight (Figure IA and IB in the online-only Data Supplement). With exception of 1 sample in the high-fiber group, which had low levels of hemoglobin A_{1c} (4.2%, 23 mmol/mol), hemoglobin A_{1c} levels were below detection (<4%, <20 mmol/mol) for all mice independently of treatment group. We also measured urine glucose (Figure ID in the online-only Data Supplement), and there was no difference between groups.

Distinguishable Renal Transcriptome

The renal transcriptome of mice fed a high-fiber diet or acetate was different from those fed a control diet (Figure 5A and 5B). In the kidney, 316 genes were differentially expressed in mice fed a high-fiber diet (FDR <0.05; Table II in the online-only Data Supplement), and 3026 genes were differentially expressed in mice that received acetate (Table III in the online-only Data Supplement) compared with those fed the control diet (Figure 5C). Two hundred forty-four genes were in common between high fiber and acetate (Figure 5C and 5D). Examples were genes previously associated with renal fibrosis[24] such as renin-angiotensin system protein activator-like 1 (*Rasal1*), fluid absorption through sodium channel regulation[25] such as cytochrome P450 family 4 subfamily α polypeptide 14 (*Cyp4a14*), and anti-inflammatory actions[26] such as cholecystokinin (*Cck*) mRNA (Figure 5D).

Several of these common genes were in the same pathways, and they formed interconnected networks (Figure III in the online-only Data Supplement). An enrichment map highlighted that both high-fiber diet (Figure 5E) and acetate supplementation (Figure IV in the online-only Data Supplement) upregulated several pathways acting on translation, mRNA metabolism, and respiratory electron chain and downregulated pathways on cholesterol and lipid metabolism and endoplasmic

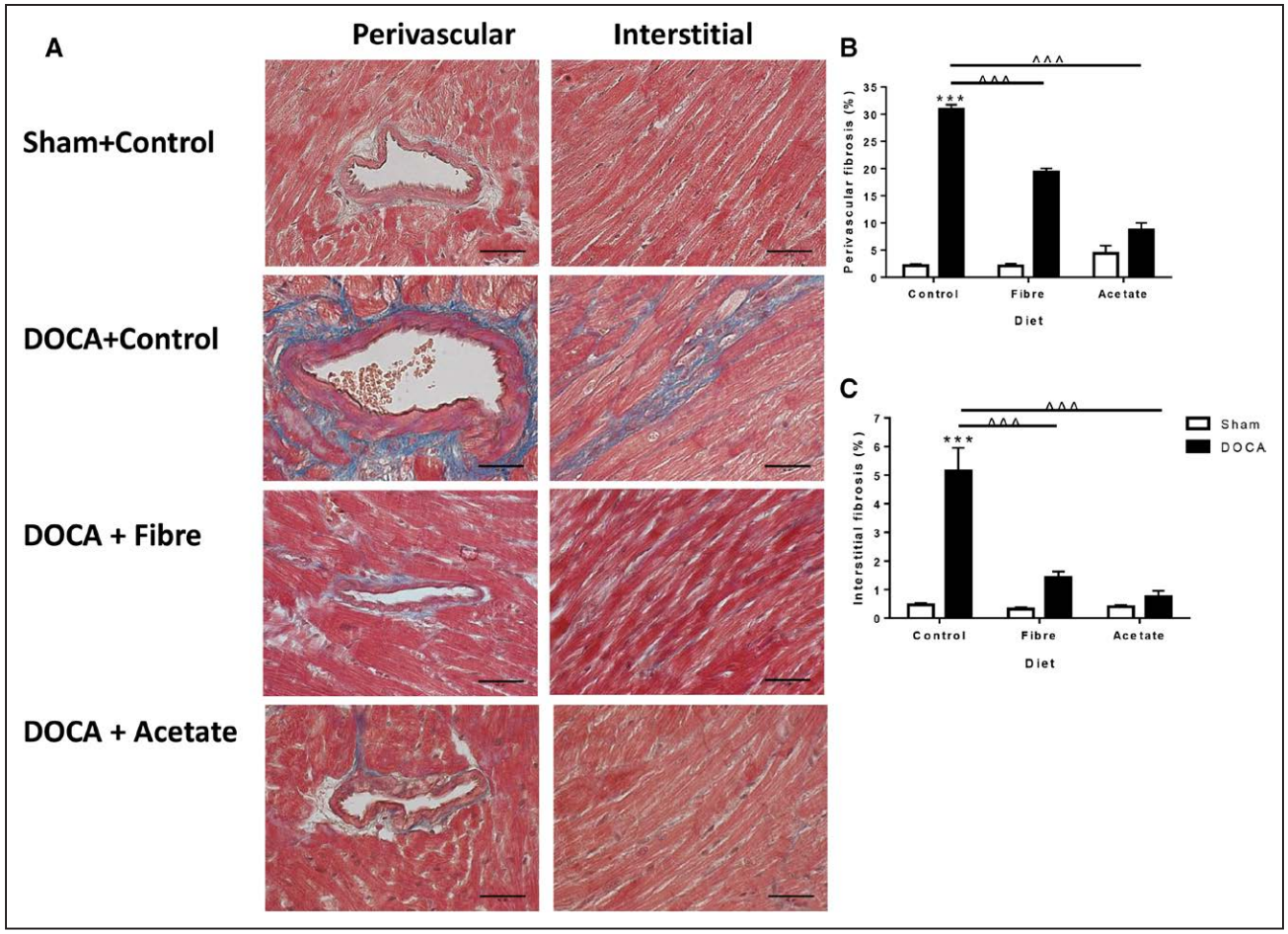

Figure 4. High fiber diet and acetate reduce cardiac fibrosis in deoxycorticosterone acetate (DOCA)–salt model. **A**, Representative histological images of Masson trichrome staining for each treatment (scale bar=40 μm). Compared with controls, DOCA-salt mice had a significant increase in cardiac (**B**) perivascular and (**C**) interstitial fibrosis, which was significantly reduced by a high-fiber diet or acetate complementation. Values are mean±SEM. ***P<0.001 vs sham-control. ^^^P<0.001 vs DOCA-control.

reticulum stress. This also included the upregulation of circadian rhythm and the borderline downregulation the renin-angiotensin system, well known in BP regulation (fiber FDR q=0.113, acetate q=0.088). Although high-fiber diet and acetate supplementation shared a significant number of genes and pathways, our data suggest that the mechanisms are not entirely the same. For example, fiber upregulated *G protein–coupled receptor* ligand binding (q=0.034) and intestinal immune network for immunoglobulin A production (q=0.088) and downregulated the proinflammatory cytokine interleukin-1 (IL-1) signaling (q=0.035), whereas acetate downregulated the metabolism of butyrate (q<0.001) and propionate (q<0.001) in the kidney (Figure V in the online-only Data Supplement).

Cardiac Transcriptome

Consistent with the renal data, the cardiac transcriptome of mice fed a high-fiber diet or acetate was different from those fed a control diet (Figure 6A and 6B). In the heart, 447 genes were differentially expressed in mice fed a high-fiber diet (FDR <0.05; Table IV in the online-only Data Supplement), and 536 genes were differentially expressed in mice that received acetate (Table V in the online-only Data Supplement) compared with those fed the control diet. One hundred ninety-two genes were in common between high fiber and acetate (Figure 6C and 6D) such as the upregulation of genes thought to have a preventive role for heart disease such as titin-cap (*Tcap*)[27] and tissue metallopeptidase inhibitor 4 (*Timp4*).[28] It is interesting to note that in both the kidney and heart, fiber and acetate downregulated the gene for early growth response protein 1 (*Egr1*) mRNA (Figure 6D), a master regulator of cardiovascular pathology.[29–31]

Several of these common genes were in the same pathways, and they formed interconnected networks (Figure VI in the online-only Data Supplement). An enrichment map highlighted that both high-fiber diet (Fig-

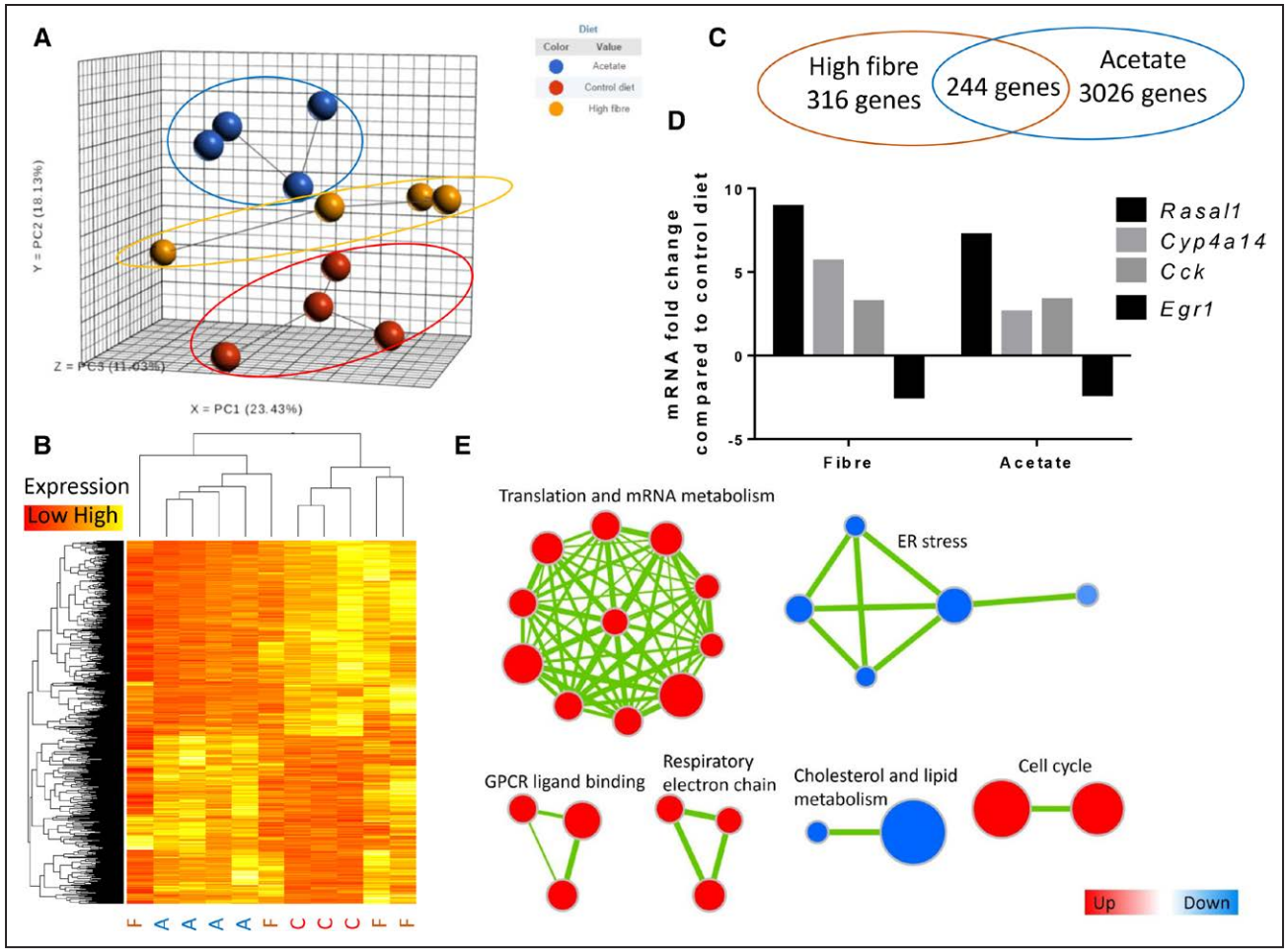

Figure 5. Diet rich in fiber and supplementation with acetate modify the renal transcriptome.
Mice were fed a high-fiber diet or water supplemented with acetate for 3 weeks. **A**, Unsupervised principal component analysis and (**B**) hierarchical clustering of the renal transcriptome showing that mice fed high fiber or acetate form a distinctive group from those fed the control diet (blue=acetate, orange= fiber, red=control). **C**, Venn diagram of the renal transcriptome, showing differentially expressed genes in common between mice that received a diet rich in fiber or acetate. **D**, Fold change of renal genes of mice treated with high fiber or acetate compared with the control diet (all false discovery rate [FDR] q<0.05, data extracted from next-generation sequencing). Significantly higher renal levels of *Rasal1*, *Cyp4a14*, and *Cck* mRNA and lower *Egr1* mRNA with fiber and acetate. **E**, Enrichment map highlighting major pathway upregulated (red) or downregulated (blue) with dietary fiber in the kidney. Shown are reactome pathways that had an FDR <0.05 in the gene-set enrichment analysis. ER indicates endoplasmic reticulum; and GPCR, G-protein–coupled receptor.

ure 6E) and acetate supplementation (Figure VII in the online-only Data Supplement) upregulated several pathways acting on cell cycle, DNA replication, translation, mRNA metabolism, and respiratory electron chain. Similar to the renal transcriptome, this included the upregulation of circadian rhythm (fiber q=0.021 and acetate q<0.001) and a borderline downregulation of mitogen-activated protein kinase signaling (fiber q=0.078 and acetate q=0.086). Fiber downregulated Wnt (q<0.001) and transforming growth factor-β signaling (borderline q=0.07), whereas acetate downregulated extracellular matrix receptor interaction (q=0.02) and the renin-angiotensin system (q=0.04; Figure VIII in the online-only Data Supplement).

DISCUSSION

In this study, we determined whether dietary fiber intake, through changes in the gut microbiota, could prevent the adverse renal and cardiac effects of CVD. Because fiber is fermented in the colon by commensal bacteria, leading to the release of the SCFA acetate into the circulation,[10] we also treated mice with acetate itself. Obesogenic diets that are low in fiber are associated with inflammation and a heightened risk of CVD.[32] Indeed, we found that supplementation with fiber and particularly acetate leads to lower body weight and no change in urine glucose or hemoglobin A_{1c}, both suggestive that the mice were not diabetic. Our data show that dietary

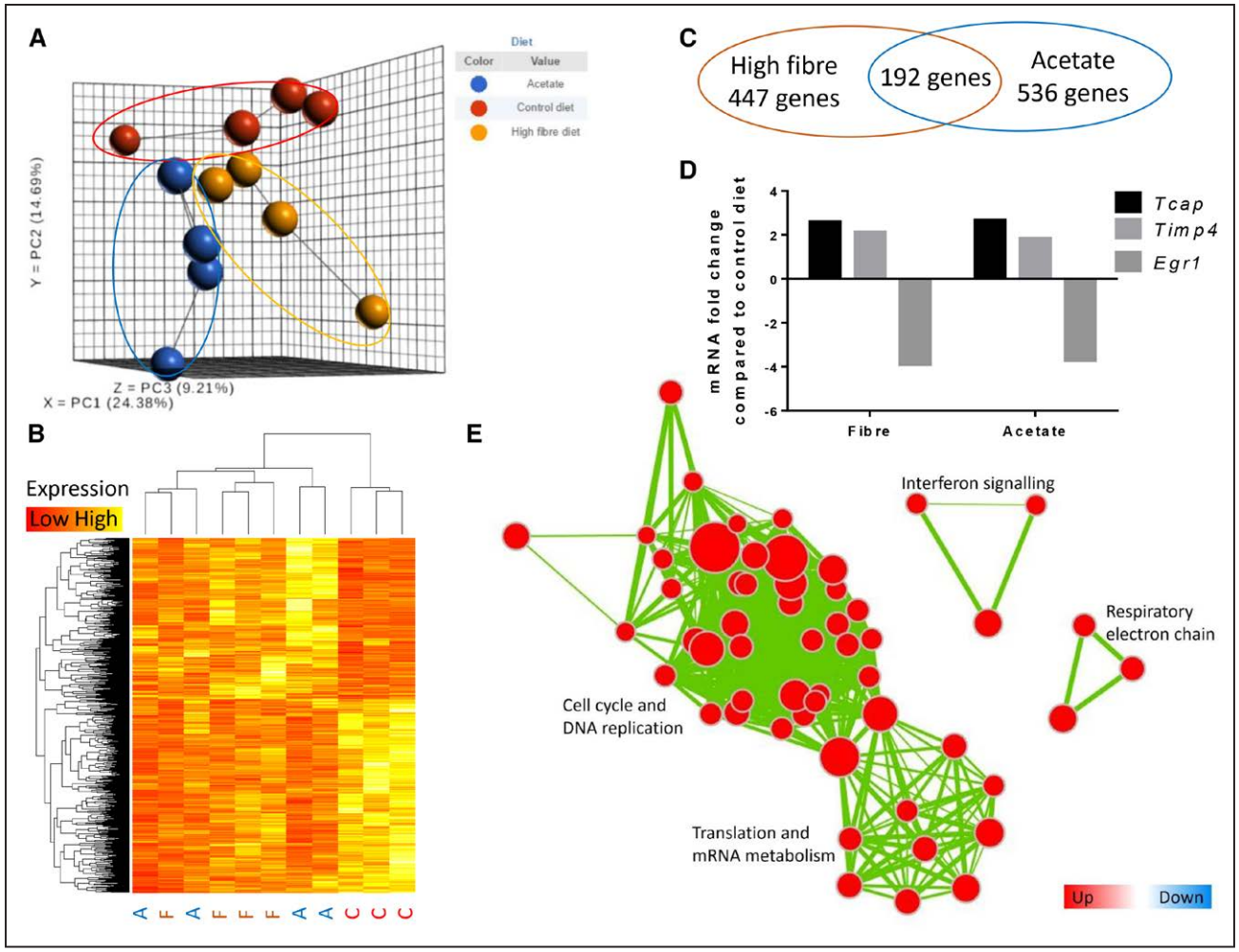

Figure 6. Diet rich in fiber and supplementation with acetate modify the cardiac transcriptome.
A, Unsupervised principal component analysis and (**B**) hierarchical clustering of the cardiac transcriptome showing that mice fed high fiber or acetate form a distinctive group from those fed the control diet (blue=acetate, orange= fiber, red=control). **C**, Venn diagram of the cardiac transcriptome showing differentially expressed genes in common between mice that received a diet rich in fiber or acetate. **D**, Fold change of cardiac genes of mice treated with high fiber or acetate compared with the control diet (all false discovery rate [FDR] q<0.05, data extracted from next-generation sequencing). Significantly higher cardiac levels of *Tcap* and *Timp4* mRNA and lower *Egr1* mRNA with fiber and acetate. **E**, Enrichment map highlighting major pathway upregulated (red) or downregulated (blue) with dietary fiber in the heart. Shown are reactome pathways that had FDR <0.05 in the gene-set enrichment analysis.

manipulations including fiber and acetate decreased the ratio between bacteria of the phyla Firmicutes and Bacteroidetes, thus improving gut dysbiosis, and increased the prevalence of acetate-producing bacteria and those of the genus *Bacteroides acidifaciens*. These changes were accompanied by a marked attenuation of the adverse actions of mineralocorticoid excess on BP and renal and cardiac structure and function, supporting a gut-kidney-heart axis. Fiber and acetate improved heart and kidney function through regulation of key pathways and genes involved in cardiovascular health, including the transcription factor *Egr1*, a master regulator of CVD through cardiac and renal fibrosis, inflammation, and cardiac hypertrophy.[29–31] Fiber also downregulated the proinflammatory IL-1 cytokine signaling in the kidney. This is consistent with our fundamental hypothesis, which was based on the recent demonstration of a strong link between dietary fiber, the gut microbiota, and the pathogenesis of inflammatory diseases.[9,10] In our study, fiber and acetate also upregulated the regulation of circadian rhythm, which is supported by its role in BP,[33] and downregulated the renin-angiotensin system in the kidney and mitogen-activated protein kinase signaling in the heart.

An inverse relationship between dietary fiber intake and the risk of CVD is well established,[10,34] as is the capacity of diets such as the Mediterranean diet to reduce the risk of incident heart disease.[35] Inflammation and the immune system have been increasingly associated with the development of CVD in general and hypertension specifically.[36] Recent interest has emerged in regard to

the ability of fiber in the diet to reduce the incidence of inflammatory conditions such as asthma[10] and inflammatory bowel disease.[13] In our study, we did not observe any changes in cells of the immune system in the spleen (data not shown). Our renal transcriptome data, however, supported that fiber lowered IL-1 signaling, an early response proinflammatory cytokine originated from monocytes and macrophages.[37] Besides the higher levels of IL-1 usually detected in hypertensive subjects,[37] the activation of the IL-1 receptor leads to sodium retention in the kidney through a mechanism that involves macrophages and nitric oxide production.[38] Moreover, fiber and acetate led to the downregulation of the transcription factor *Egr1*, considered a master regulator because it controls the expression of a wide range of genes and pathways implicated in CVD processes.[29] Particularly relevant to the present study is the role of *Egr1* in cardiac hypertrophy, renal fibrosis, and inflammation.[29–31]

A diet rich in fiber is associated with the production of substantial quantities of SCFAs such as acetate.[10] SCFAs are thought to act in the gut via metabolite-sensing receptors such as the inhibitory G-protein coupled receptor GPR43, which interacts with acetate.[13,39,40] Activation of GPR43 by acetate leads to colonic epithelial hyperpolarization, which was accompanied by a reduction in apoptosis.[11] We found, however, that *Gpr43* and other metabolite-sensing G protein-coupled receptors previously associated with SCFAs such as the olfactory receptor (Olfr78)[41] were not expressed in the kidney or heart (data not shown). *Gpr43* mRNA was also downregulated in the gut with mineralocorticoid excess independently of the treatment group (data not shown). This suggests that the mechanism is likely to be independent of the ones previously proposed in the literature.

Combined with recent evidence,[12] our study supports that the dysbiosis of the gut microbiota is associated with the development of hypertension. We are the first, however, to causally link fiber consumption and the SCFA acetate to the prevention of hypertension and renal and cardiac fibrosis and function, which supports a gut-kidney-heart axis. Because of the well-established role of gut-brain cross-talk in several central nervous diseases,[42] this raises questions as to whether a gut–sympathetic nervous system cross-talk is involved in hypertension and the contribution of SCFAs. Indeed, the SCFA propionate could regulate sympathetic nervous system activity in the sympathetic ganglion via Gpr41,[43] and metabolites produced by the gut microbiota have been linked to the regulation of central circadian rhythm.[44] This is consistent with our findings that support a role for fiber and acetate in the regulation of circadian cycle in both the kidney and heart. Our data also showed that fiber and acetate significantly increased the abundance of the bacteria *Bacteroides acidifaciens*. This bacteria was recently shown to prevent obesity and to improve insulin sensitivity in mice,[45] but whether it can prevent the development of high BP is yet to be determined.

High fiber and SCFA supplementation exerted favorable actions on the heart of DOCA-treated mice. Specifically, there was a significant reduction in heart weight and cardiac fibrosis with both interventions. The observations may have resulted from the lower BP itself, for example, because previous studies have shown that aggressive BP lowering in DOCA rats treated for a short period attenuated cardiac fibrosis but not cytokine expression.[46] We also found that a high-fiber diet was able to reduce cardiac but not renal fibrosis. This observation might be explained by the different molecular mechanisms involved in the prevention of CVD by fiber or acetate itself. However, the renal transcriptome shared 244 genes and several pathways between fiber and acetate. This includes the downregulation of the gene for *Egr1*, which deficiency protects from renal fibrosis and inflammation,[30] and the upregulation of the gene for *Rasal1*, which normalizes the proliferative activity of activated fibroblasts in the kidney.[47]

Dietary and acetate intervention had a marked effect on hypertension and cardiac fibrosis, and future studies will determine the ability of dietary intervention not just to prevent but also to reverse established disease. It is clear that from a mechanistic and public health point of view, such studies will be important because of implications for human diets and the relatively benign, cost-effective, and nonpharmacological nature of such treatments. Second, supplementation with acetate had more pronounced effects on the mineralocorticoid-excess model than the high fiber-diet. This could be a result of the already high fiber content (albeit a different type) of the standard mouse chow (Table I in the online-only Data Supplement). Indeed, in a previous study, we found that differences in phenotype were more marked when the high-fiber group was compared with a group receiving no-fiber chow instead of standard mouse chow as control.[10] It is also conceivable that acetate in the drinking water allowed higher peripheral exposure compared with the high-fiber diet. Our previously published work supported that the high-fiber diet used in the present study leads to significantly higher levels of SCFAs, particularly acetate.[10] However, these were not measured in the present study.

Taken together, the results of our study show that a high-fiber diet intervention or, in particular, supplementation with the SCFA acetate significantly attenuates the development of hypertension, cardiac hypertrophy, and cardiorenal fibrosis in the context of mineralocorticoid excess. Our data provide evidence for the importance of the gut microbiota in the pathogenesis of CVD and renal disease and propose a gut-kidney-heart axis. Last, our study provides mechanistic insights into the protective actions of dietary fiber on cardiovascular and renal disease with a specific emphasis on the potential role of gut-derived SCFAs in several genes and pathways involved in the development of CVD such as *Egr1* and *Rasal1*. Novel dietary strategies that involve fiber supplementation or specifically acetate supplementation could be used to prevent CVD.

ACKNOWLEDGMENTS

The authors thank Rachael McNally and Dr Naga Kasinadhuni from the Australian Genome Research Facility for help with the sequencing of the 16S gene, Dr Helen Kiriazis from the Mouse Cardiology Research Platform at Baker Heart and Diabetes Institute for help with the echocardiography and cardiac catheter, Prof Gavin Lambert, Dr Nina Eikelis, and Sarah Phillips from the Human Neurotransmitters Laboratory at Baker Heart and Diabetes Institute for help with experiments, Prof Geoff Head from Baker Heart and Diabetes Institute for guidance on statistical analysis, and the Monash Micro Imaging Facility for help with microscopy.

SOURCES OF FUNDING

This work was supported by a National Health & Medical Research Council of Australia Program Grant and fellowship to Prof Kaye. Dr Marques is supported by National Health & Medical Research Council of Australia and National Heart Foundation coshared Early Career Fellowships and a Foundation for High Blood Pressure Research grant and a seeding grant from the Baker Heart and Diabetes Institute. Prof El-Osta and Dr Ziemann are supported by the National Health & Medical Research Council of Australia, the Juvenile Diabetes Research Foundation, and the Diabetes Australia Research Trust. The Baker Heart and Diabetes Institute is supported in part by the Victorian Government's Operational Infrastructure Support Program. The authors acknowledge the use of Illumina sequencing at the Australian Genome Research Facility (and the support it receives from the Commonwealth of Australia).

DISCLOSURES

None.

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FOOTNOTES

Received July 18, 2016; accepted November 29, 2016.

The online-only Data Supplement, podcast, and transcript are available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.116.024545/-/DC1.

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