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**RESEARCH ARTICLE** | *Role of Gut Microbiota, Gut-Brain and Gut Liver Axes in Physiological Regulation of Inflammation, Energy Balance, and Metabolism*

# Fecal transplant from resveratrol-fed donors improves glycaemia and cardiovascular features of the metabolic syndrome in mice

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[1]*Cardiovascular Research Centre, Department of Pediatrics, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada;* [2]*The Metabolomics Innovation Centre, University of Alberta, Edmonton, AB, Canada;* [3]*Division of Gastroenterology, Department of Medicine, University of Alberta, Edmonton, AB, Canada; and* [4]*Department of Biochemistry and Biomedical Sciences and Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, ON, Canada*

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**Kim TT, Parajuli N, Sung MM, Bairwa SC, Levasseur J, Soltys CL, Wishart DS, Madsen K, Schertzer JD, Dyck JR.** Fecal transplant from resveratrol-fed donors improves glycaemia and cardiovascular features of the metabolic syndrome in mice. *Am J Physiol Endocrinol Metab* 315: E511–E519, 2018. First published June 5, 2018; doi:10.1152/ajpendo.00471.2017.—Oral administration of resveratrol attenuates several symptoms associated with the metabolic syndrome, such as impaired glucose homeostasis and hypertension. Recent work has shown that resveratrol can improve glucose homeostasis in obesity via changes in the gut microbiota. Studies involving fecal microbiome transplants (FMTs) suggest that either live gut microbiota or bacterial-derived metabolites from resveratrol ingestion are responsible for producing the observed benefits in recipients. Herein, we show that obese mice receiving FMTs from healthy resveratrol-fed mice have improved glucose homeostasis within 11 days of the first transplant, and that resveratrol-FMTs is more efficacious than oral supplementation of resveratrol for the same duration. The effects of FMTs from resveratrol-fed mice are also associated with decreased inflammation in the colon of obese recipient mice. Furthermore, we show that sterile fecal filtrates from resveratrol-fed mice are sufficient to improve glucose homeostasis in obese mice, demonstrating that nonliving bacterial, metabolites, or other components within the feces of resveratrol-fed mice are sufficient to reduce intestinal inflammation. These postbiotics may be an integral mechanism by which resveratrol improves hyperglycemia in obesity. Resveratrol-FMTs also reduced the systolic blood pressure of hypertensive mice within 2 wk of the first transplant, indicating that the beneficial effects of resveratrol-FMTs may also assist with improving cardiovascular conditions associated with the metabolic syndrome.

fecal transplant; glycemia; hypertension; metabolic syndrome; resveratrol

## INTRODUCTION

Obesity remains a global health concern, with most recent estimates indicating that more than 1.9 billion adults are either overweight or obese (1). Detrimental health outcomes of obesity include increased risk for diseases such as type 2 diabetes (T2D) and cardiovascular disease (1), which continue to be the primary cause of death in those with T2D. Furthermore, the direct underlying impacts of obesity include hypertension, hyperglycemia, high serum triglyceride levels, and low high-density lipoprotein cholesterol levels (6). Although these conditions may occur independently, it is common for individuals to present with more than one of the symptoms simultaneously, and the presence of three or more of these symptoms is used to diagnose an individual with metabolic syndrome. Thus, improving features of the metabolic syndrome such as hyperglycemia and hypertension may prove to have significant health benefits (3, 15, 23).

Resveratrol is a polyphenolic compound found in various plant-based foods that has shown promising results in the prevention of insulin resistance caused by obesity (2) and the treatment of hypertension (11). Additionally, although some clinical trials have been positive in patients with T2D (20) and patients with hypertension (34), the results have been inconclusive as a subset of clinical trials has shown no significant effect with resveratrol treatment (4, 27, 34). Although it is possible that the negative trials could be a result of differences in treatment dose or duration, it is also possible that the precise mechanism of action of resveratrol has not been clearly delineated. Thus, a better understanding the mechanisms of action of resveratrol may promote its effective use.

Owing to the low bioavailability of resveratrol, it has been postulated that a mechanism of resveratrol is through its interaction with the gut (7, 21). In fact, recent findings suggest that ingestion of resveratrol induced changes in the gut microbiome composition of obese mice (8), and these changes were associated with improvements in glucose homeostasis (30). In the current study, we further our understanding of resveratrol and show that the benefits of resveratrol occur by transferring fecal material containing nonviable microorganisms between mice. We found that sterile bacterial metabolites or other components within the feces of resveratrol-fed mice have glucose-lowering effects when transplanted in obese mice. Furthermore, we show that transplanting fecal slurry from

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resveratrol-fed mice had beneficial effects in lowering the blood pressure of hypertensive mice. Thus, the transfer of fecal-derived components from healthy resveratrol-fed donor mice may contain materials that have therapeutic potential in improving at least two symptoms associated with the metabolic syndrome.

## METHODS AND MATERIALS

*Materials.* Rodent diets were either purchased from Dyets Inc. (Bethlehem, PA) or Research Diets Inc. (New Brunswick, NJ), and trans-resveratrol was purchased from Lalilab (Durham, NC).

*Experimental animals.* The animal protocols used in this study were reviewed and approved by the University of Alberta Animal Policy and Welfare Committee, which adheres to the guidelines of the Canadian Council on Animal Care. Eight-week-old male C57BL/6N mice were acquired from Charles River Laboratories and individually housed on a 12-h light/dark cycle (0600 to 1800 h light). Mice were given ad libitum access to food and water and provided with diets for various durations based on their experimental group assignment. Mice were fed one of the following diets: *1*) chow (control, American Institute of Nutrition-93G diet(AIN); Dyets), *2*) Resv (American Institute of Nutrition-93G + 4 g/kg resveratrol; Dyets), or *3*) high-fat, high-sucrose (HFHS; 45 kcal% fat, 17 kcal% sucrose; cat. no. D-12451, Research Diets).

*Mouse model of diet-induced obesity.* To confirm that resveratrol improves insulin sensitivity during obesity, 8-wk-old C57BL/6N mice were conventionally raised and maintained on an HFHS diet for 5 wk. Change in glucose tolerance was measured through intraperitoneal glucose tolerance tests (IP-GTTs) at baseline and after every 2 wk. Body weights were monitored weekly and EchoMRIs were completed to monitor changes in body composition.

*Oral supplementation of resveratrol.* Eight-week-old C57BL/6N mice were conventionally raised and maintained on an HFHS diet for 5 wk. Mice were then randomly assigned to *1*) continue an HFHS diet or *2*) receive an HFHS diet supplemented with resveratrol (0.4% Resv ad libitum) for two more weeks.

*Fecal slurry preparation.* Eight-week-old C57BL/6N mice were conventionally raised and maintained on a chow or Resv diet for 8 wk ($n = 10$/group). Fecal pellets were collected fresh every morning during the eight weeks and homogenized in ice-cold 20% glycerol in PBS (supplemented with 0.05% L-cysteine-HCl). Pooled slurry was stored in −80°C as 300-μl aliquots until the day of gavage. Each treatment dose contained ~40 mg of fecal matter.

*Fecal microbiome transplants.* In a separate group, 8-wk-old C57BL/6N mice were subjected to an HFHS diet for 5 wk. Mice were randomly assigned to one of two groups either receiving fecal microbiome transplants (FMTs) from donor mice fed a chow ($n = 8$) or Resv ($n = 9$) diet. Following an overnight fast (*day 0*), fecal matter from donor mice was transplanted via oral gavage on *days 1*, *3*, and *5* as previously described (30). IP-GTTs were completed 3 days before the first FMT (baseline) and again on the eleventh day after the first FMT. Body weights and food intake were monitored daily during the treatment period.

*Heat-killed FMTs.* To test whether live microbes are necessary for the beneficial effects of FMTs, prepared fecal slurry was autoclaved in a sterilization pouch for 15 min to achieve a temperature of 121.1°C. Sterilized fecal slurry was cultured on brain heart infusion + cysteine, LB, and MacConkey agar plates to confirm that no live microbes were present. A separate group of 8-wk-old C57BL/6N mice was subjected to the same experimental timeline as mentioned above. Mice were maintained on an HFHS diet for 5 wk, fasted overnight (*day 0*), and received 3 oral gavages of Resv or heat-killed (HK) Resv slurry ($n = 14$/group) on *days 1*, *3*, and *5*. IP-GTTs, monitoring of body weights, and food intake were carried out as mentioned above.

*IP-GTTs.* Starting between 0800 and 0900, mice were fasted for 5–6 h and then administered intraperitoneal injections of 2 g/kg body wt glucose (dissolved in sterile 0.9% saline). Glucose levels were detected in blood collected from the tail tip using an ACCU-CHEK Advantage glucometer (Roche Diagnostics, Laval, Canada) as described previously (18). Measurements were recorded before injection (0 min) and at 10, 20, 30, 60, 90, and 120 min following the injection. IP-GTTs were completed in all treatment groups 3 days before the overnight fast as a pretreatment baseline and on *day 11* to confirm the effects of the treatment.

*Mouse model of angiotensin II-induced hypertension.* Eight-week-old C57BL/6N mice were randomly assigned into groups, and Alzet Osmotic Minipumps were implanted subcutaneously. In brief, mice received either saline or angiotensin II (Ang-II; 1.4 $mg \cdot kg^{-1} \cdot day^{-1}$) for 2 wk through a small incision on the dorsal interscapularis, forming a small subcutaneous pocket.

*Tissue cytokine measurement.* Frozen colon tissues were powdered using a mortar and pestle and homogenized in ice-cold PBS (with 0.05% Tween-20) for cytokine extraction. Samples were centrifuged at 10,000 revolutions/min for 10 min, and then the supernatant was used to measure cytokines [TNF-α, CXCL1/KC, and IL-1β) using an ELISA duo set (R&D Systems, Inc., Minneapolis, MN) as indicated in the manufacturer's protocol.

*Nuclear magnetic resonance spectroscopy of fecal metabolites.* Fresh fecal matter from chow- and Resv-fed donor mice that were fed their respective diets for 8 wk were collected, frozen in liquid $N_2$, and stored at −80°C until analysis. Approximately 100 mg of the frozen fecal matter was powderized and homogenized with 1 ml of ice-cold water. Samples were then sonicated at 4°C for 20 min and subjected to vortex shaking at 250 revolutions/min for 20 min. Following this, the samples were centrifuged twice at 15,000 × *g* for 1 h at 4°C with the supernatant being transferred to a fresh Eppendorf tube after each centrifugation. The 700-MHz Avance III (Bruker) spectrometer equipped with a 5-mm HCN Z-gradient pulsed-field gradient cryoprobe was used to acquire 1H-NMR spectra. Spectra were acquired at 25°C using the first transient of the nuclear overhauser effect spectroscopy (NOESY) pulse sequence with presaturation during the mixing time (noesy1dpr), and all free induction decays were zero-filled to 250 K data points. The singlet produced by the 4,4-dimethyl-4-silapentane-1-sulfonic acid-methyl groups was used as an internal standard for chemical shift referencing, which was set to 0 parts per million. The spectra were processed and analyzed with the Chenomx NMR Suite Professional software package version 8.1 (Chenomx Inc., Edmonton, Canada), which allows

for qualitative and quantitative analysis of NMR spectra by manually fitting spectral signatures using an internal database.

*Statistical analysis.* Blinding was not possible for these experiments and statistical methods were not used to predetermine sample sizes. Variance between all groups being tested was similar, and the Shapiro-Wilk test was applied to test for normality. Results are expressed as means ± standard error (SE). GraphPad Prism was used to perform statistical analyses, and comparisons were performed by unpaired two-tailed Student's *t*-test or one-way or two-way ANOVA and Tukey post hoc test when appropriate.

## RESULTS

*Oral supplementation of resveratrol is not sufficient to improve glucose homeostasis in obese mice.* To ascertain whether oral supplementation of resveratrol in obese mice is able to rescue impaired glucose homeostasis, a group of 8-wk-old mice was fed an HFHS diet for 5 wk and then received an HFHS ($n = 8$) or an HFHS plus 0.4% resveratrol (HFHS + Resv; $n = 9$) diet for another 2 wk (Fig. 1*A*). Interestingly, the area under the curve (AUC) for both HFHS and HFHS + Resv groups increased to a similar magnitude after 2 wk on their respective diets (Fig. 1, *B* and *C*), and no differences in body weights were observed (Fig. 1*D*). Thus, although oral supplementation of resveratrol may have protective effects when given before the onset of insulin resistance (30), our data show that 2-wk intervention with resveratrol is unable to effectively treat already exiting impairments in glucose homeostasis.

*Resveratrol-FMTs improve glucose clearance in obese mice.* To demonstrate that resveratrol-FMTs were sufficient to treat impaired glucose homeostasis in obese mice, 8-wk-old mice maintained on an HFHS diet for 5 wk then received either chow-($n = 8$) or Resv-FMTs ($n = 9$), as previously described (30). Obese mice receiving Resv-FMTs showed a marked improvement in glucose homeostasis (Fig. 2, *A* and *B*) in the absence of differences in body weights between groups (Fig. 2*C*) and demonstrate that the metabolic improvements are not secondary to weight loss. Importantly, these decreases in blood glucose were observed in the absence of changes in the fasted serum insulin levels (Fig. 2*D*) or any differences in baseline glucose levels before and after receiving FMTs (data not shown). Together, these data suggest that Resv-FMTs may rescue glucose homeostasis by improving glucose uptake in peripheral tissues through increased sensitivity to insulin and not via elevated insulin secretion.

*Resveratrol-FMTs lower inflammatory cytokine levels in the colon of obese mice.* Because we ruled out Resv-FMTs acting to improve glucose homeostasis through regulation of insulin secretion (Fig. 2*D*), we tested other potential mechanisms of action to explain the potential for insulin sensitization such as intestinal inflammation. Previous studies in mouse models of diet-induced obesity have demonstrated that alterations in the gut composition in obesity contributes to intestinal inflammation and subsequent impairments in metabolic function and insulin sensitivity (5, 29). Based on this, we investigated the impact of Resv-FMTs on the levels of inflammatory cytokines

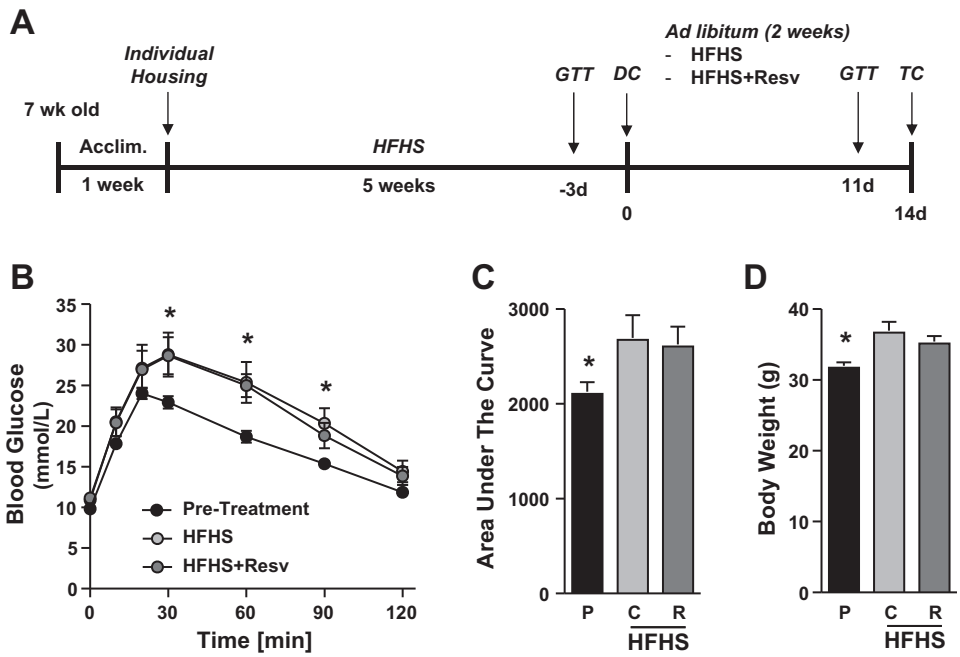


Fig. 1. Oral supplementation of resveratrol in obese mice does not improve glucose homeostasis. *A*: male C57BL/6 mice (8-wk-old) were fed an HFHS diet for 5 wk, and baseline glucose tolerance tests (GTTs) were completed 3 days before undergoing a diet change (DC; *day 0*). Following an overnight fast (F) on *day 0*, mice were either maintained on an HFHS or HFHS + 0.4% resveratrol diet for 14 days. Follow-up GTTs were completed on *day 11* (11d). Tissues were collected (TC) after a 5- to 6-h fast on *day 14* (14d). *B*: GTTs of HFHS-fed mice at baseline before (pretreatment; $n = 17$) and after randomization to receiving 2 wk of HFHS diet (HFHS; $n = 8$) or HFHS + 0.4% resveratrol diet (HFHS + Resv; $n = 9$). *C*: glucose clearance represented by the AUC of the GTTs from HFHS-fed mice before (P, $n = 17$) and after 2 wk of HFHS without (C, $n = 8$) or with (R, $n = 9$) resveratrol. *D*: body weight in all groups of mice. Values in *B–D* are shown as means ± SE $^*P < 0.05$; analyzed by two-way ANOVA with Tukey's post hoc test in *B* and analyzed by one-way ANOVA with Tukey's post hoc test in *C* and *D*. Acclim., acclimation; AUC, area under the curve; C, control; GTT, glucose tolerance test; HFHS, high-fat, high-sucrose; P, pretreatment; R, resveratrol.

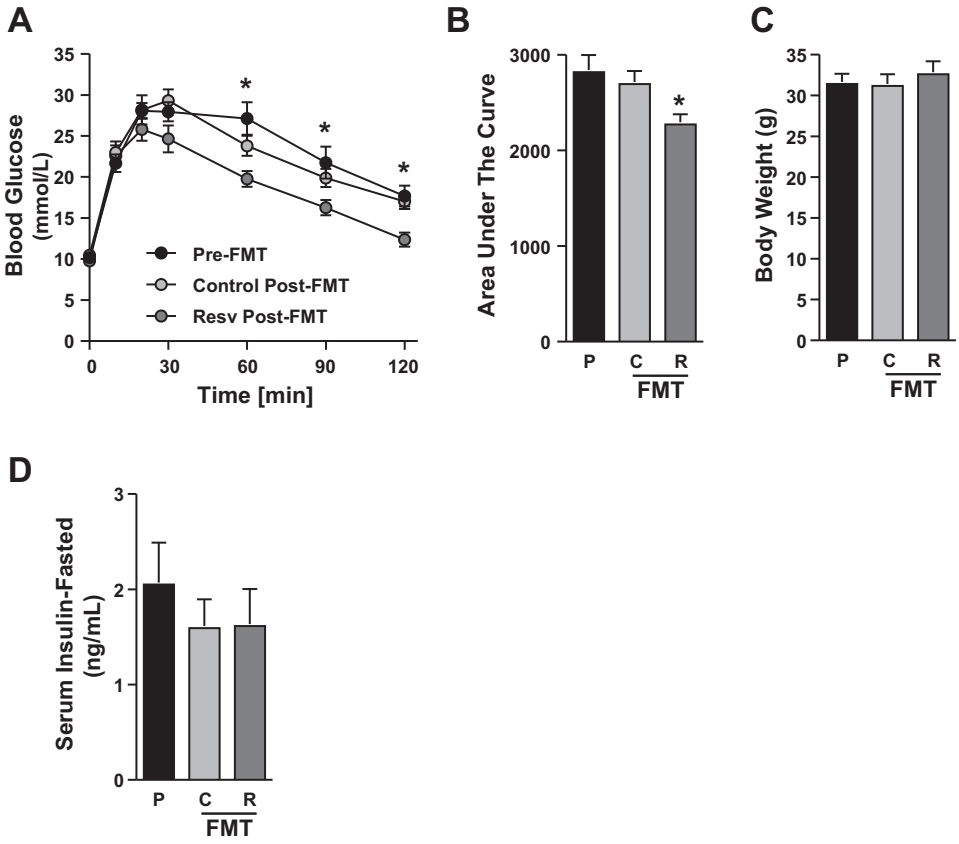


Fig. 2. Resveratrol-FMTs in obese mice are sufficient to improve glucose homeostasis. *A*: GTTs of HFHS-fed mice at baseline before receiving FMTs (pre-FMT; $n = 17$) and after randomization to receiving an FMT from chow-fed mice (control post-FMT; $n = 8$) or from chow plus resveratrol-fed donor mice (Resv post-FMT; $n = 9$). *B*: glucose clearance represented by the AUC of the GTTs from HFHS-fed mice before FMT (P, $n = 17$) and after FMT from control- FMT (C, $n = 8$) and Resv-FMT (R, $n = 9$). *C*: body weight in all groups of mice. *D*: serum insulin levels following a 5- to 6-h fast. Values in *A–D* are shown as means ± SE $*P < 0.05$; analyzed by two-way ANOVA with Tukey's post hoc test in *A* and analyzed by one-way ANOVA with Tukey's post hoc test in *B–D*. AUC, area under the curve; C, control; FMT, fecal microbiome transplant; GTT, glucose tolerance test; HFHS, high-fat, high-sucrose; P, pre-FMT; R, resveratrol; Resv, resveratrol.

in the colon of obese mice. When compared with chow-FMT recipients ($n = 7$), Resv-FMT recipients ($n = 10$) showed significantly lower levels of TNF-α, CXCL1/KC (murine IL-8 homologue), and IL-1β (Fig. 3, *A–C*), demonstrating reduced colon inflammation. In addition, comparison of the AUC of GTTs of all the mice receiving FMTs showed a positive correlation with levels of TNF-α (Fig. 3*D*). Together, these findings suggest that a mechanism of action of Resv-FMTs may be through its anti-inflammatory effects, at least at the level of the colon.

*Resv-FMTs do not alter the metabolic profile of obese mice.* With growing evidence that alterations in the gut microbiome are also associated with changes in serum metabolite (14, 19), we sought to identify potential changes in serum metabolites that could explain the observed physiological changes in obese Resv-FMT recipient mice. Analysis of 147 serum metabolites indicated no significant differences in the metabolic profiles of chow- and Resv-FMT recipients (data not shown). Furthermore, no individual metabolites were significantly different between the two groups. Although it is possible that transient changes in serum metabolites may occur immediately after transplantation of the fecal matter, no observable differences were present 9–10 days following the last FMT.

*Resveratrol-fed donor mice show few changes in fecal metabolites.* To investigate whether the observed physiological changes were mediated by the transfer of specific metabolites in the fecal matter of donor mice, we quantified potential metabolites through 1H-NMR spectroscopy. Given that administration of short-chain fatty acids (SCFAs) have shown to improve glucose homeostasis (12, 33), we quantified 23 SCFAs through NMR spectroscopy (Table 1). We found that the concentration of 4-hydroxyphenylacetate was significantly higher in the feces of resveratrol-fed donor mice ($n = 10$) compared with chow-fed donor mice ($n = 10$). Furthermore, the levels of formate, isobutyrate, and isovalerate were significantly lower in the feces of resveratrol-fed donor mice. Thus, the presence or lack of these potential SCFAs may be associated with the observed physiological benefits in obese Resv-FMT recipient mice.

*HK slurry from resveratrol-fed mice improve glucose homeostasis in obese mice.* To test whether live microbes were required for improved glucose homeostasis, a separate cohort of HFHS-fed mice underwent FMT using viable Resv-FMT or HK fecal slurry from Resv-fed mice. Consistent with our previous result (Fig. 2, *A* and *B*), Resv-FMT recipients ($n = 14$) showed improved glucose homeostasis (Fig. 4*A*). Although HK-FMT recipients ($n = 14$) showed improved glucose clearance at the 90- and 120-min time points, the comparable decrease in AUC was not statistically significant (Fig. 4, *B* and *C*). Of importance, these improvements in glucose homeostasis were not associated with changes in body weight (Fig. 4*D*), demonstrating that the effects were not secondary to weight loss. Although the fasted serum insulin levels appear slightly lower in both the Resv- and HK-FMT groups, the changes were not significant (Fig. 4*E*).

To determine if HK-FMT recipients showed different gut inflammatory profiles compared with Resv-FMT recipients, we measured the tissue cytokines of colon tissue. No significant differences were observed in the levels of TNF-α, CXCL1/KC, and IL-1β between Resv-FMT and HK-FMT recipients (data not

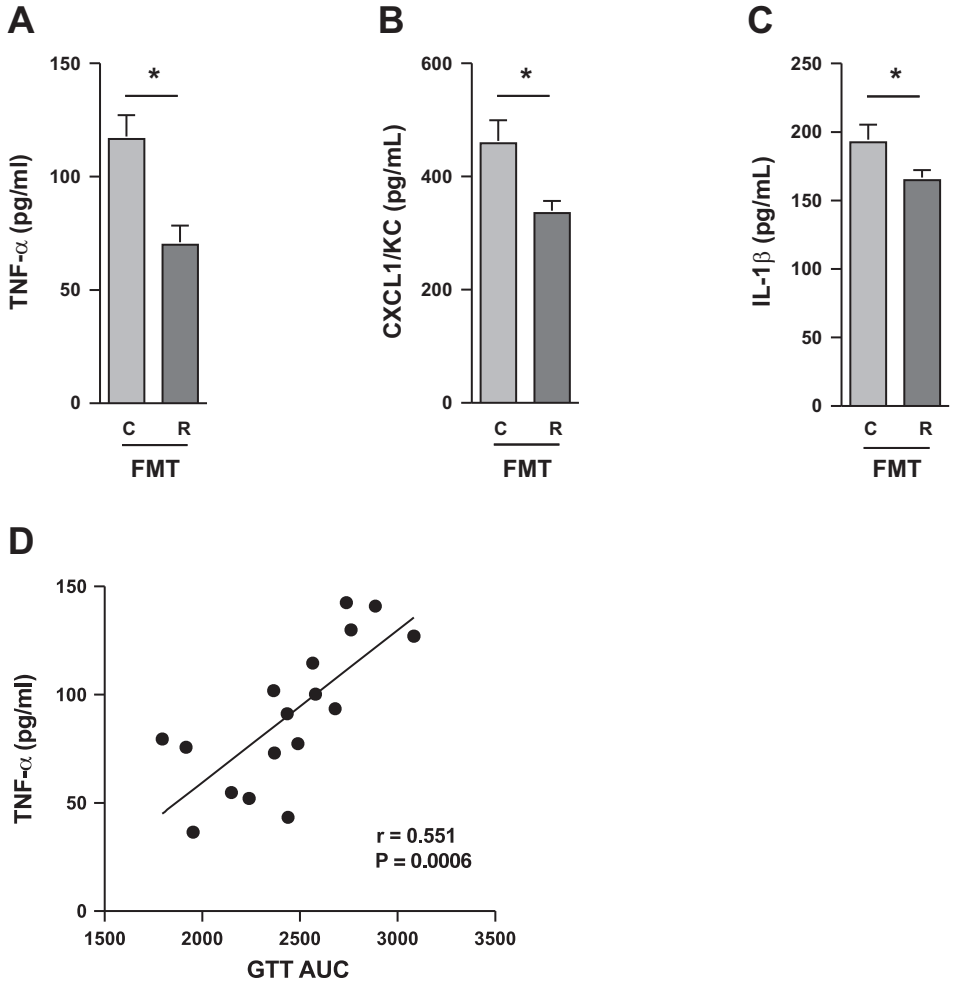


Fig. 3. Resveratrol-FMTs lower inflammatory cytokine levels in the colon of obese mice. *A–C*. Quantity of TNF-α (pg/ml; *A*), CXCL1/KC (*B*), and IL-1β (*C*) in mouse colon tissue 11 days after first FMT for control-FMT (C, $n = 7$) and Resv-FMT (R, $n = 10$) Linear regression of TNF-α quantity (pg/ml) on GTT AUC for all mice 11 days after first FMT (*D*). Values in *A–C* were analyzed by unpaired *t*-tests. AUC, area under the curve; C, control; CXCL1, chemokine (C-X-C motif) ligand 1; FMT, fecal microbiome transplant; GTT, glucose tolerance test; HFHS, high-fat, high-sucrose; P, pre-FMT; R, resveratrol; Resv, resveratrol.

shown). Although colonic cytokine levels in recipients of Resv-FMT were lower in comparison to control-FMT recipients (Fig. 3, *A–C*), these findings suggest that reduction in colonic inflammatory cytokines may be a contributing mechanism but not a prerequisite for improvements in glucose homeostasis.

*Resv-FMTs lower systolic blood pressure in mice with Ang-II-induced hypertension.* Given that the beneficial effects of oral supplementation of resveratrol has been observed in hypertension (11), we sought to elucidate the potential for Resv-FMTs to be used as a treatment for other symptoms of metabolic syndrome such as hypertension. Based on our previous mouse model of oral resveratrol treatment in Ang-II-induced hypertension (10), we subjected mice to FMT following 2 wk of Ang-II (1.4 $mg \cdot kg^{-1} \cdot day^{-1}$) infusion. Hypertensive mice receiving Resv-FMTs ($n = 13$) showed a significantly lower systolic blood pressure compared with mice receiving chow-FMTs ($n = 7$) (Fig. 5, *A* and *B*). These changes were observed in the absence of any differences in the baseline systolic blood pressure between mice receiving a saline or Ang-II infusion (Fig. 5*A*). Hypertensive mice receiving chow-FMTs had an increase in systolic blood pressure of 39.0%, whereas mice receiving Resv-FMTs show only an average of 11.7% increase during the 2-wk period (Fig. 5*C*). Because a physiological response to elevated blood pressure is cardiac hypertrophy, we also performed echocardiography along with gravimetric analysis of the hearts after euthanasia. Together with a lower systolic blood pressure compared with Ang-II-infused chow-FMT mice, echocardiographic measures of left ventricular (LV) hypertrophy (corrected LV mass; Fig. 5*D*), and LV mass/tibia length ratio (Fig. 5*E*) were lower in Resv-FMT recipients compared with chow-FMT recipients. These findings indicate that Resv-FMTs are sufficient to attenuate Ang-II induced high systolic blood pressure in hypertensive mice.

## DISCUSSION

This study demonstrates that transplantation of fecal matter from healthy Resv-fed donor mice is sufficient to improve glucose homeostasis during diet-induced obesity. Furthermore, these new findings suggest that FMTs from Resv-fed mice to obese mice is more efficacious than an oral supplementation of resveratrol during a 2-wk treatment period. Although resveratrol has protective benefits when administered orally before the onset of weight gain and subsequent impairments in glucose homeostasis (30), oral supplementation does not appear to rescue mice when administered as a treatment (i.e., after the onset of weight gain and insulin resistance). This is in agreement with most studies that have outlined the insulin-sensitizing effect of resveratrol, because the partial rescue of insulin signaling at a molecular level did not translate to physiological

Table 1. *Univariate analysis of fecal metabolites from resveratrol-fed mice*

| Metabolite | Resv, $n = 10$ | | Chow, $n = 10$ | | $P$ value | $q$ value (FDR) | Fold Change | Resv/Chow |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | | | | |
| 4-Hydroxyphenylacetate | 17.730 | 5.925 | 9.820 | 2.104 | 0.0021 | 0.0159 | 1.81 | Up |
| Acetate | 496.370 | 248.243 | 552.030 | 315.655 | 0.5787 (W) | 0.6406 | −1.11 | Down |
| Butyrate | 52.340 | 21.547 | 73.890 | 31.600 | 0.0961 (W) | 0.2686 | −1.41 | Down |
| Choline | 3.070 | 1.250 | 2.420 | 0.735 | 0.1733 | 0.3624 | 1.27 | Up |
| Creatine | 3.420 | 2.808 | 3.810 | 1.945 | 0.3845 (W) | 0.5896 | −1.11 | Down |
| Dimethyl sulfone | 1.350 | 1.072 | 1.060 | 0.813 | 0.5041 | 0.6406 | 1.27 | Up |
| Dimethylamine | 12.810 | 8.218 | 8.900 | 6.345 | 0.4270 (W) | 0.5897 | 1.44 | Up |
| Formate | 73.980 | 5.162 | 96.060 | 31.196 | 0.0015 (W) | 0.0159 | −1.30 | Down |
| Fumarate | 0.640 | 0.222 | 0.900 | 0.668 | 0.5659 (W) | 0.6406 | −1.41 | Down |
| Glucose | 794.050 | 203.554 | 521.260 | 199.543 | 0.0073 | 0.0399 | 1.52 | Up |
| Glycerol | 831.340 | 1,155.859 | 397.090 | 516.145 | 0.4359 (W) | 0.5897 | 2.09 | Up |
| Hypoxanthine | 28.330 | 16.690 | 20.260 | 7.555 | 0.2897 (W) | 0.5126 | 1.40 | Up |
| Isobutyrate | 11.760 | 3.387 | 24.840 | 7.439 | 0.0002 | 0.0055 | −2.11 | Down |
| Isovalerate | 7.250 | 2.514 | 11.850 | 4.254 | 0.0087 | 0.0399 | −1.63 | Down |
| Lactate | 64.420 | 42.441 | 35.780 | 20.595 | 0.0771 | 0.2532 | 1.80 | Up |
| Malonate | 10.150 | 7.465 | 22.920 | 16.634 | 0.1051 (W) | 0.2686 | −2.26 | Down |
| Methylamine | 5.570 | 2.437 | 3.880 | 1.277 | 0.0679 | 0.2532 | 1.44 | Up |
| Propionate | 126.300 | 40.005 | 107.130 | 24.254 | 0.2114 | 0.4052 | 1.18 | Up |
| Pyruvate | 6.950 | 4.081 | 7.960 | 4.039 | 0.5849 | 0.6406 | −1.15 | Down |
| Succinate | 18.830 | 14.917 | 22.630 | 22.583 | 0.9397 (W) | 0.9397 | −1.20 | Down |
| Uracil | 15.820 | 11.872 | 11.810 | 3.906 | 0.3322 | 0.5458 | 1.34 | Up |
| Valerate | 33.360 | 11.843 | 41.020 | 11.295 | 0.1561 | 0.3591 | −1.23 | Down |
| Xanthine | 9.360 | 6.727 | 9.500 | 6.540 | 0.9097 (W) | 0.9397 | −1.01 | Down |

Fecal metabolites of chow- or Resv-fed mice ($n = 10$/group). Using MetaboAnalyst, univariate analysis of fecal metabolites was performed by unpaired two-tailed Student's $t$-test and Wilcoxon Mann-Whitney test [$P$ values calculated with this method are indicated with "(W)"]. To consider the corrections needed for multiple comparisons, FDR $q$ values were calculated. Chow, chow-fed mice; FDR, false discovery rate; Resv, resveratrol-fed mice.

improvements in glucose homeostasis, such as improved glucose clearance during a GTT (9, 17).

Another indicator of the Resv-FMT-mediated improvements in glucose homeostasis was observed through a lower GTT curve and smaller AUC when compared with pre-FMT and control-FMT recipients (Fig. 2, *A* and *B*). These changes were observed in the absence of any significant differences between serum insulin levels of chow and Resv-FMT recipients (Fig.

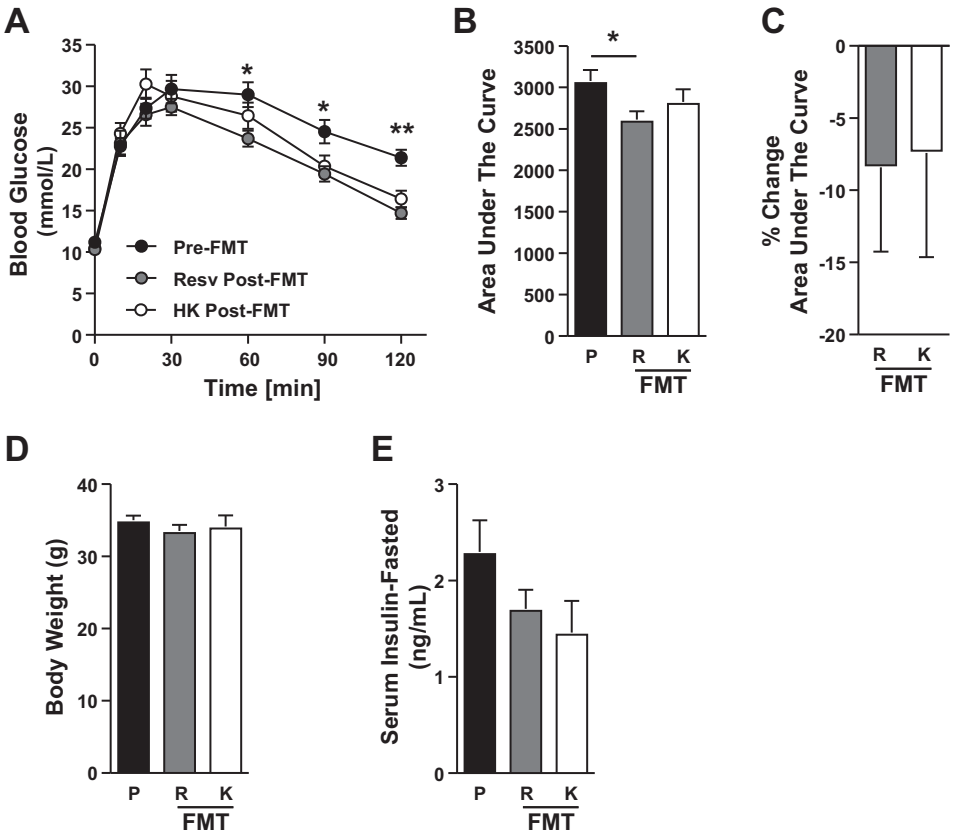


Fig. 4. Heat-killed resveratrol slurry is sufficient to improve glucose homeostasis in obese mice. *A*: GTTs of HFHS-fed mice at baseline before receiving FMTs (Pre-FMT; $n = 28$) and after randomization to receiving an FMT from resveratrol-fed mice, either Resv-FMT (Resv Post-FMT; $n = 14$) or heat-killed Resv-FMT (HK post-FMT; $n = 14$). *B*: glucose clearance represented by the AUC of the GTTs from HFHS-fed mice before FMT (P, $n = 28$) and after FMT from Resv-FMT (R, $n = 14$) and heat-killed Resv-FMT (K, $n = 14$). *C*: percent change in AUC of the GTTs following FMT from R ($n = 14$) and K ($n = 14$). *D*: body weight in all groups of mice. *E*: serum insulin levels following a 5- to 6-h fast. Values in *A*–*E* are shown as means ± SE *$P < 0.05$; analyzed by two-way ANOVA with Tukey's post hoc test in *A* and analyzed by one-way ANOVA with Tukey's post hoc test in *B*, *D*, and *E*. Data in *C* were analyzed by unpaired $t$-tests. AUC, area under the curve; C, control; FMT, fecal microbiome transplant; GTT, glucose tolerance test; HFHS, high-fat, high-sucrose; HK, heat-killed; K, heat-killed; R, Resv-FMT; P, pre-FMT; Resv, resveratrol.

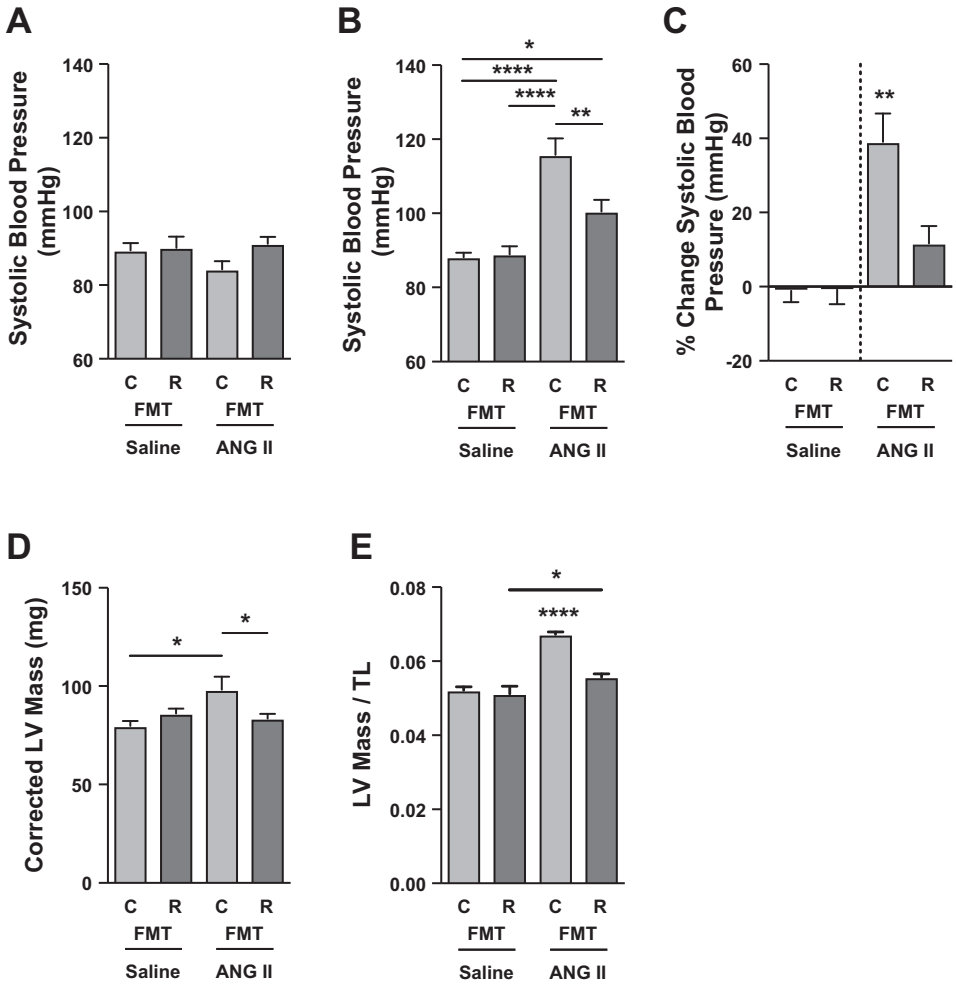


Fig. 5. Resv-FMTs in hypertensive mice are sufficient to reduce systolic blood pressure. Systolic blood pressure (mmHg) before (*A*) and after (*B*) receiving saline or angiotensin II (ANG II) infusion, and treatment with control-FMT (C) or Resv-FMT (R). Chow-saline, $n = 8$; Resv-saline, $n = 6$; chow-ANG-II, $n = 7$; Resv-ANG-II, $n = 13$. Percent change in the systolic blood pressure (*C*). Corrected left ventricular (LV) mass (*D*). LV mass-to-tibia length (mass/TL) ratio (*E*). Values in *A*–*E* are shown as means ± SE*$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$, and ****$P < 0.0001$; analyzed by one-way ANOVA with Tukey's post hoc test in *A*–*E*. FMT, fecal microbiome transplant; LV, left ventricular; Resv, resveratrol; TL, tibia length.

2*D*). Furthermore, we observed that HK Resv-FMTs had similar effects as Resv-FMTs, which also showed a significantly lower glucose level at the end point of the experiment. This suggests that improved peripheral tissue glucose handling in Resv-FMT and HK Resv-FMT mice may be attributed to an increased responsiveness to insulin. However, when we analyzed potential changes in signaling of proteins involved in insulin sensitivity and glucose regulation, we observed no significant differences in the expression or activity of Akt, AMPK, or sirtuin 1 (data not shown). Because the improvements in glucose homeostasis were not accompanied by changes in protein expression and activity, the mechanisms involved in these beneficial effects of Resv-FMT are currently unknown. This presents a limitation to our study, which may be addressed through future studies.

To investigate the mechanisms of how Resv-FMT improves glucose homeostasis during obesity, we examined whether levels of inflammatory cytokines were different between the two treatment groups. Resv-FMT recipients showed a significantly lower level of TNF-α in the colon, and there was a strong positive correlation between the AUC of GTT and levels of TNF-α in the colon tissue following FMTs for both groups. Likewise, the levels of murine IL-8 homologue CXCL1/KC and IL-1β were significantly lower in Resv-FMT mice, which are also proinflammatory cytokines involved in the pathogenesis of metabolic syndrome (24). These findings are in agreement with the growing body of evidence, which suggest that low-grade inflammation attributed to dysbiosis can induce obesity, impair insulin function and contribute to further metabolic dysfunction (5). More importantly, the reduction of these inflammatory cytokines was associated with improvements in glucose homeostasis, suggesting that reducing gut inflammation may play a role in Resv-FMT rescuing impaired glucose homeostasis during obesity. However, the degree to which gut inflammation correlates with improvements in glucose homeostasis has yet to be fully elucidated.

In addition to elucidating the mechanism of action of Resv-FMTs, another aim of this study was to determine whether intact microbes from the feces of Resv-fed mice were required to impart the beneficial effects of Resv-FMTs in obese and insulin-resistant mice. Although initial findings associated the beneficial effects of Resv-FMTs with changes in the microbial composition (30), the data presented herein indicate that improvements in glucose homeostasis in obese mice can occur even in the absence of transferring live gut microbes during FMT. Indeed, we show that HK slurry from Resv-fed donor mice show a comparable improvement in the glucose homeostasis of obese mice. Therefore, it is highly possible that nonliving microorganisms, metabolites, or other components within the feces of Resv-fed mice may be responsible for the

beneficial effects of Resv-FMTs, which is consistent with previous studies (1a, 25, 26).

Because we did not observe any significant changes in signaling proteins associated with glucose homeostasis, we analyzed serum metabolites in FMT recipient mice as a potential explanation of the observed physiological improvements. Newfound evidence also suggests that changes in the gut microbiome are associated with changes in the metabolic profiles (14, 19). Of the 147 serum metabolites we analyzed, no significant differences in individual metabolites or metabolic profiles were observed in the recipient mice (data not shown). Because our previous findings indicated 10 specific metabolites were considered major contributors to the difference in metabolic profiles (30), these new findings suggest that the beneficial effects of fecal transplants are not attributed to changes in the metabolic profile of the recipients. When we quantified SCFAs in the fecal matter of donor mice, we did not observe major changes in known SCFAs, such as butyrate, that have been shown to improve glucose homeostasis in obese mice (12, 32, 33). However, we found that the concentration of 4-hydroxyphenylacetate was significantly higher in the feces of Resv-fed donor mice compared with chow-fed donor mice. In fact, a previous study has suggested that 4-hydroxyphenylacetate [a microbial product of tyrosine degradation (16)] may be involved in improving insulin sensitivity (22). Overall, we can conclude that the delivery of butyrate does not appear to be involved in the ability of Resv-FMT to improve glucose homeostasis in obese mice; however, the role that elevated levels of 4-hydroxyphenylacetate in the feces plays in the observed physiological effects cannot be discounted.

Another symptom associated with obesity and insulin resistance is hypertension, which can also contribute to systemic inflammation in addition to its impact on cardiac and vascular remodeling (15). Similar to the findings that underscore the role of dysbiosis in insulin resistance, gut pathology has also been identified in the context of hypertension (28). Consistent with our previous work using 2-wk oral resveratrol treatment (11), FMTs from Resv-fed mice to hypertensive mice showed significantly lower systolic blood pressure as well as a lower corrected LV mass and a lower LV mass/tibia length ratio when compared with chow-FMT recipients. Although it is unclear whether dysbiosis precedes the progression of hypertension, alterations in the gut microbiome have also been shown to associate with changes in sympathetic nervous system activity and the renin angiotensin aldosterone system of the gut (28). These data suggest that, in addition to the beneficial effect on glucose homeostasis, Resv-FMTs are also sufficient to attenuate high systolic blood pressure in a mouse model of hypertension.

Overall, given that resveratrol supplementation has multifaceted beneficial effects that range from antimicrobial and anti-inflammatory, to activating various signaling pathways (31), it is unlikely that the beneficial effects of FMTs from Resv-fed mice are attributed to one specific physiological or molecular mechanism. However, we provide evidence that Resv-FMTs are equally effective in addressing impaired glucose homeostasis during obesity as well as hypertension. Together, these data improve our understanding of the mechanism of resveratrol, and further shed light on the potential for Resv-FMTs to be used as a metabolic therapy that can treat symptoms underlying the metabolic syndrome.

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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

T.T.K., N.P., M.M.S., and J.R.B.D. conceived and designed research; T.T.K., N.P., M.M.S., S.C.B., J.L., C.-L.M.S., and D.S.W. performed experiments; T.T.K., N.P., M.M.S., D.S.W., and J.R.B.D. analyzed data; T.T.K., N.P., M.M.S., and J.R.B.D. interpreted results of experiments; T.T.K., N.P., and J.R.B.D. prepared figures; T.T.K., K.M., J.D.S., and J.R.B.D. drafted manuscript; T.T.K., N.P., M.M.S., K.M., J.D.S., and J.R.B.D. edited and revised manuscript; T.T.K. and J.R.B.D. approved final version of manuscript.

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