The gut microbiome regulates host glucose homeostasis via peripheral serotonin

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The gut microbiome is an established regulator of aspects of host metabolism, such as glucose handling. Despite the known impacts of the gut microbiota on host glucose homeostasis, the underlying mechanisms are unknown. The gut microbiome is also a potent mediator of gut-derived serotonin synthesis, and this peripheral source of serotonin is itself a regulator of glucose homeostasis. Here, we determined whether the gut microbiome influences glucose homeostasis through effects on gut-derived serotonin. Using both pharmacological inhibition and genetic deletion of gut-derived serotonin synthesis, we find that the improvements in host glucose handling caused by antibiotic-induced changes in microbiota composition are dependent on the synthesis of peripheral serotonin.

microbiome | glucose homeostasis | microbe–host interactions | serotonin

Intervention studies using germ-free (GF) mice or antibiotic-associated microbiota perturbation have demonstrated a causal role of the gut microbiome in regulating host metabolism (1–4). Treatment of mice with antibiotics improves host glucose tolerance, and reduces fat mass and obesity (5, 6), while colonization of GF mice with microbiota from obese mice (1) and humans (4) conveys glucose intolerance in the host. How this occurs remains unknown. Resident cells within the gut wall are leading candidates, as their location enables them to convey microbial signals to the host. Approximately 90% of total body serotonin (5-hydroxytryptophan [5-HT]) is synthesized in nonneuronal cells lining the gut wall, called enterochromaffin (EC) cells (7). The gut microbiome signals to EC cells through microbial metabolites, including short-chain fatty acids and secondary bile acids (8–10), with mucosal 5-HT substantially reduced in GF and antibiotic-treated mice due to decreased EC cell numbers and reduced 5-HT biosynthesis (10). EC cells provide all circulating 5-HT via the rate-limiting enzyme of nonneuronal 5-HT synthesis, tryptophan hydroxylase 1 (TPH1). The 5-HT regulates glucose homeostasis (11–13), with depletion conveying protection against diet-induced obesity and glucose intolerance (14). Circulating 5-HT is also increased in obese humans (15). In this study, we assessed the host metabolic profile, including glucose homeostasis, of mouse models of antibiotic (Abx)-associated microbiota perturbation in combination with either genetic or pharmacological 5-HT depletion to determine whether the gut microbiome affects host metabolism through its effects on gut 5-HT.

An intraperitoneal (i.p.) glucose tolerance test (IPGTT) was used to examine the links between peripheral 5-HT and the gut microbiome on host glucose homoeostasis. Comparisons within the same animal over time were used to remove potentially confounding interanimal variance. Glucose tolerance was unchanged after 28 d in vehicle-treated control mice (Fig. 1*A*) but improved significantly in mice treated with LP533401 (Fig. 1*B*), Abx (Fig. 1*C*), or combined LP533401 and Abx treatment (Fig. 1*D*). Importantly, these positive effects of inhibiting 5-HT synthesis and antibiotic-associated microbiota perturbation on glucose tolerance were not additive, as seen using paired comparisons within each mouse over time (Fig. 1*E*), demonstrating their interdependence. Importantly, all treatments had similar effects in reducing both serum (Fig. 1*F*) and colonic mucosal (Fig. 1*G*) 5-HT levels. The effects of 5-HT inhibition and antibiotic-associated microbiota perturbation on glucose homeostasis are not due to differences in energy expenditure (Fig. 1*H*), substrate use (Fig. 1*I*), activity (Fig. 1*J*), or body weight (Fig. 1*K*).

In mice fed a control chow diet, the genetic deletion of Tph1 ($Tph1^{-/-}$) improved glucose clearance compared to controls ($Tph1^{+/+}$) (Fig. 2 *A* and *B*). This effect differed from previous results in which there was no difference in glucose tolerance between chow-fed $Tph1^{-/-}$ and $Tph1^{+/+}$ mice and was likely due to the greater glucose load (4 mg/kg compared to 2 mg/kg; ref. 14) and shorter fasting period (4 h compared to 24 h; ref. 16). Importantly, 28 d of Abx treatment improved glucose clearance in $Tph1^{+/+}$ but not $Tph1^{-/-}$ mice (Fig. 2 *A* and *B*). There was no difference in insulin sensitivity between groups before or after treatment with Abx (Fig. 2*C*). No difference in energy expenditure (Fig. 2*E*), substrate use (Fig. 2*F*), physical activity (Fig. 2*G*), or body weight (Fig. 2*H*) was observed between $Tph1^{+/+}$ and $Tph1^{-/-}$ mice treated with Abx.

The outcomes of our study address a question which has long been unanswered. We used genetic and pharmacological models to provide evidence that gut-derived serotonin is the link through which the gut microbiome affects host glucose metabolism. The key evidence supporting this conclusion is that the combination of depleted EC cell 5-HT and gut antibiotic-associated microbiota perturbation did not show any additive effect compared to the individual treatments alone, demonstrating that the gut microbiome and EC cell 5-HT act via the same pathway to influence host glucose metabolism. If the microbiome were regulating host glucose homeostasis via another route, we would have

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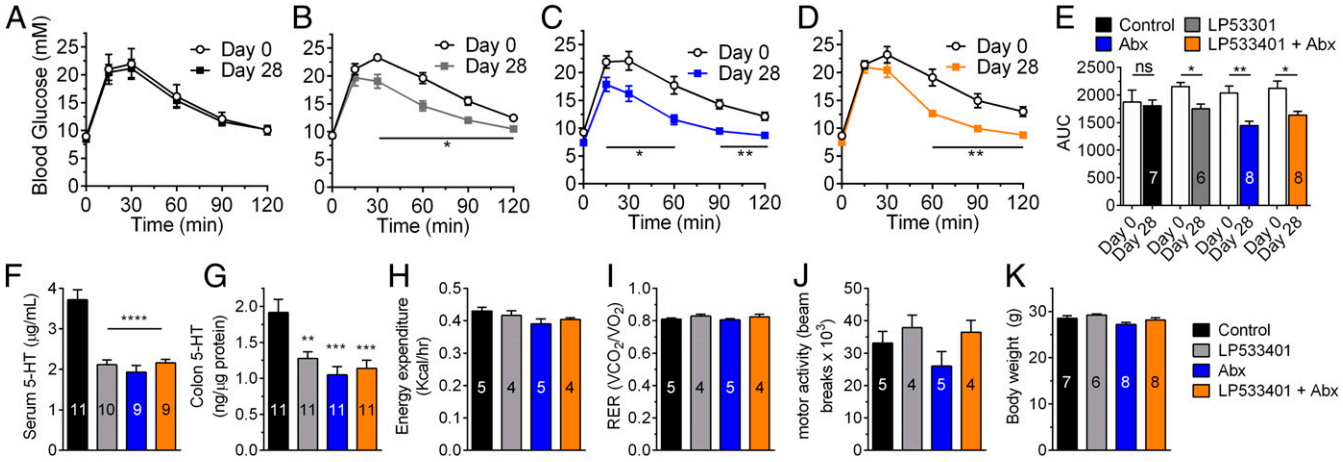

Fig. 1. Antibiotics improve host glucose handling by reducing gut-derived 5-HT. Paired day 0 and day 28 comparisons of blood glucose, following an IPGTT, in (*A*) control, (*B*) LP533401-treated, (*C*) Abx-treated, and (*D*) combined LP533401- and Abx-treated mice. (*E*) Paired comparisons of GTT area under the curve (AUC) at day 0 and day 28. (*F*) Serum and (*G*) colonic 5-HT levels at day 28. (*H*) Energy expenditure, (*I*) respiratory exchange ratio (RER), (*J*) horizontal motor activity, and (*K*) body weight at day 28. Data are shown as mean ± SEM; ns, not significant; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. Numbers in bars are individual mice per group.

seen improved glucose tolerance in the LP533401 + Abx group compared to Abx alone or in the Tph1$^{-/-}$ day 0 vs. day 28 comparisons. These impacts of antibiotic-associated microbiota perturbation in the presence or absence of gut-derived 5-HT on glucose handling are not driven by potentially confounding factors such as altered basal energy expenditure, physical activity, substrate use, or body weight. This is consistent with previous findings from mice with gut-specific *Tph1* ablation, which exhibit no change in energy expenditure compared to wild-type (WT) mice (17), and with other studies using LP533401 on a standard chow diet (17, 18), which show no change in total body weight. Our finding is indicative that, on a control chow diet, the gut microbiome controls glucose homeostasis through regulation of EC cell serotonin synthesis.

Methods

Animal Housing and Breeding. Male C57BL/6 were bred and maintained at the South Australian Health and Medical Research Institute, and Tph1$^{+/+}$ and Tph1$^{-/-}$ mice (14, 19) were maintained at McMaster University (both under specific-pathogen–free conditions). All mice were fed water and standard chow diet ad libitum and were housed under 12-h light–dark cycle. Experiments were performed in accordance with the South Australian Health and Medical Research Institute and the McMaster Animal Care Committee (Animal Utilization Protocol 16-12-41) guidelines.

Animal Experiments. At 8 wk to 12 wk of age, mice were randomized and separated into groups and placed on an antibiotic mixture (1 g/L ampicillin, 0.5 g/L neomycin; Sigma) to induce a dysbiosis model (20, 21) or on control water for 28 d. Mice were weighed daily in the morning. Mice in the TPH inhibitor group received a daily oral gavage of LP533401 (30 mg/kg; Dalton Pharma Services) as previously described (22), while control mice received a vehicle gavage.

Glucose Tolerance Test. Mice were fasted for a period of 4 h by housing in bedding- and feed-free cages with fasting trays. Baseline fasting blood glucose was measured via tail vein bleed. Glucose (4 g/kg; Tph1$^{+/+}$ and Tph1$^{-/-}$, 2 g/kg; C57/BL6) was administered via i.p. injection, and blood glucose levels were taken at 15-min intervals for 2 h.

Metabolic Cage Analysis. Animals were placed in Promethion (C57/BL6 mice; Sable Systems International) and Comprehensive Lab Animal Monitoring

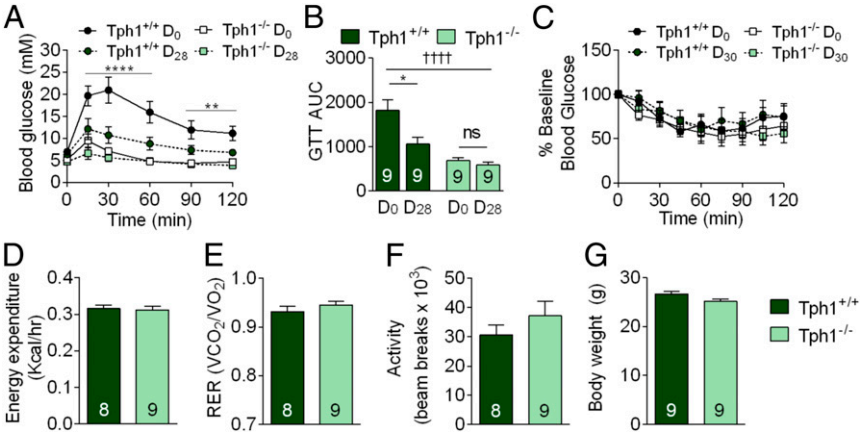

Fig. 2. Antibiotic-induced improvements in glucose tolerance require Tph1. (*A*) Paired day 0 and day 28 blood glucose and (*B*) area under the curve following i.p. glucose injection (4 mg/kg) in Tph1$^{+/+}$ and Tph1$^{-/-}$mice. (*C*) Paired day 0 and day 30 blood glucose following i.p. insulin injection (0.75 U/kg) in Tph1$^{+/+}$ and Tph1$^{-/-}$mice. Mean (*D*) energy expenditure, (*E*) RER, and (*F*) horizontal motor activity measured from days 32 to 34. (*G*) Body weight measured at day 36. Data are shown as mean ± SEM; **P* < 0.05; ***P* < 0.01; *****P* < 0.0001; ††††*P* < 0.001 genotype. Numbers in bars are individual mice per group.

System (Tph1 WT and Tph1 knockout mice; Columbus Instruments) metabolic cages to assess oxygen consumption, carbon dioxide emission, and resting energy expenditure. Mice were acclimatized over a 24-h period before data were drawn.

The 5-HT Measurements. Serum and colonic mucosal tissue serotonin was detected by ELISA (BA E-5900; Labor Diagnostika Nord) as previously described (15, 23).

Statistical Analysis. Data were analyzed using PRISM (GraphPad, v7.0). Statistical significance was reported when $P < 0.05$ and was determined using a paired 2-tailed *t* test for single comparisons, or 1-way ANOVA and Tukey's post hoc test for multiple comparisons.

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1. P. J. Turnbaugh *et al*., An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–1031 (2006).
2. F. Bäckhed *et al*., The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 15718–15723 (2004).
3. A. Vrieze *et al*., Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* **143**, 913–916.e7 (2012).
4. V. K. Ridaura *et al*., Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* **341**, 1241214 (2013).
5. F. Bäckhed, J. K. Manchester, C. F. Semenkovich, J. I. Gordon, Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 979–984 (2007).
6. B. M. Carvalho *et al*., Modulation of gut microbiota by antibiotics improves insulin signalling in high-fat fed mice. *Diabetologia* **55**, 2823–2834 (2012).
7. V. Erspamer, Pharmacology of indole-alkylamines. *Pharmacol. Rev.* **6**, 425–487 (1954).
8. A. M. Martin, E. W. Sun, G. B. Rogers, D. J. Keating, The influence of the gut microbiome on host metabolism through the regulation of gut hormone release. *Front. Physiol.* **10**, 428 (2019).
9. A. M. Martin *et al*., "Cellular regulation of peripheral serotonin" in *Serotonin*, P. M. Pilowsky, Ed. (Academic Press, Boston, MA, 2019), pp. 137–153.
10. J. M. Yano *et al*., Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* **161**, 264–276 (2015).
11. A. M. Martin *et al*., The diverse metabolic roles of peripheral serotonin. *Endocrinology* **158**, 1049–1063 (2017).
12. J. M. Yabut *et al*., Emerging roles for serotonin in regulating metabolism: New implications for an ancient molecule. *Endocr. Rev.* **40**, 1092–1107 (2019).
13. R. L. Young, A. L. Lumsden, D. J. Keating, Gut serotonin is a regulator of obesity and metabolism. *Gastroenterology* **149**, 253–255 (2015).
14. J. D. Crane *et al*., Inhibiting peripheral serotonin synthesis reduces obesity and metabolic dysfunction by promoting brown adipose tissue thermogenesis. *Nat. Med.* **21**, 166–172 (2015).
15. R. L. Young *et al*., Augmented capacity for peripheral serotonin release in human obesity. *Int. J. Obes.* **42**, 1880–1889 (2018).
16. N. Paulmann *et al*., Intracellular serotonin modulates insulin secretion from pancreatic beta-cells by protein serotonylation. *PLoS Biol.* **7**, e1000229 (2009).
17. G. Sumara, O. Sumara, J. K. Kim, G. Karsenty, Gut-derived serotonin is a multifunctional determinant to fasting adaptation. *Cell Metab.* **16**, 588–600 (2012).
18. V. K. Yadav *et al*., Pharmacological inhibition of gut-derived serotonin synthesis is a potential bone anabolic treatment for osteoporosis. *Nat. Med.* **16**, 308–312 (2010).
19. F. Côté *et al*., Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13525–13530 (2003).
20. P. D. Cani *et al*., Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* **57**, 1470–1481 (2008).
21. M. A. Lynn *et al*., (2018) Early-life antibiotic-driven dysbiosis leads to dysregulated vaccine immune responses in mice. *Cell Host Microbe* **23**, 653–660.e5.
22. C. M. Oh *et al*., Regulation of systemic energy homeostasis by serotonin in adipose tissues. *Nat. Commun.* **6**, 6794 (2015).
23. A. M. Martin *et al*., The nutrient-sensing repertoires of mouse enterochromaffin cells differ between duodenum and colon. *Neurogastroenterol. Motil.* **29**, e13046 (2017).