Brief Communication Title: Exercise training modifies gut microbiota in normal and diabetic mice

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ABSTRACT

Cecal microbiota from Type 2 diabetic (*db/db*) and control (*db/*$^{+}$) mice was obtained following 6 weeks of sedentary or exercise activity. qPCR analysis revealed a main effect of exercise, with greater abundance of select Firmicutes species and lower *Bacteroides/Prevotella* spp. in both normal and diabetic exercised mice compared to sedentary counterparts. Conversely, *Bifidobacterium* spp. was greater in exercised normal but not diabetic mice (exercise x diabetes interaction). How exercise influences gut microbiota requires further investigation.

Keywords: microbiota, diabetes, exercise

Introduction

A complex and reciprocal relationship exists between the gut microbiota and whole body energy metabolism. The gut microbial phenotype is determined by the interaction of genetic and environmental factors, including disease state and lifestyle behaviors. For example, obese individuals may exhibit a greater proportion of Firmicutes and lower *Bacteroidetes* (Zhang et al. 2013; Ley et al. 2006); however, this is not uniformly observed (Duncan et al. 2008), and likely reflects the complex spectrum of metabolic health. Other diseases associated with obesity also exhibit dysbiosis – for example, type 2 diabetes is associated with alterations in the abundance of certain microbial species (e.g. increase in *Lactobacillus* species and reduction in *Roseburia*); this microbial signature may coincide with metabolic functions such as enhanced sugar and branched chain amino acid transport, reduced butyrate production, and increased oxidative stress, as assessed by metagenomic analysis (Tilg and Moschen 2014). Fortunately, lifestyle changes resulting in weight loss can modify the microbial profile, such that obese individuals more closely resemble lean humans (Ley et al. 2006).

Exercise is a front-line and cost-effective treatment for metabolic diseases. A recent study in elite rugby players suggests that athletes have greater gut microbial diversity compared to sedentary individuals (Clarke et al. 2014); this greater diversity is generally associated with better metabolic health (Le Chatelier et al. 2013). Therefore, the benefits of exercise on metabolic systems may be mediated in part by effects on the gut microbiota; however, whether and how exercise beneficially modifies the microbial

profile is unclear. The purpose of this study was to determine if exercise influences the gut microbial profile in normal and diabetic mice.

Materials and Methods

Ethical approval was granted by the University of Calgary Animal Care Committee and conforms to the Canadian Council for Animal Care. Six-week-old male type 2 diabetic *db/db* (C57BL/KsJ-lepr*db*/lepr*db*) and *db/*$^{+}$ (heterozygote; control) littermates (Jackson Laboratories; Bar Harbor, ME) were randomized to sedentary (Sed) or exercise (Ex) groups for 6 weeks (n= 9-10/treatment). db/db mice have mutant copies of the leptin receptor gene and exhibit the type 2 diabetic obese phenotype from an early age, whereas the lean littermates (db/+) have one mutant copy and are normal non-diabetic animals (Belke et al. 2000; Belke et al. 2004; Hummel et al. 1966). Mice were maintained on a 12:12hr light-dark cycle, housed 4-5 per cage within the same treatment group, and consumed standard chow (Purina, Richmond, IN; #5020). At sacrifice, a tail blood sample was obtained for glucose measurement via glucose meter (Bayer Diagnostics, Whippany, NJ). Mice were euthanized with ~0.5–0.8 mL of sodium pentobarbital followed by cardiac puncture.

Exercise Training

Exercise consisted of low-intensity treadmill running (Lafayette Instruments, Lafayette, IN) 5 days/week. Exercise speed was chosen relative to genotype and based on the physical capacity of the mice (the *db/db* mice were obese and had deterioration of

muscle mass), such that both groups received a moderate-to-high intensity exercise stimulus. *Db/*$^{+}$ mice exercised for 60 min/session at 4.79 m/min (287m/session) and *db/db* mice for 66 min/session at 2.87 m/min (189m/session). Exercise was ceased for 48h and mice were not fasted prior to sacrifice.

Gut Microbiota Analysis

Cecal matter was collected from individual mice at sacrifice and stored at -80ºC. Total bacterial DNA was extracted using FastDNA Spin Kit for Feces (MP Biomedicals, Lachine, QC) and quantified using the Nanodrop 2000 (Thermo Fisher Scientific, Asheville, NC). Microbiota were quantified using quantitative PCR (qPCR) with SYBR Green, an iCycler (BioRad Inc., Mississauga, ON) and group specific primers according to our previous protocols (Bomhof et al. 2014; Parnell and Reimer 2012). Primers were chosen to represent members of the major phyla of the murine gut microbiome, similar in many respects to the human gut microbiome, to provide a broad coverage of the total microbial signal (Ley et al. 2005; Huttenhower and Human Microbiome Project Consortium 2012). Purified template DNA from the reference strains (ATCC, Manassas, VA) was used to generate standard curves for each primer set using 10-fold serial dilutions of DNA. Standard curves were normalized to copy number of 16S rRNA genes as previously described (Bomhof et al. 2014). Data is expressed as log10 16S rRNA gene copies per mg cecal material.

Statistical Analysis

Data were analyzed with SPSS (v.21; IBM Software, Armonk, NY) by 2-way ANOVA with testing for simple effects to determine the main effects of exercise and diabetic state and their interaction, as well as significant differences between groups, respectively. ANCOVA analysis was also performed, using body weight and fasting glucose as covariates. Values are presented as mean ± SE.

Results

As expected, diabetic mice were heavier at sacrifice (Sed-*db/db* 57.6±1.5g and Ex-*db/db* 56.7±1.4g) than *db/*$^{+}$ littermates (Sed-*db/*$^{+}$ 34.9±0.6g and Ex-*db/*$^{+}$ 31.8±1.1g) ($P<0.001$), and had higher blood glucose ($P< 0.001$). However, there was a trend towards an interaction between diabetic state and exercise training ($P=0.070$), such that glucose was higher in Ex-*db/*$^{+}$ than Sed-*db/*$^{+}$ (12.9±1.9 vs. 8.1±0.6 mmol/L), but similar in Ex-*db/db* compared to Sed-*db/db* (20.4±2.4 vs. 21.9±1.7 mmol/L), a finding we have previously observed in the *db/db* model (Shearer et al. 2011).

Gut Microbiota

The interaction between diabetic state and exercise training affected the cecal abundance of total bacteria, *Enterobacteriaceae* and *Bifidobacterium* spp. (**Table 1**). Specifically, total bacteria and *Enterobacteriaceae* were similar in *db/*$^{+}$ mice regardless of exercise, but lower with exercise in *db/db* mice. *Bifidobacterium* spp. was greater in exercised non-diabetic mice, but the presence of diabetes negated this effect, such that abundance was lower with exercise in *db/db* mice. Diabetes was independently

associated with greater abundance of *Clostridium* cluster XI. Exercise was independently associated with lower abundance of *Bacteroides/Prevotella* spp. and *Methanobrevibacter* spp., and greater *Lactobacillus* spp. and *Clostridium leptum*. Both diabetes and exercise affected *Clostridium* cluster I, with greater abundance in exercised animals and lesser in diabetic mice.

Adjusting for body weight and blood glucose

Because obesity and glucose intolerance are associated with microbiome aberrations (Zhang et al. 2013; Ley et al. 2006), we performed ANCOVA analysis, adjusting for body weight and blood glucose. After adjustment, most of the differences attributed to exercise remained significant, specifically lower *Bacteroides/Prevotella* spp. (P=0.015) and *Methanobrevibacter* spp. (P<0.001), and higher *Clostridium* cluster I (P=0.001). Similarly, the differential pattern of *Bifidobacterium* spp. and *Enterobacteriaceae* between exercise-trained *db/*$^{+}$ and *db/db* mice remained significant (P<0.001 and P=0.027, respectively). The higher abundance of *Lactobacillus* spp. (P=0.082) and *Clostridium leptum* (P=0.075) in exercise-trained *db/*$^{+}$ and *db/db* animals were numerically but not significantly different.

Discussion

The field of exercise and microbiota is in its infancy, with limited reports to date. Here, we found that exercise affected more cecal microbial groups (*Bacteroides/Prevotella*,

Methanobrevibacter, and *Lactobacillus* spp., and *Clostridium* leptum and cluster I) than did diabetes alone (*Clostridium* clusters I and XI).

The effect of exercise on gut microbiota is conflicting. In the limited studies available, exercise in normal rats was associated with higher Bacteroidetes and lower Firmicutes in fecal matter (Evans et al. 2014; Queipo-Ortuño et al. 2013). These phyla differences might be simplistically interpreted as positive based on human studies describing this pattern as beneficial, however the data are conflicting and the field is rapidly moving towards species-level profiling with pyrosequencing to further our understanding of phylum level shifts (Zhang et al. 2013; Ley et al. 2006; Geurts et al. 2011). Conversely, the opposite was observed in exercised mice exposed to polychlorinated biphenyls (Choi et al. 2013). The most compelling insight comes from recent observational work comparing the fecal bacterial profile of male elite rugby players to healthy subjects (Clarke et al. 2014). Surprisingly, athletes had lower Bacteroidetes and greater Firmicutes than controls, similar to our own observations in exercised mice. These observations are intriguing, considering that lower Bacteroidetes and greater Firmicutes are sometimes observed in detrimental metabolic states such as obesity and diabetes (Zhang et al. 2013; Ley et al. 2006; Geurts et al. 2011). However, recent studies in rats have shown that exercise is associated with greater butyrate levels (Matsumoto et al. 2008), which may confer metabolic benefits (Canani et al. 2011). Therefore, elevations in butyrate-producing members of the Firmicutes phylum (Guilloteau et al. 2010) (e.g. *Clostridium leptum*, observed herein) may actually be advantageous. The findings by

Clarke et al. (2014) are intriguing, but offer only preliminary insight, as the study was observational in nature and major factors (diet and exercise) were not controlled for.

Perhaps most interesting is the interaction of diabetic state and exercise on *Bifidobacterium* spp., where exercise was associated with greater abundance in non-diabetic but lower abundance in diabetic mice. Queipo-Ortuño et al. (2013) have also previously reported greater *Bifidobacterium* in cecal matter in non-diabetic rats with exercise (Queipo-Ortuño et al. 2013). By contrast, Evans et al. (2014) found lower *Bifidobacterium* in fecal matter of exercised rats fed a low-fat diet compared to sedentary animals, while exercise had no impact on *Bifidobacterium* levels in rats fed a high-fat diet (Evans et al. 2014). This suppressive effect of high-fat feeding on *Bifidobacterium* in rats has been reported previously (Cani et al. 2007). Rescuing *Bifidobacterium* levels by feeding oligofructose (Cani et al. 2007) or supplementing with either a single strain (Chen et al. 2012) or multispecies probiotic (Asemi et al. 2013) has been associated with improvements in insulin sensitivity and reduced inflammation in high-fat fed rats as well as patients with type 2 diabetes. While still preliminary, taken together the evidence suggests that exercise may beneficially affect *Bifidobacterium* levels in normal rats, but may not be as effective a stimulus in metabolically challenged states.

The mechanisms for how non-dietary aspects of the host environment influence gut microbiota are still unclear, but, as highlighted by a recent review (De Palma et al. 2014), these factors clearly play a role. For example, psychological and physical stress

are associated with gastrointestinal disorders, leading to the recent suggestion this may occur in part via modification of the gut bacteria, possibly through systemic mechanisms like catecholamines (Lyte et al. 2011). How exercise specifically affects gut microbiota is not yet clear, but may involve modification of the intestinal immune system (Viloria et al. 2011) or movement of substrates through the colon by increasing intestinal transit time, though this is not uniformly observed (Evans et al. 2014; Choi et al. 2013).

Here, we are limited in reporting differences in select microbial species only at the end of exercise training, but baseline microbiota and longitudinal changes are clearly factors that should be considered in future work. In the study by Clarke et al. in rugby players (Clarke et al. 2014), differences could be due to inherent properties of the core microbiome of fit individuals versus an effect of exercise. Therefore, intervention studies are needed to determine how training affects the microbial profile within an individual. Further, technological advances in gene sequencing have allowed recent studies to highlight differences in individual species and insight into deeper compositional changes, which are not possible with the qPCR methods utilized here. Nevertheless, using qPCR to analyze selected taxonomic groups via well-established and validated methods, we describe for the first time the interaction between exercise and the diabetic state on gut microbiota. The data suggests that exercise exerts independent effects on microbial species in diabetic and non-diabetic animals. The mechanisms for these independent effects, and the metabolic implications of these changes, require further investigation.

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REFERENCES

Asemi, Z., Zare, Z., Shakeri, H., Sabihi, S.-S., and Esmaillzadeh, A. 2013. Effect of Multispecies Probiotic Supplements on Metabolic Profiles, Hs-CRP, and Oxidative Stress in Patients with Type 2 Diabetes. Ann. Nutr. Metab. **63**(1-2): 1–9. doi:10.1159/000349922.

Belke, D.D., Larsen, T.S., Gibbs, E.M., and Severson, D.L. 2000. Altered Metabolism Causes Cardiac Dysfunction in Perfused Hearts from Diabetic (db/db) Mice. Am. J. Physiol. Endocrinol. Metab. **279**(5): E1104–13. http://www.ncbi.nlm.nih.gov/pubmed/11052966.

Belke, D.D., Swanson, E.A., and Dillmann, W.H. 2004. Decreased Sarcoplasmic Reticulum Activity and Contractility in Diabetic Db/db Mouse Heart. Diabetes. **53**(12): 3201–8. http://www.ncbi.nlm.nih.gov/pubmed/15561951.

Bomhof, M.R., Saha, D.C., Reid, D.T., Paul, H.A., and Reimer, R.A. 2014. Combined Effects of Oligofructose and Bifidobacterium Animalis on Gut Microbiota and Glycemia in Obese Rats. Obesity (Silver Spring). **22**(3): 763–71. doi:10.1002/oby.20632.

Canani, R., Costanzo, M., and Leone, L. 2011. Potential Beneficial Effects of Butyrate in Intestinal and Extraintestinal Diseases. World J. Gastroenterol. **17**(12): 1519–28. doi:10.3748/wjg.v17.i12.1519.

Cani, P.D., Neyrinck, a. M., Fava, F., Knauf, C., Burcelin, R.G., Tuohy, K.M., Gibson, G.R., and Delzenne, N.M. 2007. Selective Increases of Bifidobacteria in Gut Microflora Improve High-Fat-Diet-Induced Diabetes in Mice through a Mechanism Associated with Endotoxaemia. Diabetologia. **50**(11): 2374–83. doi:10.1007/s00125-007-0791-0.

Chen, J., Wang, R., Li, X.-F., and Wang, R.-L. 2012. Bifidobacterium Adolescentis Supplementation Ameliorates Visceral Fat Accumulation and Insulin Sensitivity in an Experimental Model of the Metabolic Syndrome. Br. J. Nutr. **107**(10): 1429–34. doi:10.1017/S0007114511004491.

Choi, J.J., Eum, S.Y., Rampersaud, E., Daunert, S., Abreu, M.T., and Toborek, M. 2013. Exercise Attenuates PCB-Induced Changes in the Mouse Gut Microbiome. Environ. Health Perspect. **121**(6): 725–30. doi:10.1289/ehp.1306534.

Clarke, S.F., Murphy, E.F., O'Sullivan, O., Lucey, A.J., Humphreys, M., Hogan, A., Hayes, P., et al. 2014. Exercise and Associated Dietary Extremes Impact on Gut Microbial Diversity. Gut., June, 1–8. doi:10.1136/gutjnl-2013-306541.

De Palma, G., Collins, S.M., Bercik, P., and Verdu, E.F. 2014. The Microbiota-Gut-Brain Axis in Gastrointestinal Disorders: Stressed Bugs, Stressed Brain or Both? J. Physiol. **592**(Pt 14): 2989–97. doi:10.1113/jphysiol.2014.273995.

Duncan, S.H., Lobley, G.E., Holtrop, G., Ince, J., Johnstone, a M., Louis, P., and Flint, H.J. 2008. Human Colonic Microbiota Associated with Diet, Obesity and Weight Loss. Int. J. Obes. (Lond). **32**(11): 1720–24. doi:10.1038/ijo.2008.155.

Evans, C.C., LePard, K.J., Kwak, J.W., Stancukas, M.C., Laskowski, S., Dougherty, J., Moulton, L., et al. 2014. Exercise Prevents Weight Gain and Alters the Gut Microbiota in a Mouse Model of High Fat Diet-Induced Obesity. PLoS One. **9**(3): e92193. doi:10.1371/journal.pone.0092193.

Geurts, L., Lazarevic, V., Derrien, M., Everard, A., Van Roye, M., Knauf, C., Valet, P., et al. 2011. Altered Gut Microbiota and Endocannabinoid System Tone in Obese and Diabetic Leptin-Resistant Mice: Impact on Apelin Regulation in Adipose Tissue. Front. Microbiol. **2**(July): 149. doi:10.3389/fmicb.2011.00149.

Guilloteau, P., Martin, L., Eeckhaut, V., Ducatelle, R., Zabielski, R., and Van Immerseel, F. 2010. From the Gut to the Peripheral Tissues: The Multiple Effects of Butyrate. Nutr. Res. Rev. **23**(2): 366–84. doi:10.1017/S0954422410000247.

Hummel, K.P., Dickie, M.M., and Coleman, D.L. 1966. Diabetes, a New Mutation in the Mouse. Science. **153**(3740): 1127–28. http://www.ncbi.nlm.nih.gov/pubmed/5918576.

Huttenhower, C., and Human Microbiome Project Consortium. 2012. Structure, Function and Diversity of the Healthy Human Microbiome. Nature. **486**(7402): 207–14. doi:10.1038/nature11234.

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., et al. 2013. Richness of Human Gut Microbiome Correlates with Metabolic Markers. Nature. **500**(7464): 541–46. doi:10.1038/nature12506.

Ley, R.E., Bäckhed, F., Turnbaugh, P., Lozupone, C.A., Knight, R.D., and Gordon, J.I. 2005. Obesity Alters Gut Microbial Ecology. Proc. Natl. Acad. Sci. U. S. A. **102**(31): 11070–75. doi:10.1073/pnas.0504978102.

Ley, R.E., Turnbaugh, P.J., Klein, S., and Gordon, J.I. 2006. Microbial Ecology: Human Gut Microbes Associated with Obesity. Nature. **444**(7122): 1022–23. doi:10.1038/4441022a.

Lyte, M., Vulchanova, L., and Brown, D.R. 2011. Stress at the Intestinal Surface: Catecholamines and Mucosa-Bacteria Interactions. Cell Tissue Res. **343**(1): 23–32. doi:10.1007/s00441-010-1050-0.

Matsumoto, M., Inoue, R., Tsukahara, T., Ushida, K., Chiji, H., Matsubara, N., and Hara, H. 2008. Voluntary Running Exercise Alters Microbiota Composition and Increases N-Butyrate Concentration in the Rat Cecum. Biosci. Biotechnol. Biochem. **72**(2): 572–76. doi:10.1271/bbb.70474.

Parnell, J.A., and Reimer, R.A. 2012. Prebiotic Fibres Dose-Dependently Increase Satiety Hormones and Alter Bacteroidetes and Firmicutes in Lean and Obese JCR:LA-Cp Rats. Br. J. Nutr. **107**(4): 601–13. doi:10.1017/S0007114511003163.

Queipo-Ortuño, M.I., Seoane, L.M., Murri, M., Pardo, M., Gomez-Zumaquero, J.M., Cardona, F., Casanueva, F., and Tinahones, F.J. 2013. Gut Microbiota Composition in Male Rat Models under Different Nutritional Status and Physical Activity and Its Association with Serum Leptin and Ghrelin Levels. PLoS One. **8**(5): e65465. doi:10.1371/journal.pone.0065465.

Shearer, J., Ross, K.D., Hughey, C.C., Johnsen, V.L., Hittel, D.S., and Severson, D.L. 2011. Exercise Training Does Not Correct Abnormal Cardiac Glycogen Accumulation in the Db/db Mouse Model of Type 2 Diabetes. Am. J. Physiol. Endocrinol. Metab. **301**(1): E31–39. doi:10.1152/ajpendo.00525.2010.

Tilg, H., and Moschen, A.R. 2014. Microbiota and Diabetes: An Evolving Relationship. Gut. **63**(9): 1513–21. doi:10.1136/gutjnl-2014-306928.

Viloria, M., Lara-Padilla, E., Campos-Rodríguez, R., Jarillo-Luna, A., Reyna-Garfias, H., López-Sánchez, P., Rivera-Aguilar, V., Salas-Casas, A., Berral de la Rosa, F.J., and García-Latorre, E. 2011. Effect of Moderate Exercise on IgA Levels and Lymphocyte Count in Mouse Intestine. Immunol. Invest. **40**(6): 640–56. doi:10.3109/08820139.2011.575425.

Zhang, X., Shen, D., Fang, Z., Jie, Z., Qiu, X., Zhang, C., Chen, Y., and Ji, L. 2013. Human Gut Microbiota Changes Reveal the Progression of Glucose Intolerance. PLoS One. **8**(8): e71108. doi:10.1371/journal.pone.0071108.

Table 1: Cecal microbiota of normal (*db/*$^{+}$) or diabetic (*db/db*) mice following 6 weeks of sedentary or exercise-training

Bacteria	*db/*$^{+}$		*db/db*		2-way ANOVA P values		
	Sedentary (n=10)	**Exercise** (n=10)	**Sedentary** (n=9)	**Exercise** (n=10)	**Diabetes**	**Exercise**	**Diabetes x Exercise**
Total bacteria	7.69 ± 0.04 [a]	7.70 ± 0.04 [a]	7.72 ± 0.03 [a]	7.54 ± 0.04 [b]	0.14	0.043	**0.026**
Enterobacteriaceae	4.38 ± 0.02 [a]	4.37 ± 0.03 [a]	4.44 ± 0.02 [a]	4.31 ± 0.03 [b]	0.94	0.009	**0.021**
Bifidobacterium spp.	4.99 ± 0.14 (0.4) [a]	5.70 ± 0.03 (1.6) [b]	5.06 ± 0.10 (0.4) [a]	4.70 ± 0.12 (0.3) [c]	<0.001	0.10	**<0.001**
Bacteroides/Prevotella spp.	6.93 ± 0.09 (27.7) [a]	6.79 ± 0.05 (17.4) [a]	6.87 ± 0.13 (24.9) [a]	6.49 ± 0.08 (13.5) [b]	0.063	**0.007**	0.19
Methanobrevibacter spp.	3.95 ± 0.02 [a]	3.88 ± 0.02 [b]	3.98 ± 0.03 [a]	3.84 ± 0.03 [b]	0.96	**<0.001**	0.24
Firmicutes							
Lactobacillus spp.	5.00 ± 0.09 (0.3)	5.15 ± 0.09 (0.4)	4.99 ± 0.07 (0.3)	5.19 ± 0.03 (0.6)	0.84	**0.034**	0.76
Clostridium leptum (C-IV)	6.35 ± 0.05 (3.6) [a]	6.58 ± 0.07 (6.9) [b]	6.41 ± 0.04 (4.0) [a]	6.51 ± 0.11 (7.7) [ab]	0.93	**0.038**	0.37
Clostridium coccoides (C-XIVa)	7.24 ± 0.07 (51.8)	7.30 ± 0.03 (57.3)	7.30 ± 0.05 (48.2)	7.20 ± 0.08 (51.2)	0.76	0.67	0.19
Clostridium cluster (C-I)	5.19 ± 0.06 (0.2) [a]	5.49 ± 0.05 (0.4) [b]	5.11 ± 0.04 (0.2) [a]	5.30 ± 0.09 (0.4) [c]	**0.052**	**0.001**	0.40
Clostridium cluster (C-XI)	2.91 ± 0.03 [a]	3.00 ± 0.04 [a]	3.06 ± 0.05 [b]	3.03 ± 0.04 [a]	**0.030**	0.48	0.16
Roseburia spp.	4.59 ± 0.12 (0.2)	4.60 ± 0.09 (0.1)	4.64 ± 0.05 (0.1)	4.68 ± 0.08 (0.2)	0.49	0.77	0.86

Data presented as mean + SE. Data represent Log 16S rRNA gene copies per mg sample. The number in brackets indicates the relative abundance (%) of bacterial taxa per total bacteria normalized to genomic copies; contributions of <0.1% are not provided. Data analyzed by 2-way ANOVA, with testing for simple effects; labeled values for a variable without a common superscript letter are different (P<0.05).