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## **Exercise Attenuates PCB-Induced Changes in the Mouse Gut Microbiome**

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**Running title:** Changes in the gut microbiome by exercise and PCBs

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**Abbreviations:** HC-AN, hierarchical clustering-average-neighbor; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; OTU, operational taxonomic unit; PAH, polycyclic aromatic hydrocarbons; PAM, prediction analysis for microarrays; PCB, polychlorinated biphenyl; PCoA, principal component analysis

## ABSTRACT

**Background:** The gut microbiome is a dynamic bacterial community that interacts with the host and closely relates to human health by regulating energy metabolism and immune functions. Recent reports also point to the role of the gut microbiome in risk assessment to environmental toxicants.

**Objectives:** To investigate the effects of polychlorinated biphenyls (PCBs) and exercise on the composition and structure of the gut microbiome.

**Methods:** Mice exercised voluntarily for 5 weeks, followed by the exposure to a mixture of environmentally relevant PCB congeners (PCB153, PCB138 and PCB180; total PCB dose, 150  $\mu\text{mol/kg}$ ) for 48 h. The microbiome was assessed by determination of 16S rRNA.

**Results:** Oral exposure to PCBs significantly altered the abundance of the gut microbiome in mice primarily by decreasing the levels of Proteobacteria. The activity level correlated with a substantial shift in abundance, biodiversity, and microbiome composition. Importantly, exercise attenuated PCB-induced changes in the gut microbiome.

**Conclusions:** This study provides the first evidence that oral exposure to PCBs can induce substantial changes in the gut microbiome, which may then influence their systemic toxicity. Importantly, these changes can be attenuated by behavioral factors, such as voluntary exercise.

## INTRODUCTION

The gut microbiome is a collection of bacteria that reside in the host gut. It is estimated that as many as  $10^{14}$  bacterial cells are in human gut (Ley et al. 2006), accounting for 15,000 - 36,000 bacterial species (Frank et al. 2007). The main bacterial phyla composing the human gut microbiome are the Gram-negative Bacteroidetes and Proteobacteria and the Gram-positive Actinobacteria and Firmicutes (Frank et al. 2007). Each host has a unique composition of gut microbiota, which implies highly individual responses to environmental stressors and suggests a role for gut microbiota in future personalized health strategies (Kinross et al. 2011).

Recent evidence implicated the gut microbiome in the development of a wide range of disorders including obesity, diabetes, metabolic dysfunctions, vascular disease, and inflammatory bowel disease (IBDs) (Kinross et al. 2011). Strong evidence also indicates the critical role of the gut microbiota in drug metabolism and toxicity, energy metabolism, immune functions, and post-surgical recovery (Holmes et al. 2011; Kinross et al. 2011; Tilg and Kaser 2011). Moreover, the gut microbiome was shown to regulate psychiatric health and influence etiopathology of autism (Bravo et al. 2011; Lyte et al. 2006). Chronic administration of *Lactobacillus rhamnosus* induced anxiolytic and antidepressant effects by modulating the expression of GABA receptors in the brain (Bravo et al. 2011). Infection with *Citrobacter rodentium* was shown to induce anxiety-like behaviors via vagal sensory regulation (Lyte et al. 2006).

Despite these diverse effects on human health, the influence of the microbiome on the toxicity of environmental pollutants and its role in risk assessment are largely unknown (Betts 2011). It was recently suggested that preabsorptive metabolism can modify toxicity of environmental

pollutants, influencing their health effects. The most compelling evidence illustrating this phenomenon was obtained in studies on biotransformation of heavy metals by the gut microbiome. For example, it was reported that anaerobic microbiota of mouse cecum converts arsenate into oxy- and thio-arsenicals (Pinyayev et al. 2011). Exposure to mercury was also demonstrated to alter the bacterial community in the terrestrial isopod (*Porcellio scaber*) gut (Lapanje et al. 2007). Importantly, gram-negative fecal bacteria were shown to be involved in biotransformation of mercury (Liebert et al. 1997). Experiments on germ-free mice also provided evidence that the gut microbiome can regulate the expression of cytochrome P450 enzymes, which are involved in metabolism of a variety of xenobiotics, including environmental chemicals (Claus et al. 2011). Indeed, human colon microbiota can transform polycyclic aromatic hydrocarbons (PAH) to estrogenic metabolites (Van de Wiele et al. 2005). These findings are significant because PAH toxicity has been linked to estrogenicity of the compounds, thus suggesting that PAH bioactivation in the colon should be taken into consideration, when evaluating risk assessment (Van de Wiele et al. 2005). The role of the gut microbiota, its variability in particular on the disposition of environmental chemicals in the human body, and its contribution to the development of obesity and diabetes, have also recently been recognized (Snedeker and Hay 2012).

The present study was designed based on the recent evidence implicating the role of the gut microbiome in risk assessment of environmental chemicals. We examined whether exposure to polychlorinated biphenyls (PCBs) could affect the abundance and composition of the gut microbiome. In addition, there is an emerging interest in the role of behavioral factors in modulating toxicity of environmental pollutants. While the role of nutrition has been explored (e.g., [Majkova et al. 2008]), the impact of exercise on the health effects of toxicants is not

known. Because exercise can influence the outcomes of disorders known to be associated with alterations of the gut microbiome (Martin 2011), we hypothesized that physical activity may affect the composition of the gut microbiota and thus influence the impact of environmental toxicants. Our results indicate that PCBs can induce profound changes in the microbial composition of the gut and that exercise can attenuate PCB-induced effects on intestinal microbiome.

## **METHODS**

### **Animals and voluntary exercise**

All animal protocols were approved by the Institutional Committee on Animal Care and abided the NIH guidelines. The animals were treated humanely and with regard for alleviation of suffering. Male C57BL/6 (11-13 month old; Charles River Laboratories, Wilmington, MA) mice were housed under 12:12 h light/dark conditions with access to food and water *ad libitum*. Mice were randomly assigned to either exercised or sedentary groups (n=6 per group). Mice were housed individually in plastic cages equipped with a running wheel (Coulbourn Instruments, Whitehall, PA). Sedentary mice were housed in the same cages with a locked wheel. Running activity was monitored 24 h/day and 7 days/week and analyzed using Clocklab and Matlab software (Actimetrics, Wilmette, IL, and Natick, MA, respectively). Mice were allowed to exercise for five weeks, including one week adaptation to running and solitary housing.

Mice averaged 10-12 km per 24 h. The average speed was 1.12 km/h and time spent on running was  $10.3 \pm 0.33$  h/day. Exercised mice had decreased body weight compared with sedentary mice by 30% on average. No changes in gut transit or stool frequency were noted at the end of the exercise period among the exercised and sedentary mice.

## **PCB exposure**

Study design is shown in Figure 1. At the end of the exercise period, mice were administered with tocopherol-stripped safflower oil (5 ml/kg; Dyets Inc., Bethlehem, PA) in order to adjust to the vehicle used for PCB treatment. The feces were collected for two days and analyzed for control/background microbiome data. Mice were then treated with an environmentally relevant mixture of PCB congeners, namely PCB138, PCB153, and PCB180 (AccuStandard, New Haven, CT), mixed at the molar ratio of 1.7:3.2:1 in tocopherol-stripped safflower oil (vehicle). The feces were collected again for two days to obtain sufficient sample size for microbiome assessment. Each mouse served as its own control for PCB treatment to minimize the influence of individual variations on the gut microbiome. The type of PCB congeners and their relative ratio were selected based on PCB content in contaminated food (Ashley et al. 2000). Total dose of PCBs of 150  $\mu\text{mol/kg}$  administered to mice results in 5  $\mu\text{M}$  PCB level in plasma (Choi et al. 2010), which is comparable to plasma levels of PCBs in acutely exposed human population (Jensen 1989; Wassermann et al. 1979). All treatments were performed via oral gavage using an 18 G gavage needle, 3 cm long, curved, 21/4 mm ball diameter (Popper and Sons, New Hyde Park, NY).

## **Analysis of the gut microbiome**

All analyses were performed using PhyloChip Arrays. Bacterial 16S rRNAs were amplified with primer sets, 27F.1 (5'-AGRGTTTGATCMTGGCTCAG-3') and 1492R.jgi (5'-GGTTACCTTGTTACGACTT-3'). Gene amplification was accomplished by 35 cycles of polymerase chain reaction (PCR) with denature step at 95°C for 30 sec, annealing step at 50°C for 30 sec, and extension step at 72°C for 2 min using TaKaRa Ex Taq system (Takara Bio Inc., Japan). For each sample, amplified products were concentrated using a centrifuge filtration

method and quantified by electrophoresis using an Agilent 2100 Bioanalyzer® (Agilent Technologies, CA). PhyloChip Control Mix was added to each amplified product. The combined PCR products and controls were fragmented, biotin labeled, and hybridized to the PhyloChip Array (version G3). PhyloChip arrays were washed, stained, and scanned using a GeneArray® scanner (Affymetrix, OH). Each scan was captured using standard Affymetrix software (GeneChip® Microarray Analysis Suite). Hybridization values and the fluorescence intensity for each taxon were calculated as a trimmed average, with maximum and minimum values removed before averaging.

### **Statistical analysis**

Data processing and multivariate statistical analyses were performed with the PhyCA-Stats™ analysis software package (Second Genome, San Bruno, CA). Probe intensities were background subtracted and scaled to PhyloCip™ Control Mix. Subsequently, hybridization scores (HybScores) were calculated as  $\log_2(\text{mean probe fluorescence intensity}) \times 1000$ . OTUs were defined by high 16S rRNA gene sequence similarity, with the majority demonstrating >99% intra-OTU concordance. Prior to classification analysis, data reduction was performed via multiple filtering steps as previously reported (Hazen et al. 2010), and taxa-sample intersections were then calculated using abundance (AT) and binary matrices (BT). Pairwise BT and AT dissimilarity scores were computed using the Unifrac distance metric (Lozupone et al. 2006) and weighted Unifrac (Wunifrac) distance metric, respectively. The Wunifrac metric considers OTU abundance in addition to phylogenetic distance between OTUs. Hierarchical clustering via average-neighbor (HC-AN) and principal coordinate analysis (PCoA) were used to graphically summarize inter-sample relationships according to AT and BT dissimilarity scores. Unsupervised classification using the nearest shrunken centroid method (Tibshirani et al. 2002)

as implemented in Prediction Analysis of Microarrays (PAM) was used to identify OTU's with the most significant differences in abundance between comparison groups. Also, a randomization/monte carlo permutation-based test (Adonis test) was used for significance testing of differences between discrete and continuous variables.

## RESULTS

### **PCB exposure decreases the abundance of the gut microbiota**

We first analyzed the effects of PCB exposure on the gut microbiome in sedentary mice. The Welch test revealed that exposure to PCB mixture significantly (Student's *t*-test,  $p < 0.05$ ) altered abundance of 1,223 bacterial taxa in these mice, including 1,133 taxa with decreased abundance and only 90 taxa with increased abundance. As a result of these changes, the overall abundance of bacteria significantly diminished in PCB-exposed mice by 2.2%. Bacterial taxa that decreased abundance to the greatest extent (for up to 5.6 fold) as the result of PCB treatment are listed in Table 1. These taxa belonged primarily to phylum Proteobacteria; however, the classes and families were diverse. The group of bacterial taxa that increased abundance in response to PCB exposure was relatively modest, and the changes did not exceed 2 fold. Bacterial taxa that increased abundance to the highest level after PCB treatment are listed in Table 2. They belonged to several difference phyla, including Bacteroidetes, Actinobacteria, Verrucomicrobia, Firmicutes, and Bacteroidetes.

Interestingly, exposure to PCB mixture did not alter biodiversity of the gut microbiome. All 11,229 taxa were detected in at least one sample from sedentary mice. Following PCB exposure, this number changed to 10,798, which did not reach statistical significance.

### **Exercise alters the composition of the gut microbiome**

The structure of the gut microbiome analyzed by weighted unifrac distance was significantly different between the exercised and sedentary mice (Adonis test,  $p < 0.05$ ). PCoA with unweighted unifrac distance with given presence/absence metrics also showed prominent categorization of the composition of the gut microbiome when comparing the exercised and sedentary mice (Figure 2A).

Among detected bacterial taxa, 93 taxa were present exclusively in exercised or sedentary mice. Specifically, 67 taxa were detected only in the exercised and not the sedentary group (Supplemental Material, Table S1), while 26 taxa were unique to sedentary mice (Supplemental Material, Table S2).

A group of 2,510 taxa showed differences in abundance between the exercised and sedentary mice. These taxa were then analyzed by PCoA with weighted unifrac distance, which indicated significant differences in the composition of the microbial communities between the exercised and sedentary mice (Figure 2B). Hierarchical clustering-average-neighbor (HC-AN) analysis based on weighted unifrac distance confirmed a shift of the composition of the gut microbiome related to physical activity (exercised vs. sedentary mice) (Figure 2C).

Further examination using the PAM identified 10 taxa with substantially different abundance between the exercised and sedentary mice (Table 3). The taxa that were more abundant in the exercised group were in the class Bacilli composing a phylum Firmicutes. The majority of these bacterial species comprised the order Lactobacillales. The taxa that were decreased in the exercised group belonged to phyla Tenericutes, Bacteroidetes, and Firmicutes. The most striking

change in exercised mice was a decrease in *Erysipelotrichaceae* bacterium C11\_K211 from phylum Tenericutes, which decreased dramatically in exercised as compared to sedentary mice.

### **Exercise attenuates PCB-induced alterations of the gut microbiome composition**

Comparison of the gut microbiome among all experimental groups (sedentary and exercised mice with or without PCB exposure) identified that 1,568 bacterial taxa were differentially abundant in at least one out of these groups. These taxa were then analyzed for dissimilarity between the groups using PCoA with weighted unifrac. The analysis indicated significant differences between the composition of the gut microbiome before and after PCB exposure in sedentary mice. Importantly, exercise altered PCB-mediated effects on the gut microbiome, as indicated by a loss of bacterial clustering (Figure 3A). This phenomenon was subsequently confirmed by HC-AN analysis (Figure 3B).

When analyzing the number of bacterial taxa, 10,799 taxa were detected in sedentary mice exposed to PCB mixture, compared to 13,383 taxa in the exercised mice treated with PCBs. While there appeared to be a tendency toward increased biodiversity in the exercise plus PCB group, these changes were not significant. In contrast, abundance of bacterial species was significantly elevated by 2.9% (Student's *t*-test,  $p < 0.05$ ) in exercised mice exposed to PCBs as compared to sedentary mice treated with PCBs, providing additional evidence that exercise can protect against PCB-mediated alterations in the gut microbiota. One of the important findings indicates that exercise prevented PCB-induced a decrease in abundance of Proteobacteria, which was observed in sedentary mice (Figure 3C).

## DISCUSSION

In view of recent evidence indicating that gut bacteria can be involved in the preabsorptive metabolism of heavy metals and organic chemicals, the gut microbiome has been proposed to play a role in the assessment of health risks associated with environmental chemicals (Betts 2011; Snedeker and Hay 2012). Therefore, our demonstration that even short-term exposure to an environmentally relevant PCB mixture resulted in profound changes in the gut microbiome in mice is highly significant. The most striking change in the intestinal microbial profiles was a decrease in the overall abundance of bacterial species. While these results are the first report on the effects of PCBs on the gut microbiome, a decrease in bacterial abundance observed in the present study corresponds with the finding that PCB-contaminated soil is characterized by a shift in structure and abundance of bacterial community (Petric et al. 2011). It was observed that incubation of soil slurries with higher-chlorinated PCB congeners, e.g., PCB28, PCB77, and Aroclor 1242, resulted in lower bacterial numbers (Correa et al. 2010). Even though the gut and soil provide completely different bacterial environments, it appears that exposure to PCBs can elicit environmental stress on the structure and composition of bacterial communities resulting in diminished bacterial abundance. In our studies, these changes selectively affected bacterial phylotypes belonging to Proteobacteria. Such changes may be most relevant to immune functions of the host, as the gut microbiome was shown to play important roles in mucosal immunity and interactions with intestinal and colonic epithelial cells, dendritic cells, and T and B immune cells. It is well established that microbiota composition has functional effects on T effector- and T regulatory-cell balance, immune responsiveness, and homeostasis (Kelly and Mulder 2012). Thus, it is likely that alterations of the gut microbiota compromise a novel mechanism leading to immunological alterations, which develop in response to exposure to

PCBs (Maule et al. 2005; Weisglas-Kuperus et al. 2004). In addition, alterations of the gut microbiome can affect PCB-induced disruption of the intestinal barrier and translocation of lipopolysaccharides (LPS) into the blood stream as previously observed by our group (Choi et al. 2012; Choi et al. 2010). In view of these facts, it is important that exercise protected against PCB-induced gut microbiome changes. There were no statistical differences in bacterial community structure in the exercised mice before and after PCB treatment. Thus, exercise provided protection against PCB induced changes in the gut bacterial community structure. In particular, exercise prevented PCB-induced a decrease in abundance of Proteobacteria, which was observed in sedentary mice.

While the data reported in the present paper are novel, the effects of physical activity on several other aspects of gut physiology (e.g., peristalsis, (Song et al. 2012)) and pathology have been recognized. Exercise was demonstrated to decrease the risk of developing several intestinal diseases, including colon cancer (Friedenreich et al. 2006), IBD, irritable bowel syndrome (IBS) (Lustyk et al. 2001), and other disorders that are accompanied by changes in the gut microbiome (De Hertogh et al. 2012; Nelson et al. 2011; Walker et al. 2011).

The mechanisms of exercise-mediated changes in gut ecology are not known; however, they are likely to be mediated by altering the host factors that influence the intestinal microenvironment. For example, it was reported that physical activity can increase excretion of the primary bile acids to the gastrointestinal track (Meissner et al. 2011) and suppress the formation of secondary bile acids (Hagio et al. 2010). The primary bile acids, such as cholic, deoxycholic, or chenodeoxycholic acids have established antimicrobial activity, which is mediated by the reduction in internal pH levels of bacteria, dissipation of their transmembrane electrical potential, and disturbances of membrane integrity, leading to leakage of ions and cell death (Kurdi et al.

2006). In support of this hypothesis, it was demonstrated that cholic acid induced substantial changes in the cecal microbiome composition by stimulating the growth of Firmicutes at the expense of Bacteroidetes and outgrowth of several bacteria in the classes Clostridia and Erysipelotrichi (Islam et al. 2011). Thus, the antimicrobial activity of the bile acids may elicit selective pressure on the bacterial communities in exercised mice, leading to a shift of the gut microbiome structure as observed in the present study.

Short chain fatty acids (SCFAs) may be another factor that regulates the gut microbiome in response to exercise. Indeed, voluntary running exercise was shown to increase butyrate concentration in the rat cecum as compared to the sedentary rats. This effect was directly linked to the beneficial effect of exercise on the gut microbiota and the development of gastrointestinal disorders (Matsumoto et al. 2008). SCFAs, e.g. butyrate and acetate, are known to increase colonic epithelial cell proliferation and decrease the risk of colorectal cancer. Their influence on the composition of microbial environment was linked to a decrease in pH in the gut (Wong et al. 2006). Nevertheless, the effects of butyrate infusion on the rumen gut microbiome was relatively minor as compared to the results of the present study as only 19 genera and 43 bacterial taxa were significantly affected in response to butyrate (Li et al. 2012), suggesting that this SCFA may be only one of several factors involved in exercise-mediated changes in the gut microbiome. Treatment with SCFAs may also affect the host-related intestinal factors, as butyrate was shown to promote cell differentiation, cell-cycle arrest, inhibit the enzyme histone deacetylase, and decrease the transformation of primary to secondary bile acids as a result of colonic acidification (Wong et al. 2006).

Finally, exercise may influence the composition of the gut microbiome by altering the intestinal immune system. Physical activity was shown to increase expression of IgA and cytokines such

as IL-6 and TNF- $\alpha$  (Viloria et al. 2011). These changes in the intestinal immune system may lead to secondary alternations of the host-bacterial interaction and induce selective pressure on bacterial selection.

Because the development of chronic diseases related to the exposure to environmental toxicants are associated with age and the benefits of exercise are also emphasized in older individuals, the present study was performed on aged mice. Older mice are characterized by higher body mass as compared to younger animals. In fact, the average body weight was  $46.8 \pm 1.4$  g and a 5 week voluntary exercise regimen resulted in the reduction of the body weight by ~30%. Recent evidence indicated a strong association of the intestinal microbiome with the development of obesity. Genetically obese *ob/ob* mice were characterized by a major decrease in the abundance of Bacteroidetes and an increase in Firmicutes as compared to lean *ob/+*, wild-type littermates, and lean *ob/+* mothers fed the same diets (Ley et al. 2005). Similar changes were observed in wild-type mice fed a high fat/high polysaccharide diet (Turnbaugh et al. 2008) and in obese humans (Ley et al. 2005). An increase in Firmicutes (such as Lactobacilli) and a decrease in Bacteroidetes in obesity were confirmed in another human study (Armougom et al. 2009). Overweight pregnant patients had reduced abundance of Bifidobacteria and Bacteroidetes and increased abundance of selected Firmicutes (e.g., *Staphylococcus*) and Proteobacteria (e.g., *Enterobacteriaceae*) (Santacruz et al. 2010). In line with these reports, it is relevant that we detected increased abundance of several Firmicutes, primarily *Enterococcaceae* (e.g., *E. faecium*), in the exercised mice.

Although Enterococci are commensal bacteria, they are also important nosocomial pathogens that cause bacteremia, endocarditis and other infections in humans. Some strains are resistant to multiple antibiotics and possess virulence factors such as adhesins, invasins, pili and haemolysin

(Willems et al. 2011). Nevertheless, *E. faecium* isolates from clinical outbreaks belong to different types than *E. faecium* from animals, food, and humans in the community (Franz et al. 2011). In fact, several enterococci, including *E. faecium* strains, are used as probiotics in the form of pharmaceutical preparations. They are administered to treat diarrhea, antibiotic-associated diarrhea, IBS, to lower cholesterol levels, and/or improve host immunity (Franz et al. 2011).

The most striking change in the gut microbiome of exercised mice was a decrease by more than 300 fold in the abundance of *Erysipelotrichaceae*. This family plays an important role in metabolic disorders and energy metabolism (Chen et al. 2012). *Erysipelotrichaceae* were enriched in obese humans and mice as well as in mice fed a high-fat diet. In addition, the abundance of the family *Erysipelotrichaceae* was increased in patients with colorectal cancer. Thus, our data are consistent since the number of *Erysipelotrichaceae* decreased in exercised mice that also lost a substantial amount of body weight; this is associated with their role in energy production and adiposity (Claus et al. 2011; Goodman et al. 2011; Zhang et al. 2009).

## CONCLUSIONS

We demonstrate for the first time that oral exposure to a mixture of environmentally relevant PCB congeners significantly altered the abundance of the gut microbiome by decreasing the levels of Proteobacteria. These results suggest that the gut microbiome may be one of the primary targets of PCB-induced toxicity in subjects exposed orally to these environmental toxicants. Importantly, we indicate that PCB-induced alterations of the gut microbiome are attenuated by voluntary exercise.

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**Table 1.** The 10 bacterial taxa with the greatest decrease in abundance following exposure to the PCB mixture.<sup>a</sup>

Phylum	Class	Order	Family	Species	GenBank Accession ID	Fold Change <sup>b</sup>
Proteobacteria	Gammaproteobacteria	Pseudomonadales	<i>Pseudomonadaceae</i>	<i>Pseudomonas plecoglossicida</i> <i>str. CGMCC 2093</i>	EF645247	-5.6
Proteobacteria	Gammaproteobacteria	Pseudomonadales	<i>Pseudomonadaceae</i>		FJ901066	-4.8
Proteobacteria	Betaproteobacteria	Burkholderiales	<i>Comamonadaceae</i>		GQ007353	-4.4
Proteobacteria	Betaproteobacteria	Burkholderiales	<i>Comamonadaceae</i>		GQ108141	-4.4
Proteobacteria	Gammaproteobacteria	Pseudomonadales	<i>Pseudomonadaceae</i>	<i>Pseudomonas plecoglossicida</i> <i>str. R18</i>	DQ095882	-4.3
Proteobacteria	Betaproteobacteria	Burkholderiales	<i>Comamonadaceae</i>		GQ008724	-4.3
Proteobacteria	Betaproteobacteria	Burkholderiales	<i>Comamonadaceae</i>		GQ100754	-4.1
Proteobacteria	Gammaproteobacteria	Pseudomonadales	<i>Pseudomonadaceae</i>	<i>Pseudomonas putida</i> <i>str. SRI156</i>	EU826028	-4.0
Firmicutes	Bacilli	Lactobacillales	<i>Streptococcaceae</i>	<i>Streptococcus infantis</i>	GQ077246	-4.0
Proteobacteria	Betaproteobacteria	Burkholderiales	<i>Comamonadaceae</i>		EF520494	-4.0

<sup>a</sup> Sedentary mice only. -

<sup>b</sup>The fold change is the relative bacterial abundance in PCB-exposed mice as compared to vehicle-treated control. The values were calculated by - comparing the fluorescence intensities derived from hybridization scores. -

**Table 2.** The 10 bacterial taxa with the greatest increase in abundance following exposure to the PCB mixture. <sup>a</sup>

Phylum	Class	Order	Family	Species	GenBank Accession ID	Fold Change <sup>b</sup>
Bacteroidetes	Sphingobacteria	Sphingobacteriales	<i>Saprospiraceae</i>	<i>Candidatus Aquirestis calciphila</i>	AY863078	1.9
Actinobacteria	Actinobacteria	Actinomycetales	<i>Corynebacteriaceae</i>		GQ083745	1.7
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	<i>Verrucomicrobiaceae</i>		FM242339	1.7
Firmicutes	Bacilli	Bacillales	<i>Staphylococcaceae</i>	<i>Staphylococcus epidermidis</i>	GQ087061	1.7
Beacteriodetes	Bacteroidia	Bacteroidales	<i>Bacteroidaceae</i>	<i>Bacteroides thetaiotaomicron str. 8669</i>	AY895200	1.6
Cyanobacteria	Chloroplast	Chlorophyta	<i>Ulvophyceae</i>		FJ203465	1.6
Actinobacteria	Actinobacteria	Actinomycetales	<i>Corynebacteriaceae</i>		GQ039749	1.5
Bacteriodetes	Bacteroidia	Bacteroidales	<i>Porphyromonadaceae</i>		AB231049	1.5
Actinobacteria	Actinobacteria	Actinomycetale	<i>Corynebacteriaceae</i>	<i>Tropheryma whipplei</i>	AF190688	1.5
Actinobacteria	Actinobacteria	Actinomycetales	<i>Corynebacteriaceae</i>		GQ055950	1.5

<sup>a</sup>Sedentary mice only. -

<sup>b</sup>The fold change is the relative bacterial abundance in PCB-exposed mice as compared to vehicle-treated control. The values were calculated by - comparing the fluorescence intensities derived from hybridization scores. -

**Table 3.** Prediction analysis for microarrays (PAM)-selected distinctive bacterial taxa presenting differentially in the mouse gut microbiome.<sup>a</sup>

Phylum	Class	Order	Family	Species	GenBank Accession ID	Fold Change <sup>b</sup>
Firmicutes	Bacilli	Lactobacillales	<i>Enterococcaceae</i>	<i>Enterococcus faecium</i>	EF533987	24.1
Firmicutes	Bacilli	Lactobacillales	<i>Enterococcaceae</i>	<i>Enterococcus faecium</i>	AY692451	15.7
Firmicutes	Bacilli	Bacillales	<i>Staphylococcaceae</i>	<i>Staphylococcus gallinarum</i>	DQ350835	12.1
Firmicutes	Bacilli	Lactobacillales	<i>Enterococcaceae</i>	<i>Escherichia coli</i> O157:H7	FJ675223	7.6
Firmicutes	Bacilli	Lactobacillales	<i>Enterococcaceae</i>	<i>Enterococcus faecium</i>	FJ378658	7.4
Firmicutes	Bacilli	Lactobacillales	<i>Streptococcaceae</i>	<i>Streptococcus pseudopneumoniae</i>	GQ000464	8.7
Firmicutes	Bacilli	Bacillales	<i>Bacillaceae</i>	<i>Bacillus trypoxylicola</i>	AB434284	5.8
Tenericutes	Erysipelotrichi	Erysipelotrichales	<i>Erysipelotrichaceae</i>	<i>C11_K211</i>	DQ015346	-361
Firmicutes	Clostridia	Clostridiales	<i>Ruminococcaceae</i>		EU453081	-6.4
Bacteroidetes	Bacteroidia	Bacteroidales	<i>Bacteroidaceae</i>	<i>Bacteroides clarus</i>	AB490801	-8.6

<sup>a</sup>Exercised versus sedentary mice. -

<sup>b</sup>The fold change is the relative bacterial abundance in exercised as compared to sedentary mice. The values were calculated by comparing the - fluorescence intensities obtained from hybridization scores. -

## FIGURE LEGENDS

**Figure 1.** Experimental design indicating treatment and sampling times. Note that the figure is not scaled to reflect the time lapse. -

**Figure 2.** Exercise alters the structure and composition of the gut microbiome. Mice ran voluntarily for 5 weeks. Bacterial taxa were analyzed in feces by the PhyloChip assay. (A) Principal Coordinate Analysis (PCoA) based on unweighted unifrac distance between exercised and sedentary mice. Opened circle; sedentary mice, closed circle; exercised mice. PCoA1; 33% of variation, PCoA2; 15% of variation. (B) PCoA and (C) hierarchical clustering-average-neighbor (HC-AN) analysis based on weighted unifrac distance between exercised and sedentary mice of the 2,510 taxa with significant abundance differences across at least one of the categories. Opened circles; sedentary mice, closed circles; exercised mice. PCoA1; 84% of variation, PCoA2; 6% of variation. -

**Figure 3.** Exercise prevents PCB-induced alterations of the gut microbiome. Mice were subjected to voluntary exercise and exposed to PCBs as in Figure 1. (A) PCoA and (B) HC-AN analysis based on weighted unifrac distance of the 1,568 taxa with significant abundance differences across at least one of the categories. Opened circles, sedentary mice before PCB treatment; closed circles, sedentary mice after PCB treatment; opened squares, exercised mice before PCB treatment; closed squares, exercised mice after PCB treatment. PCoA1; 71% of variation, PCoA2; 11% of variation. Vehicle, tocopherol-stripped safflower oil. (C) Exercise protects against PCB-induced a decrease in Proteobacteria in sedentary mice. The values are mean  $\pm$  SEM of pooled taxa belonging to the phylum Proteobacteria. \*Statistically significant as compared to control mice -

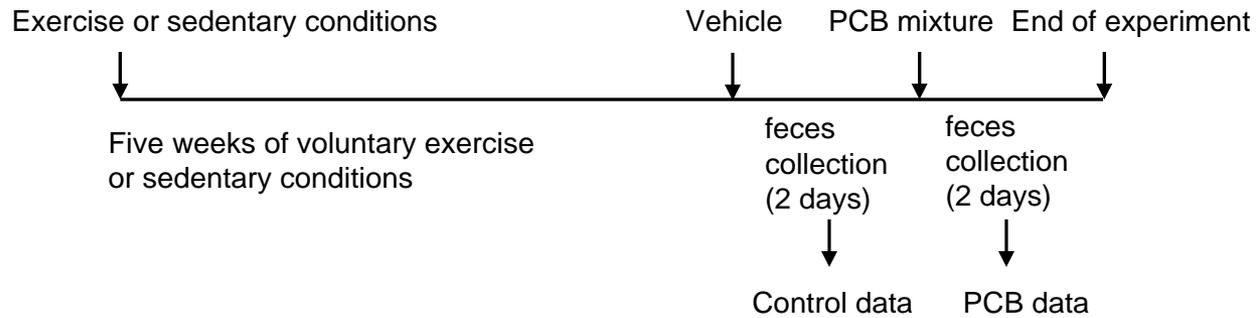


Figure 1

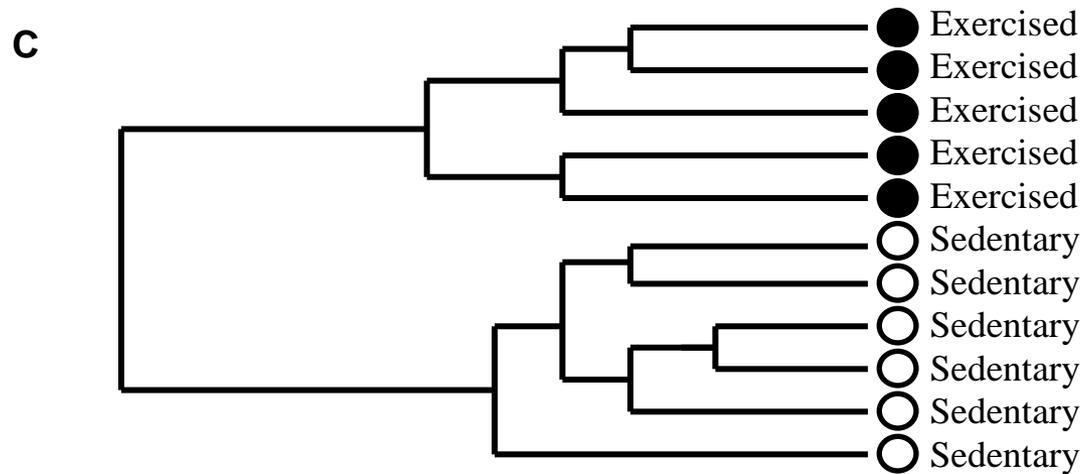
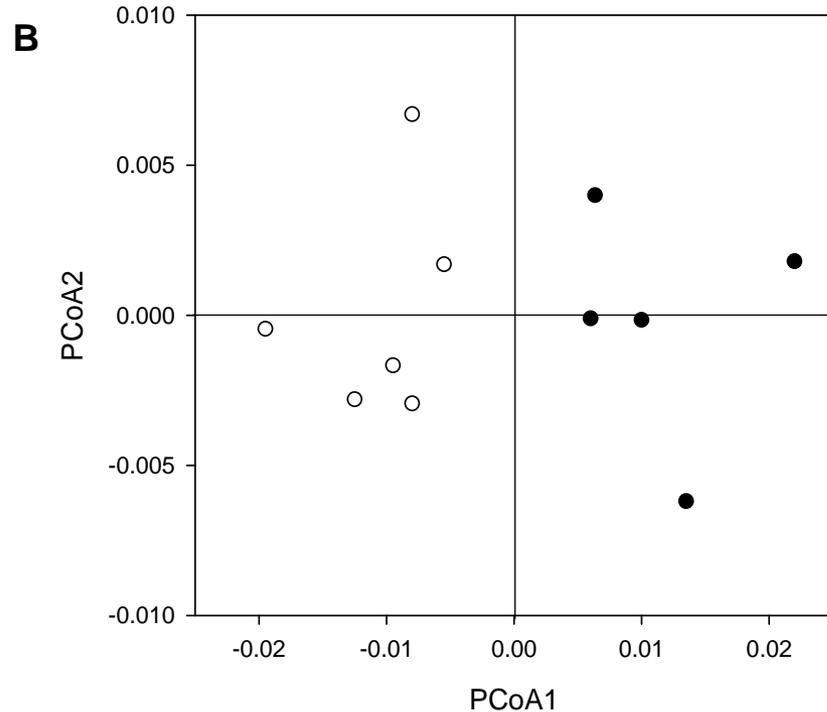
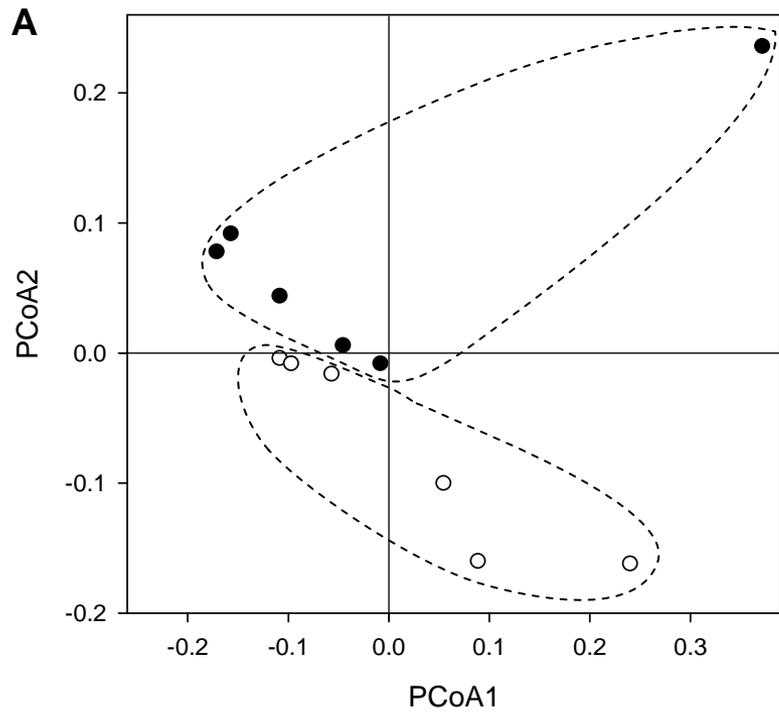


Figure 2

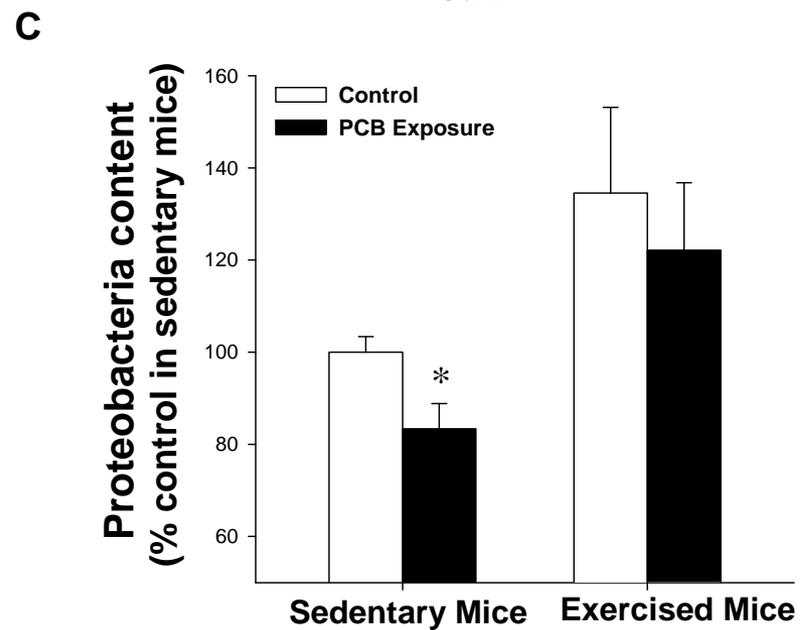
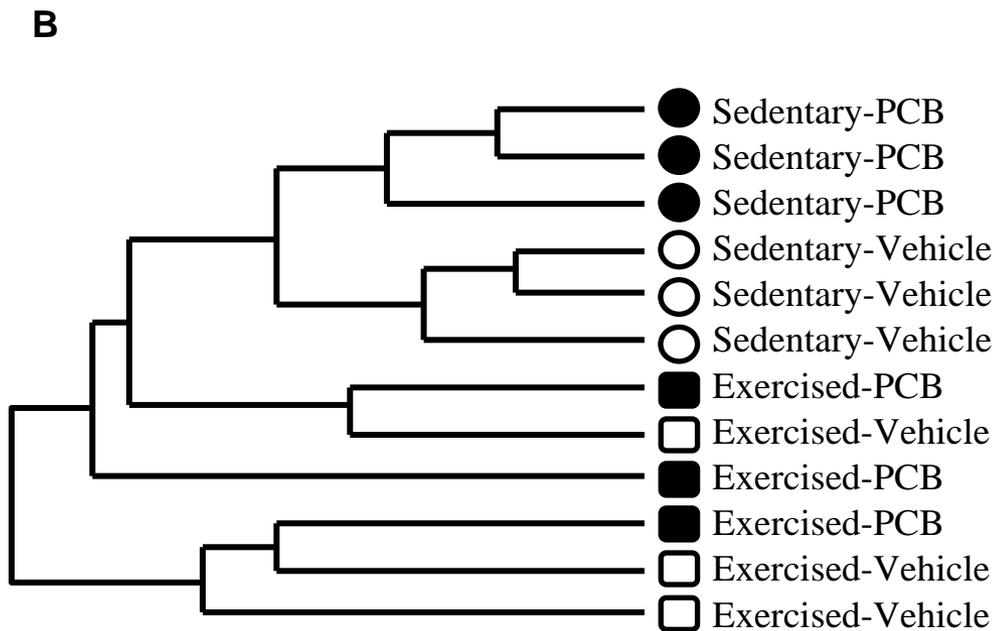
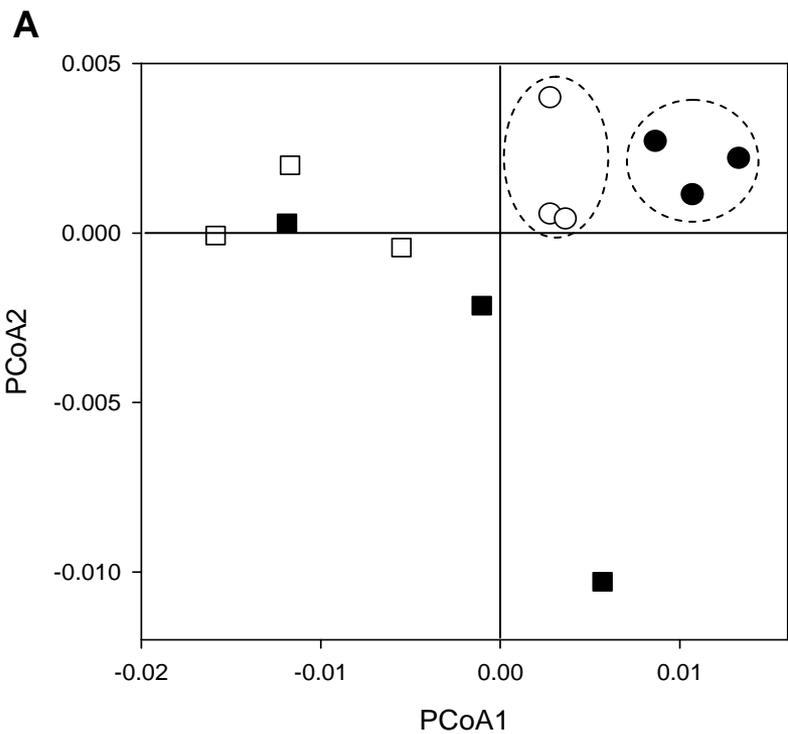


Figure 3