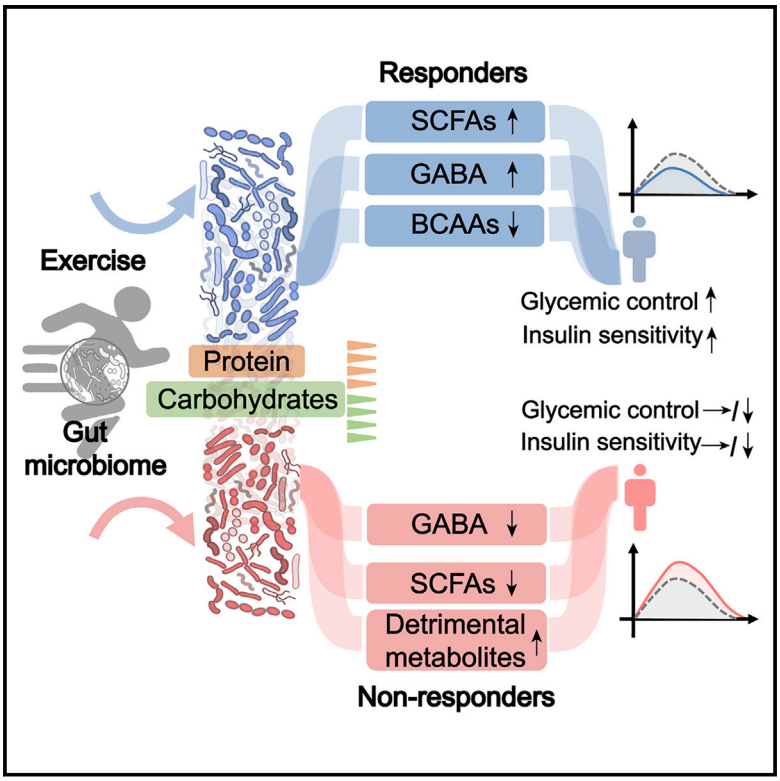
Clinical and Translational Report

Cell Metabolism

Gut Microbiome Fermentation Determines the Efficacy of Exercise for Diabetes Prevention

Graphical Abstract



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In Brief

Liu et al. identify the gut microbiota as an important determinant in the responsiveness of individuals with prediabetes to exercise for the improvement of glucose metabolism and insulin sensitivity. These findings may help in the implementation of a personalized lifestyle intervention for diabetes prevention.

Highlights

- A high variability in glycemic response to exercise in subjects with prediabetes exists
- Responders and non-responders exhibit differential alterations of the gut microbiota
- Gut microbiota from responders confers the metabolic benefits of exercise in mice
- Baseline microbiome features accurately predict personalized exercise responses

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Gut Microbiome Fermentation Determines the Efficacy of Exercise for Diabetes Prevention

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SUMMARY

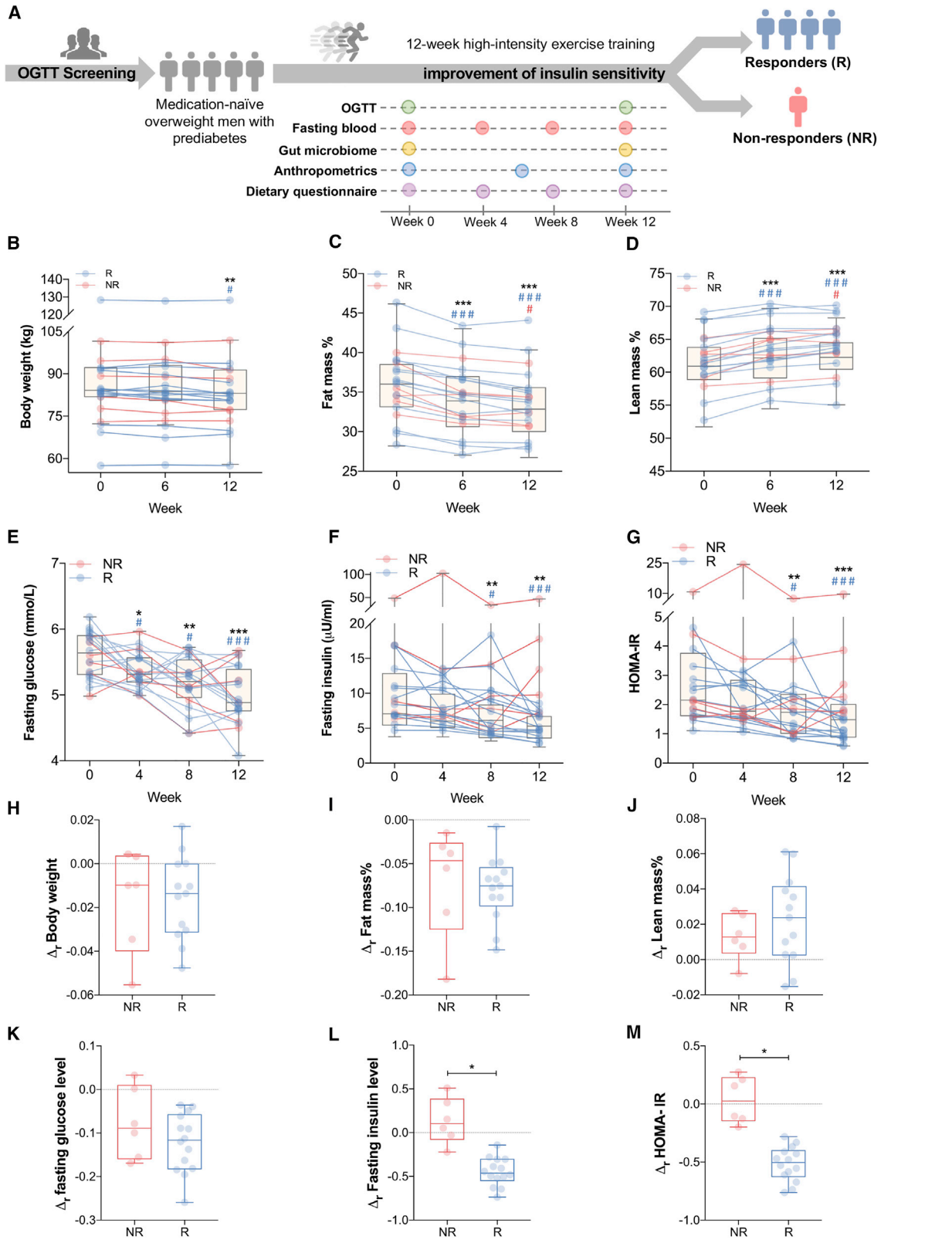
Exercise is an effective strategy for diabetes management but is limited by the phenomenon of exercise resistance (i.e., the lack of or the adverse response to exercise on metabolic health). Here, in 39 medication-naive men with prediabetes, we found that exercise-induced alterations in the gut microbiota correlated closely with improvements in glucose homeostasis and insulin sensitivity (clinicaltrials.gov entry NCT03240978). The microbiome of responders exhibited an enhanced capacity for biosynthesis of short-chain fatty acids and catabolism of branched-chain amino acids, whereas those of non-responders were characterized by increased production of metabolically detrimental compounds. Fecal microbial transplantation from responders, but not non-responders, mimicked the effects of exercise on alleviation of insulin resistance in obese mice. Furthermore, a machine-learning algorithm integrating baseline microbial signatures accurately predicted personalized glycemic response to exercise in an additional 30 subjects. These findings raise the possibility of maximizing the benefits of exercise by targeting the gut microbiota.

INTRODUCTION

Exercise is a cost-effective lifestyle intervention for the prevention and treatment of obesity, type 2 diabetes (T2D) and its complications, which are the leading causes of morbidity and mortality worldwide (Zheng et al., 2018). Despite the well-recognized benefits of exercise on metabolic homeostasis, the biomarkers and molecular transducers conferring its pleotropic effects remain poorly understood. Clinical implementation of exercise for diabetes management is still in its infancy, in part due to the high variability in physiological response to standardized exercise regimen. A large proportion of individuals, ranging from 7% to 69%, do not respond (non-responders) or even respond adversely to exercise in terms of insulin sensitivity and glucose homeostasis (Böhm et al., 2016). Though genetic predispositions and epigenetic modifications have been proposed to be potential contributors (Sparks, 2017),

Context and Significance

Exercise is the most cost-effective lifestyle intervention for the prevention and treatment of diabetes. However, its clinical implementation is hindered by the phenomenon of exercise resistance (i.e., non-response). Here, Aimin Xu and his colleagues show that the heterogeneous response to exercise in subjects with prediabetes with respect to changes in insulin sensitivity and glucose metabolism is linked to differential alterations of the gut microbiota. The microbiome of the responders exhibits enhanced capacity for the generation of short-chain fatty acids and increased breakdown of branched-chain amino acids, whereas the microbiome of non-responders is associated with an increased production of metabolically detrimental compounds. These results uncover the gut microbiota and their metabolites as important mediators of the benefits of exercise.



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neither the pathomechanisms nor potential predictors for the heterogeneity of exercise responsiveness have been clarified so far.

A growing body of evidence suggests that dysbiosis of gut microbiota plays an important role in the pathogenesis of insulin resistance and T2D (Bouter et al., 2017) through multiple mechanisms, including increased gut permeability and low-grade endotoxemia, changes in production of short-chain fatty acids (SCFAs) and branched-chain amino acids (BCAAs), and perturbation of bile acid metabolism (Utzschneider et al., 2016). Compositional and functional changes of gut microbiota have been observed in individuals with T2D and prediabetes (Allin et al., 2018; Qin et al., 2012), whereas fecal microbial transplantation from healthy donors into patients with metabolic syndrome results in increased microbial diversity and improved glycemic control, as well as insulin sensitivity (Kootte et al., 2017).

Recently, a modulatory effect of exercise on gut microbiota in both humans and animals has been observed. The microbiome of professional athletes exhibits higher diversity and more favorable metabolic capacity compared to sedentary counterparts (Barton et al., 2018; Clarke et al., 2014). Mice receiving exercise training also display favorable changes in the composition of gut microbiota, including reduced *Bacteroidetes*, but augmented *Firmicutes* and *Proteobacteria* (Choi et al., 2013). However, whether and how alterations in gut microbiota are functionally involved in the metabolic benefits of exercise remain obscure.

To address the above questions, we conducted a well-controlled exercise intervention in medication-naive overweight men with prediabetes, followed by comprehensive metagenomics and metabolomics analysis, and a functional interrogation in mice using fecal microbial transplantation to explore the roles of differentially shaped gut microbiota by exercise in glucose metabolism and insulin sensitivity. Furthermore, we developed a machine-learning algorithm that integrated baseline microbial signatures and evaluated its predictive performance for personalized exercise responsiveness in an independent validation cohort.

RESULTS AND DISCUSSION

Heterogeneous Glycemic Responses of Individuals with Prediabetes to High-Intensity Training

Eligible participants were randomized to either sedentary control or 12-week supervised exercise training (Figure S1), in which exercise responsiveness was further evaluated (Figure 1A). All participants were recommended to maintain their diet routine during the study period, which was closely monitored to ensure that no significant difference existed among all these subjects (Tables 1 and S1; Figure S2A).

After exercise intervention, a modest but significant reduction in body weight and adiposity, together with obvious improvements in insulin sensitivity, lipid profiles, cardiorespiratory fitness, and levels of adipokines functionally related to insulin sensitivity had been achieved in the whole exercise group (Table S1). However, in contrast to a homogenous change in body compositions (Figures 1B–1D), a high interpersonal variability in the alterations of fasting glucose, insulin, and the homeostatic model assessment of insulin resistance (HOMA-IR) was observed (Figures 1E–1G), suggesting a highly heterogeneous response of the cohort with respect to glucose homeostasis and insulin sensitivity. Therefore, we further classified the participants into responders (n = 14) and non-responders (n = 6), depending on whether they could demonstrate a decrease of HOMA-IR greater than 2-fold technical error, which is a threshold for true physiological adaptation (Álvarez et al., 2017; Hopkins, 2000). Notably, despite a homogeneous baseline characteristic (Table 1) and a similar degree of reduction in body weight and fat percentage between these two sub-groups (Figures 1H–1J), responders showed a remarkable 42.70% and 49.60% decrease in fasting insulin and HOMA-IR index, respectively (Figures 1L and 1M), as well as a striking 116.29% increase of Matsuda index (a comprehensive evaluation of both hepatic and peripheral insulin sensitivity derived from oral glucose tolerance test [DeFronzo and Matsuda, 2010]), whereas no obvious improvement or even deterioration in glucose homeostasis and insulin sensitivity was observed in non-responders (Table 1; Figures 1K–1M). Considering the important role of the gut microbiota in regulating glucose homeostasis and insulin sensitivity, we next explored whether it was involved in the heterogeneous metabolic effects of exercise in our cohort.

A Modest but Distinguishable Change of Gut Microbiota by Exercise Intervention in All Participants

We performed shotgun metagenome sequencing of fecal samples collected before and after the 12-week exercise regimen and generated 176G of high-quality sequencing data with an average of 29 million paired-end reads per sample. In contrast to the observation in murine models (Evans et al., 2014), no significant difference in alpha or beta diversity between the microbiome before and after exercise was observed (Figures S2B–S2D), possibly due to a more diversified environment in humans. Furthermore, we performed a compositional analysis and found that the relative abundances of 6 species, belonging to *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*, respectively, were significantly altered after exercise (Figure 2A). Moreover, species falling into the *Bacteroides* genus and *Clostridiales* order, most of which are involved in the production of SCFAs, underwent a significant strain-level genomic variation by exercise (Figure 2B). Importantly, none of the alterations observed above could be detected in sedentary controls sharing similar metabolic characteristics (Table S1; Figure S3). Co-abundance

Figure 1. High Interpersonal Variability in the Improvement of Insulin Sensitivity

(A) Schematic diagram of the study design.

(B–G) Boxplots (with median) showing the dynamic changes of (B) body weight and (C) fat mass and (D) lean mass body compositions at 0, 6, and 12 weeks of exercise intervention, as well as (E) fasting glucose, (F) fasting insulin and (G) HOMA-IR at 0, 4, 8, and 12 weeks of exercise, respectively. Lines connect the same subject at different time points. $^{\#}p < 0.05$ and $^{\#\#\#}p < 0.001$ by repeated-measures ANOVA within Responders or non-responders; and $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ by repeated-measures ANOVA within all subjects.

(H–M) The relative change of (H) body weight, (I) fat mass and (J) lean mass body compositions, (K) fasting glucose, (L) fasting insulin, and (M) HOMA-IR over 12 weeks of exercise intervention. Data were shown as mean ± SEM. $^{*}p < 0.05$ by repeated-measures two-way ANOVA.

Table 1. Changes in Clinical Parameters of Prediabetic Individuals in Response to 12-Week Exercise Intervention

	0-Week			12-Week			p Value[c]
Characteristics	Responders	Non-responders	p Value[a]	Responders	Non-responders	p Value[b]	Between Group Difference in Relative Change
Age (years)	43.29 ± 3.27	36.00 ± 4.55	0.228	–	–	–	–
BMI	28.78 ± 1.08	29.82 ± 1.75	0.603	28.65 ± 1.09	29.66 ± 1.78	0.850	0.613
Fat mass%	36.13 ± 1.33	35.78 ± 1.22	0.878	33.82 ± 1.22	33.53 ± 1.22	0.888	0.882
Lean mass%	60.98 ± 1.24	61.57 ± 1.10	0.776	63.26 ± 1.11	63.67 ± 1.09	0.856	0.799
WHR	0.95 ± 0.02	0.94 ± 0.01	0.730	0.93 ± 0.02	0.92 ± 0.01	0.761	0.702
Fasting glucose (mM)	5.65 ± 0.09	5.51 ± 0.13	0.426	4.95 ± 0.11	5.08 ± 0.21	0.311	0.980
2-h glucose (mM)	8.24 ± 0.33	8.46 ± 0.81	0.757	6.15 ± 0.39	6.64 ± 0.45	0.520	0.578
Fasting insulin (μU/mL)	10.37 ± 1.31	16.12 ± 6.67	0.522	5.70 ± 0.77	17.04 ± 6.26	3.84E−04	0.030
Matsuda index	5.83 ± 1.09	3.24 ± 0.78	0.097	11.01 ± 1.91	3.81 ± 0.78	0.002	0.017
Triglycerides (mM)	2.30 ± 0.21	2.41 ± 0.30	0.690	1.91 ± 0.19	1.86 ± 0.21	0.859	0.807
Total cholesterol (mM)	5.26 ± 0.31	5.60 ± 0.27	0.524	4.76 ± 0.26	5.49 ± 0.24	0.103	0.247
HDL-c (mM)	1.19 ± 0.05	1.10 ± 0.08	0.374	1.19 ± 0.06	1.26 ± 0.06	0.460	0.938
LDL-c (mM)	3.43 ± 0.27	3.81 ± 0.23	0.406	3.11 ± 0.22	3.71 ± 0.20	0.133	0.212
Systolic blood pressure (MmHg)	130.54 ± 4.74	131.33 ± 5.84	0.923	128.31 ± 4.47	128.50 ± 2.67	0.956	0.944
Diastolic blood pressure (MmHg)	80.00 ± 2.71	81.33 ± 2.04	0.759	78.08 ± 2.77	75.83 ± 3.94	0.452	0.912
Resting heart rate (bpm)	75.00 ± 2.02	83.00 ± 4.91	0.087	70.46 ± 1.92	80.83 ± 3.65	0.081	0.024
$VO_{2\ Max}$ (mL/kg/min)	25.27 ± 1.30	26.83 ± 1.38	0.485	31.42 ± 1.20	31.03 ± 1.85	0.861	0.755
Leg press (kg)	239.23 ± 11.79	226.67 ± 15.85	0.547	282.69 ± 17.97	270.83 ± 14.52	0.894	0.608
Chest press (kg)	32.46 ± 2.17	34.40 ± 3.41	0.642	38.92 ± 2.30	39.50 ± 2.97	0.886	0.546
hs-CRP (mg/L)	3.68 ± 0.46	3.39 ± 0.94	0.565	2.53 ± 0.32	2.59 ± 0.67	0.461	0.880
FGF21 (pg/mL)	380.95 ± 80.34	349.46 ± 79.59	0.921	235.78 ± 42.35	260.34 ± 55.79	0.746	0.843
Adiponectin (μg/mL)	7.15 ± 1.12	5.38 ± 0.84	0.417	10.46 ± 1.85	7.17 ± 1.20	0.595	0.375
Leptin (ng/mL)	12.50 ± 2.05	12.55 ± 1.58	0.611	8.98 ± 1.65	8.93 ± 1.93	0.781	0.694
Total energy (Kcal/d)	1,694.74 ± 59.99	1,567.84 ± 82.97	0.250	1,685.20 ± 51.93	1,585.59 ± 79.62	0.308	0.137
Carbohydrate (g)	270.21 ± 8.94	256.26 ± 11.69	0.386	266.96 ± 9.64	244.04 ± 10.55	0.178	0.177
% of Energy intake	64.43 ± 1.42	64.33 ± 1.26	0.968	63.86 ± 0.93	62.83 ± 2.81	0.659	0.708
Protein (g)	70.51 ± 3.57	69.01 ± 4.52	0.813	70.24 ± 2.30	71.81 ± 4.65	0.738	0.933
% of Energy intake	16.64 ± 0.46	17.50 ± 0.67	0.318	16.36 ± 0.25	17.33 ± 0.67	0.104	0.147
Fat (g)	35.76 ± 3.00	33.31 ± 2.86	0.629	39.86 ± 2.63	37.43 ± 5.80	0.663	0.490
% of Energy intake	18.93 ± 1.17	18.17 ± 1.22	0.704	20.07 ± 0.99	19.83 ± 2.81	0.920	0.747
Fiber (g)	9.30 ± 0.85	8.23 ± 1.32	0.500	9.25 ± 0.56	8.10 ± 0.54	0.235	0.238

BMI, body mass index; HDL-c, high-density lipoprotein cholesterol; hs-CRP, high-sensitive C-reactive protein; LDL-c, low-density lipoprotein cholesterol; WHR, waist-hip ratio. Responders (n = 14) and Non-responders (n = 6). Data are shown as means ± SEM.

[a]Determined by independent Student's t test.

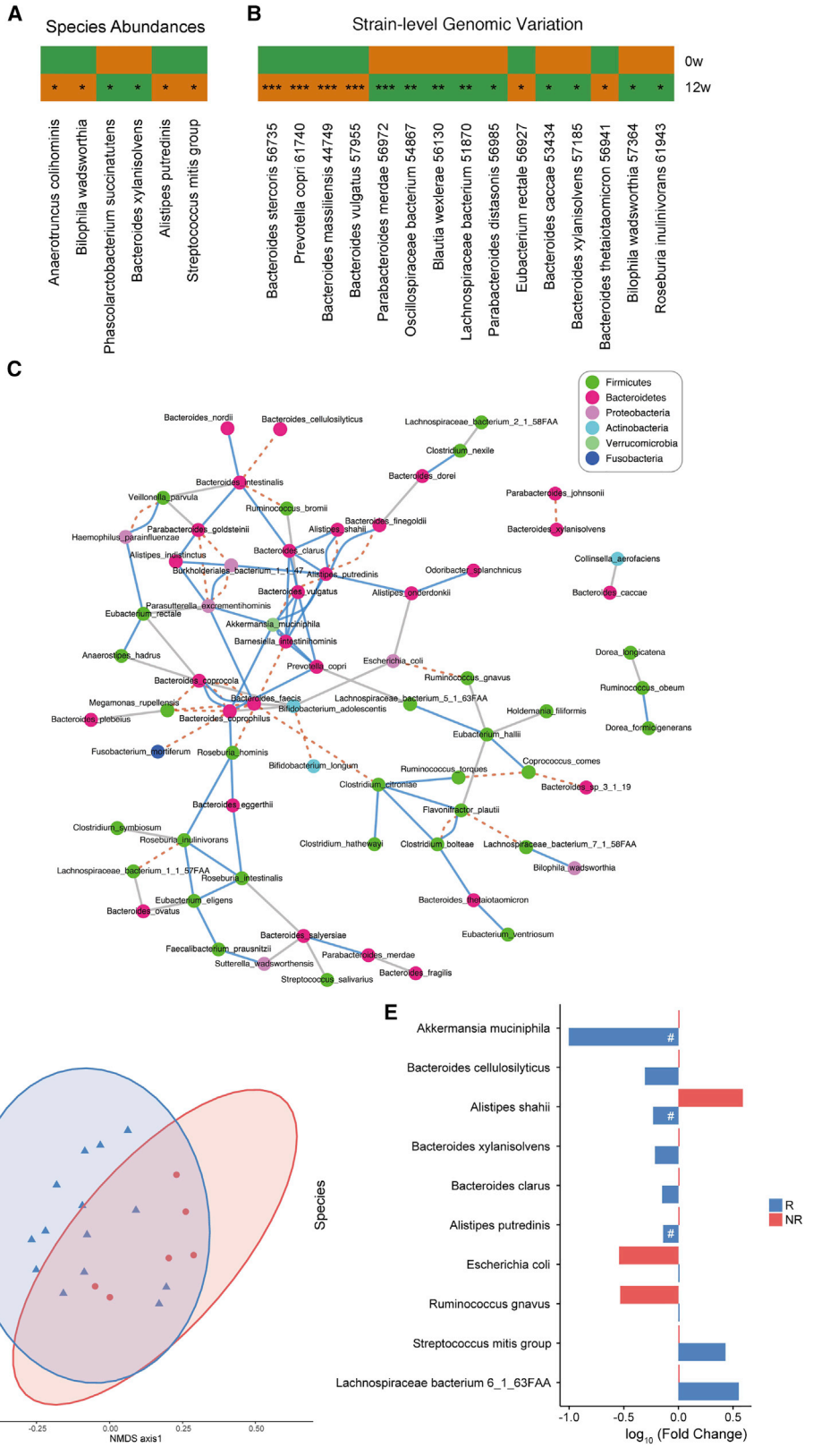
[b]Determined by ANCOVA model controlling for baseline measurements.

[c]Determined by repeated-measures two-way ANOVA.

network analysis further showed that species not necessarily sharing taxonomic similarities may co-occur when adapting to exercise intervention, as evidenced by increased network density and enhanced interactions centralizing around the significantly altered species, such as *Alistipes putredinis* (Figure 2C). In addition, the number of positive connections among those butyrate-producing genera within *Firmicutes* was obviously promoted, the decreased abundance of which is reported to be associated with obesity and T2D (Forslund et al., 2015). Furthermore, a few inter-phylum connections, such as between *Bacteroides* (*Bacteroidetes*) and *Clostridium* (*Firmicutes*) had also been identified (Figure 2C), suggesting an enhanced community interaction after exercise intervention.

Differential Alterations of Gut Microbiota between Exercise Responders and Non-responders

As there was a high interpersonal variability in the alteration of gut microbiota (Figures S4A–S4C), we next interrogated whether the heterogeneous responses to exercise in glucose homeostasis and insulin sensitivity were related to differential changes in gut



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microbiota between responders and non-responders. Similar to the observation in the whole exercise group, no remarkable difference in either alpha or beta diversity before and after exercise were identified in responders or non-responders (Figures S4D–S4L). However, we observed a significant segregation of dynamic alterations of gut microbiota between the two sub-groups (Figure 2D). Compared to responders, the microbial profiles of non-responders after 12-week exercise training shared more similarity with those of the sedentary controls ($p < 0.001$ for comparison of between-group weighted UniFrac distance), suggesting a maladaptation of gut microbiota in non-responders. Notably, we observed that the significant decrease of *Bacteroides xylanisolvens* and increase of *Streptococcus mitis group* found in all participants only occurred in responders, but not non-responders. Contrary to an increase of *Alistipes putredinis* in the whole group, this bacterium was found to be reduced in responders (Figure 2E; Table S2). In addition, responders were characterized by a 3.5-fold increase of *Lanchospiraceae bacterium* (a butyrate-producer), whereas non-responders were featured by nearly a 70% decrease in *Ruminococcus gnavus*, which has been reported to alleviate growth and metabolic impairments caused by transplanting microbiota from undernourished donors (Blanton et al., 2016). Moreover, *Alistipes shahii*, previously reported to be associated with inflammation and enriched in obese Japanese (Andoh et al., 2016), decreased by 43% in responders, but increased by 3.88-fold in non-responders (Figure 2E). When taking the growth dynamics of bacteria into consideration, responders were characterized by a decreased replication rate of *Prevotella copri*, a main bacterium responsible for the production of BCAAs and a contributor to insulin resistance (Pedersen et al., 2016), as well as an increased growth rate of several species in *Bacteroides* genus, most of which are propionate producers (Rivière et al., 2016) (Table S3). Collectively, these findings suggest that exercise intervention exerts differential modulatory effects on microbial compositions in responders and non-responders.

Associations of Taxonomic Alterations with Changes in Clinical Parameters Induced by Exercise Intervention

We next performed partial correlation analysis to investigate whether exercise-induced compositional changes in microbiota were associated with improvements in clinical parameters independent of body weight, fat mass, and visceral fat. We found that after adjustment for body weight and adiposity, associations between alterations of microbial species and improvements in insulin sensitivity-related indexes and a cluster of other metabolic features remained significant (Figure 3). At the community level, alteration in the gut microbiota was significantly associated with the percentage reduction of HOMA-IR ($p < 0.01$, ADONIS). Among the 19 species significantly correlated with the improvements of glucose homeostasis and insulin sensitivity, *Ruminococcus gnavus*, *Alistipes shahii*, *Streptococcus mitis group*, *Eubacterium hallii*, and *Escherichia coli* showed the strongest associations (Figure 3). Consistently, most of these species were also found to be differentially altered between responders and non-responders (Figure 2E). Taken together, the above findings imply that distinct changes of these species may underlie the difference in the improvement of glycemic homeostasis in response to a standard exercise regimen.

Exercise Promotes Divergent Functional Shifts in Gut Microbiome between Exercise Responders and Non-responders

To further understand how exercise-induced changes of gut microbiota modulate host metabolism, we annotated microbial genes to Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology (KOs). In total, 214 KOs were significantly increased, whereas 14 KOs were decreased after 12-week exercise training, most of which fall into the "carbohydrate metabolism" and "amino acid metabolism" functional pathways. Moreover, a total of 10 pathways were found to be significantly altered by exercise training, such as "methane metabolism" and "carbon metabolism." Notably, exercise responders and non-responders exhibited a clear segregation of microbial functional variations (Figure 4A) and divergent pathway enrichment (Figure 4B). Consistent with an enhanced coordination and communication within the microbial community (Figure S5), responders were dominated by genes involved in quorum sensing, a regulatory system that allows bacteria to share information about density and to adjust gene expression accordingly (Miller and Bassler, 2001). In line with the findings in professional athletes (Barton et al., 2018), pathways involved in DNA replication and amino acid metabolism were preferentially enhanced in responders. Moreover, distinct responses with respect to glycan biosynthesis and lipid metabolism also existed between the two subgroups (Figure 4B).

Interestingly, although genes participating in several pathways belonging to microbial proteolysis were enriched in both subgroups, amino acid fermentation was shifted to production of colonic gases or metabolically detrimental compounds in non-responders, but to biosynthesis of SCFAs in responders (Figure 4C). Abundances of genes involved in the production of phenolic derivatives (indole and p-cresol) and sulfate from aromatic and sulfur-containing amino acids (SAAs) respectively were selectively augmented in non-responders, providing ample

Figure 2. Exercise Promotes Differential Alteration of Gut Microbiota in Responders and Non-responders

(A and B) Alterations of gut microbiota in response to exercise intervention in all participants. Significant changes in (A) the relative abundances of bacterial species and (B) strain-level genomic variations before and after exercise intervention in all participants. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ by Wilcoxon signed-rank test. Green and orange indicate low and high relative abundance in (A) and low and high Jaccard distance in (B) respectively.

(C) Co-abundance network before and after exercise intervention in all participants. The edges indicate statistically significant ($p < 0.05$) Spearman correlations of >0.6 or <-0.6 between species present in at least 60% samples. The nodes are colored based on their affiliated phyla. Orange dashed and blue edges indicate positive correlations at baseline and after exercise, respectively. Gray edges indicate negative correlations.

(D and E) Participants were further classified into responders (R, blue) and non-responders (NR, red) based on the relative improvement of HOMA-IR. (D) Non-metric multidimensional scaling analysis plot of taxonomic variation induced by exercise training in R and NR, respectively. $p = 0.008$ by ADONIS test between R and NR. (E) Significantly altered species ($p < 0.05$) caused by exercise intervention in R and NR, respectively. Fold change was defined as the ratio of relative microbial abundance after exercise to those at baseline and the log fold changes were set to zero if not statistically significant. #FDR < 0.25.

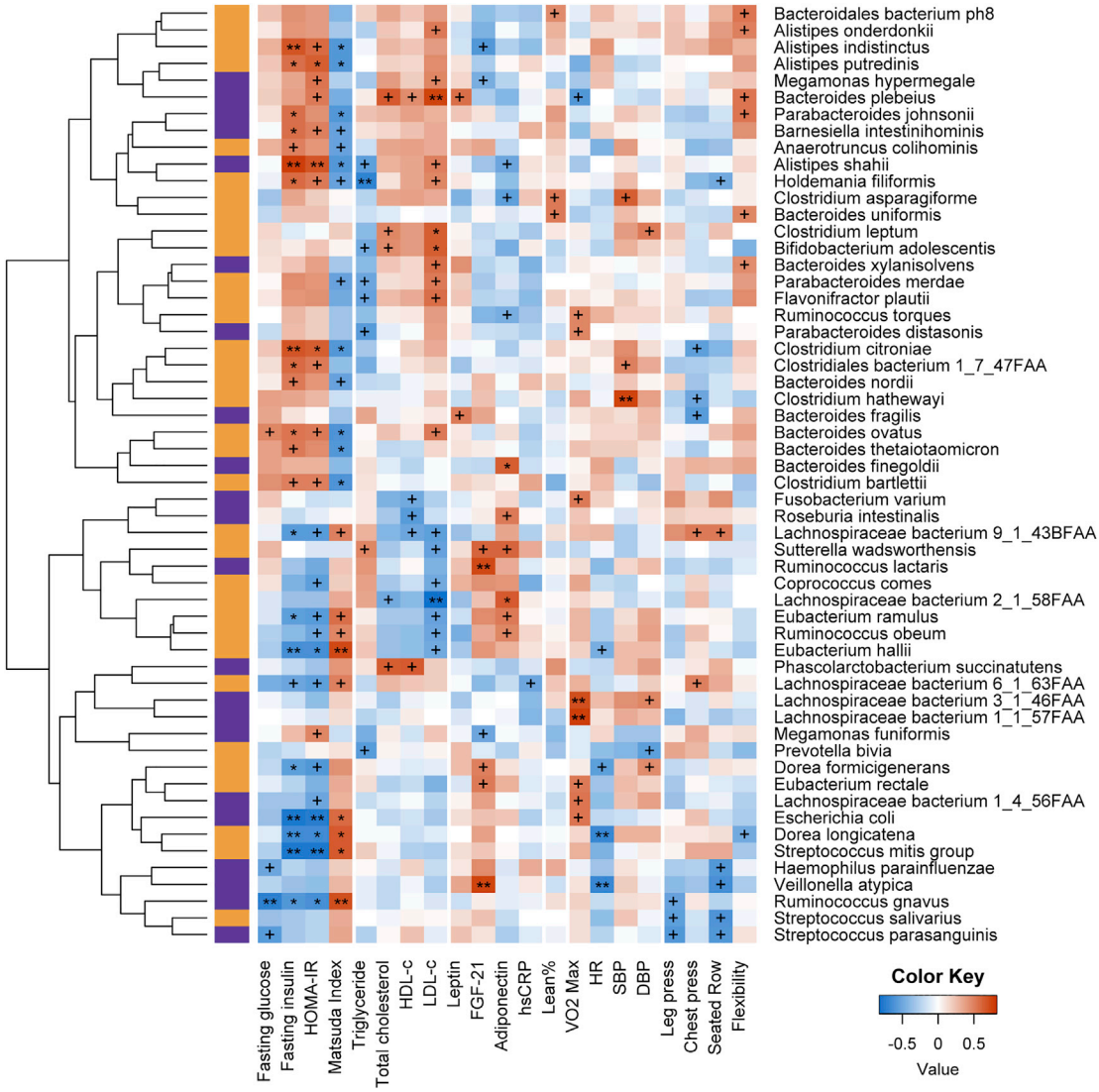

Figure 3. Exercise-Induced Alterations of Microbial Species Are Closely Associated with Improvements of Clinical Indices Independent of Body Weight and Adiposity

Heatmap of the Spearman's correlation coefficients between changes in different clinical indices and taxonomic alterations caused by exercise intervention after adjustment for body weight, fat mass, and waist-to-hip ratio. $^{+}p < 0.05$, *FDR < 0.1, and **FDR < 0.05. Yellow and purple in the far left column indicate increased and decreased relative abundance, respectively. Only species with significant correlations (at least one based on FDR or two based on raw p value) were shown.

substrates for the formation of indoxyl sulfate and p-Cresyl sulfate (toxic metabolites which promote oxidative stress and inflammation (Vanholder et al., 2014)). Additionally, capacities for production of glutamate and SAAs were also significantly elevated specifically in the microbiome of non-responders (Figure 4C, lower panel). Given the inhibitory effects of these metabolites on colonocyte mitochondrial bioenergetic activity (Andriamihaja et al., 2015), adipose tissue lipolysis and antioxidant defense system (Bunyan et al., 1976; Stone et al., 2014), such changes might attenuate the ameliorative effects of exercise on insulin resistance. In contrast, genes participating in the catabolism of BCAAs, the rise of which promote insulin resistance (Newgard et al., 2009), were significantly increased only in responders. Moreover, genes encoding enzymes responsible for the degradation of amino acids that maintain insulin sensitivity, such as glycine (the decrease of which usually occurs before clinical manifestations of T2D [Adeva-Andany et al., 2018]), were highly abundant in non-responders. However, based on significantly higher abundance of L-threonine ammonia-lyase (K01754), it appeared that glycine was transformed into 2-oxobutanoate, a substrate for propionate biosynthesis in responders. Similarly, higher abundance of L-ornithine ammonia-lyase (K01750) in non-responders and acylamide amidohydrolase (K01426) in responders respectively indicate that arginine was transformed into proline (a detrimental end product) in non-responders, but into γ-aminobutyric acid (GABA, a modulator of glucose homeostasis [Purwana et al., 2014]) in responders (Figure 4C), suggesting a more favorable utilization of amino acids in those who enjoyed a better

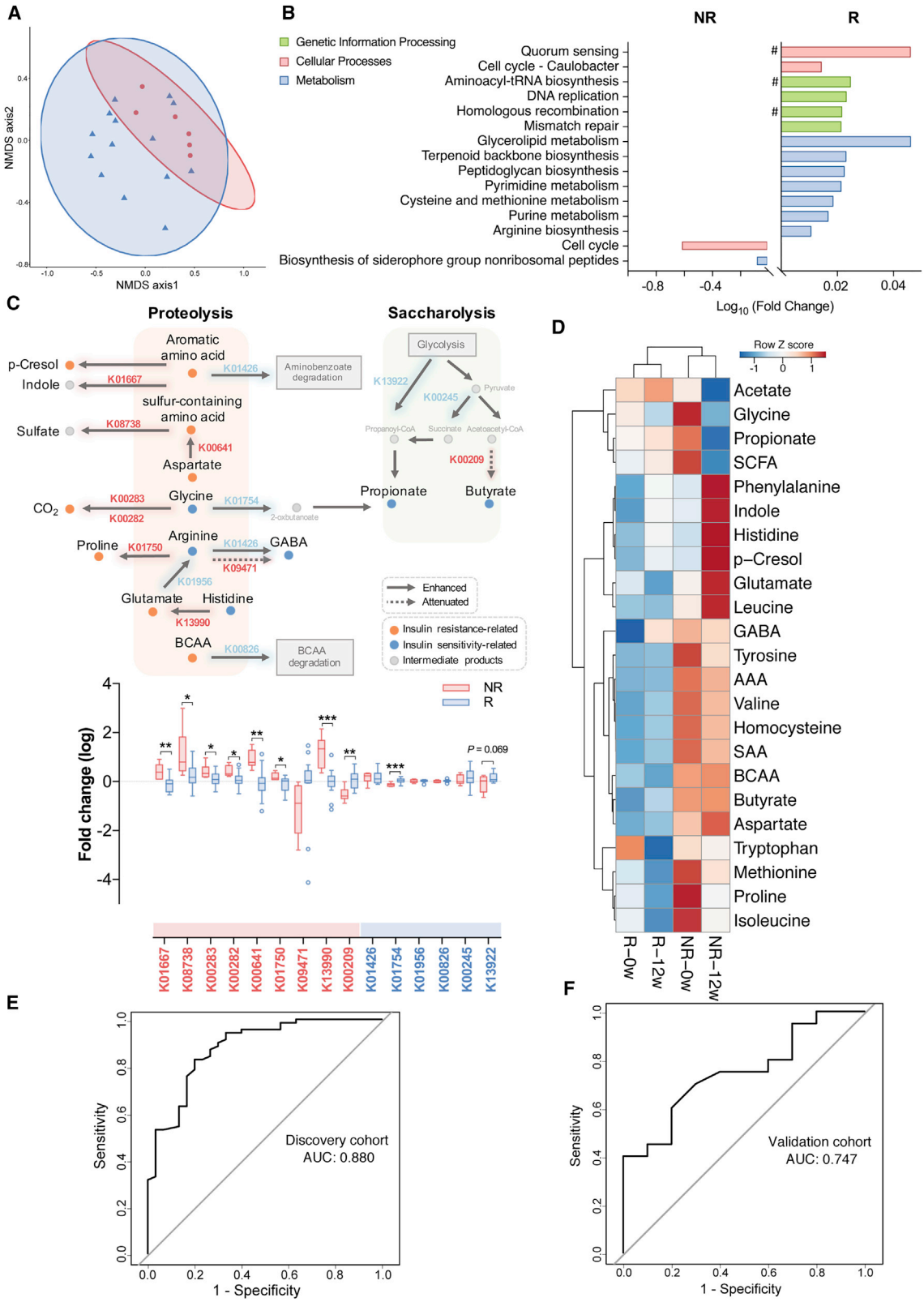

Figure 4. Exercise Promotes Distinct Functional Shifts of Gut Microbiota and Microbial Metabolites in Responders and Non-responders

(A) NMDS analysis plot of functional alterations caused by exercise intervention in responders (R, Blue) and non-responders (NR, Red), respectively. p = 0.011 by ADONIS test between R and NR.

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metabolic flexibility. Furthermore, exercise intervention led to differential changes in saccharolytic fermentation in the two subgroups (Figure 4C). Genes involved in propionate biosynthesis from glycolytic products, were significantly increased only in responders, whereas genes responsible for the biosynthesis of butyrate were selectively attenuated in non-responders. Taken together, these data demonstrate that differential capacity for carbohydrate fermentation and amino acid catabolism shaped by exercise might contribute to the difference in the amelioration of insulin resistance in the two subgroups.

Distinct Sets of Microbial Metabolites between Exercise Responders and Non-responders

As the fecal metabolome provides a functional readout of microbial activity and can be used as an intermediate phenotype mediating host-microbiome interaction (Zierer et al., 2018), we next performed targeted metabolomics analysis of fecal samples to interrogate whether exercise-induced changes in gene abundance of gut microbiota led to distinct alterations of microbial metabolites in the two subgroups. This analysis showed that the trend of changes in the metabolites of amino acid catabolism and carbohydrate fermentation was largely consistent with the altered patterns of genes encoding the metabolic enzymes (Figures 4C and 4D). Specifically, abundances of amino acids that hamper insulin sensitivity, such as BCAAs and aromatic amino acids (AAAs, especially tryptophan), were significantly decreased by exercise intervention only in responders, which was in line with the selective enhancement of the capacity for BCAAs and AAAs degradation. Additionally, in agreement with the metagenomics data that linked the functional repertoire to increased capacity for biosynthesis of SCFAs and GABA in responders, fecal abundances of propionate and GABA were significantly increased in responders, but showed an opposite trend of changes in non-responders by 12-week exercise intervention. On the other hand, consistent with an enhanced capacity for the synthesis of glutamate but impaired capacity for its conversion into GABA in non-responders (Figure 4C), a significant increase of glutamate but decrease of GABA occurred selectively in non-responders after exercise intervention (Figure 4D). Consistent with the alterations in fecal metabolites, increased circulating levels of SCFAs, but decreased concentrations of BCAAs and AAAs were observed only in responders after exercise intervention, whereas the circulating levels of these metabolites in non-responders displayed an opposite trend of changes (Table S4). Taken together, differential alterations of these microbial metabolites may underlie the distinct metabolic responses to exercise intervention in responders and non-responders.

Considering the differential alterations between responders and non-responders at taxonomic and metabolomics levels by exercise intervention, we next interrogated whether subtle differences in baseline microbial signatures could account for the divergent changes and whether it could be integrated into an algorithm to predict the individualized exercise responsiveness. To this end, a random forest algorithm integrating baseline microbial features was first developed in the discovery cohort and achieved an area under the receiver operating characteristic (ROC) curve (AUC) of 0.880 (Figure 4E). The most informative features contributing to this classifier included *Bacteroides xylanisolvens*, *Bacteroides cellulosilyticus*, and GABA (Figure S6), whose abundances were significantly different between responders and non-responders at baseline, suggesting that in response to a standard exercise regimen, subtle differences in microbial signature might initiate distinct metabolic cascades.

Second, the performance of this model was further evaluated in an independent validation cohort of 30 medication-naive individuals with prediabetes sharing similar characteristics with those in the discovery cohort (Table S5). Participants in this validation cohort were subjected to the same training protocol as those in the discovery cohort and had a similar non-response rate to exercise with respect to improvement in insulin sensitivity (30% in the discovery cohort and 33.3% in the validation cohort, respectively). Notably, the classification model, derived solely using the discovery cohort, achieved an AUC value of 0.747 (Figure 4F) for the discrimination between responders and non-responders in this independent validation cohort.

Exercise-Conditioned Microbiota from Responders Ameliorate Glucose Intolerance and Insulin Resistance in Obese Mice

To further examine the causal relationship between differentially shaped microbiota and changes in glucose metabolism and insulin sensitivity by exercise intervention, conventional antibiotics-treated mice were transplanted with microbiota from two responders and two non-responders collected at both baseline and after exercise intervention (Figure 5A). A number of microbial species, such as *Alistipes shahii*, *Alistipes putredinis* and *Ruminococcus gnavus*, demonstrated a similar trend of changes between recipient mice and human donors (Figure S7). Mice colonized with microbiota from responders and non-responders after exercise displayed a similar trend of changes in body composition, oxygen consumption, and respiratory exchange ratio compared to those receiving microbiota from the same donors taken at baseline (Figures 5B–5D). On the other hand, significant reductions in glucose and insulin levels, as well as obvious improvements in glucose disposal during glucose and insulin tolerance tests were observed only in mice transplanted with microbiota from responders, but not non-responders after exercise training (Figures 5E–5I). Moreover, hyperinsulinemic-euglycemic clamp analysis showed remarkable increases in glucose infusion rate (as measured by the amount of exogenous glucose required

(B) Significantly altered pathways ($p<0.05$) induced by exercise intervention in R and NR, respectively. The bars were colored based on the 1st level affiliated pathway class in the KEGG pathway maps. The log fold changes were set to zero if not statistically significant. #FDR < 0.15.

(C) Illustration of distinct carbohydrate and amino acids fermentation in R and NR (upper) and the relative changes of corresponding KOs (lower). Blue and red indicate significant alterations selectively in R and NR respectively. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ by Wilcoxon rank-sum test between R and NR.

(D) Heatmap showing the microbial metabolites in fecal samples from R and NR before and after exercise intervention. The colors changing from blue to red indicate higher abundance.

(E and F) The receiver operating characteristic (ROC) curves and area under curve (AUC) of the microbiome-based algorithm for the discrimination between responders and non-responders in (E) discovery and (F) validation cohort.

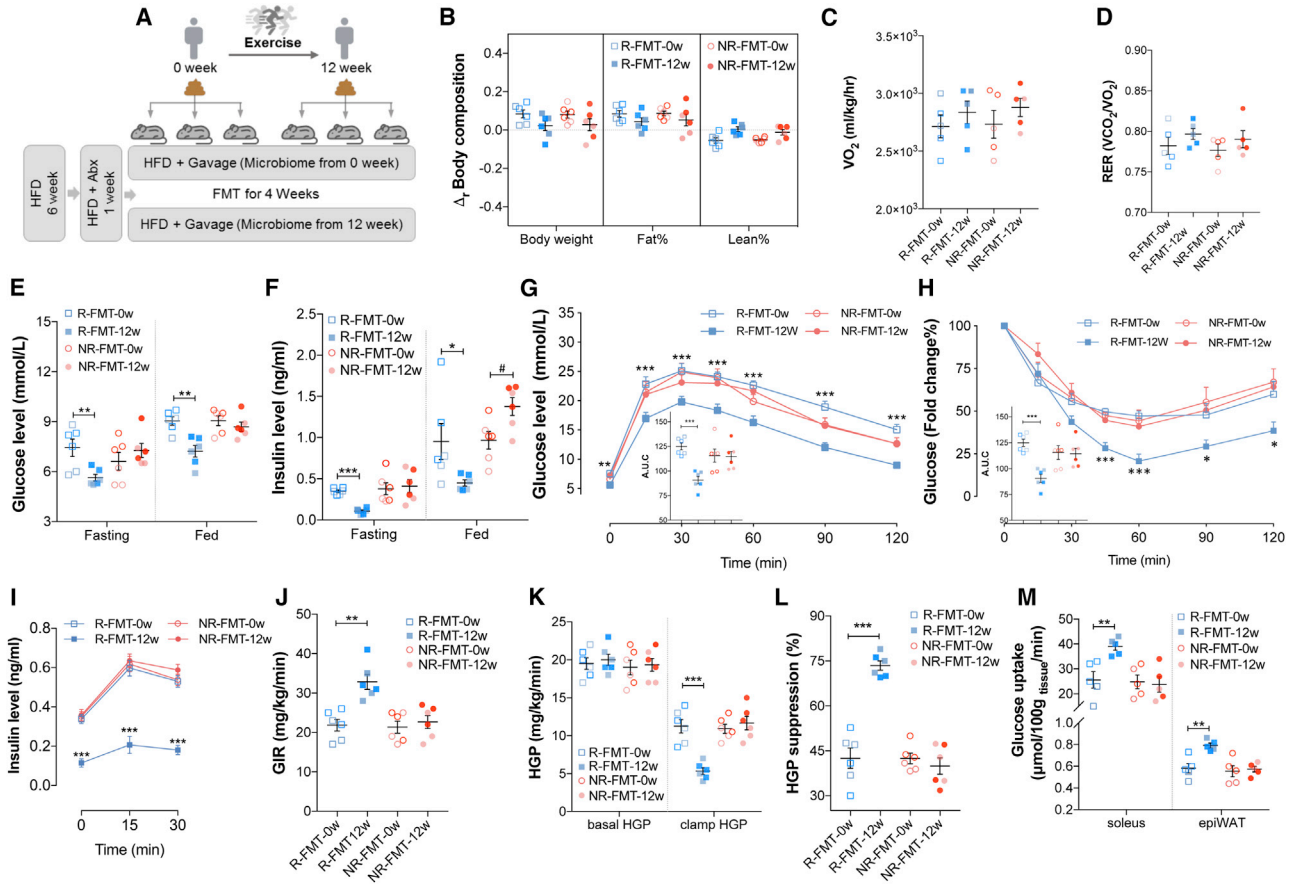

Figure 5. Transplantation of Human Fecal Microbiota into Mice Mimics the Effect of Exercise on Glucose Homeostasis and Insulin Sensitivity

(A) Schematic diagram showing the study design for fecal microbial transplantation.

(B) Relative changes of body compositions.

(C and D) Recipient mice were subjected to indirect calorimetry analysis for the determination of (C) oxygen consumption and (D) respiratory exchange ratio after 4 weeks of microbial colonization.

(E and F) Glucose (E) and insulin (F) levels at both fasting and fed status.

(G and H) Glucose tolerance test (G) and insulin tolerance test (H) with area under the curve (AUC).

(I) Insulin concentration after an oral glucose load.

(J–M) Hyperinsulinemic-euglycemic clamping studies were performed in recipient mice after 4 weeks of microbial colonization. (J) glucose infusion rate, (K) hepatic glucose production (HGP) under basal and insulin-stimulated conditions, (L) percent suppression of HGP by insulin, and (M) glucose uptake in soleus muscle (left) and epididymal adipose tissue (right).

Data were expressed as mean ± SEM (n = 6 mice/group). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ between R-FMT-0w and R-FMT-12w; $^{\#}p < 0.05$ between NR-FMT-0w and NR-FMT-12w by unpaired Student's t test. Dots with different colors represent individual mice receiving FMT from different human donors.

for maintaining euglycemia) as well as in insulin-stimulated suppression of hepatic glucose production and glucose uptake in soleus muscle and epididymal adipose tissue in mice colonized with microbiota from responder donors after exercise compared to those receiving microbiota from the same donors collected at baseline (Figures 5J–5M). In contrast, these parameters remained unchanged in mice gavaged with microbiota from exercise non-responders. Collectively, these findings suggest that gut microbiota from responders confers the metabolic benefits of exercise on glucose homeostasis and insulin sensitization in peripheral tissues.

In line with the distinct sets of microbial metabolites observed in humans, mice receiving fecal microbial transplantation (FMT) from responders after exercise demonstrated a significantly increased abundance of circulating SCFAs, but decreased levels of BCAAs and AAAs compared to those receiving FMT from the same donors collected at baseline, whereas opposite changes in these metabolites were observed in mice gavaged with microbiota from non-responders after exercise in comparison to those colonized with microbiota from the same donors collected at baseline (Table S6). Furthermore, supplementation with BCAAs dampened the beneficial effects of FMT from exercise responders on the alleviation of glucose dysregulation and insulin resistance (Figures 6A–6F), whereas replenishment with SCFAs in mice colonized with microbiota from non-responders partially rescued the non-responsiveness in glucose homeostasis and insulin sensitivity (Figures 6G–6L). Taken together, these findings further support the biological relevance of these differentially altered microbial metabolites in the heterogeneous responses to exercise intervention.

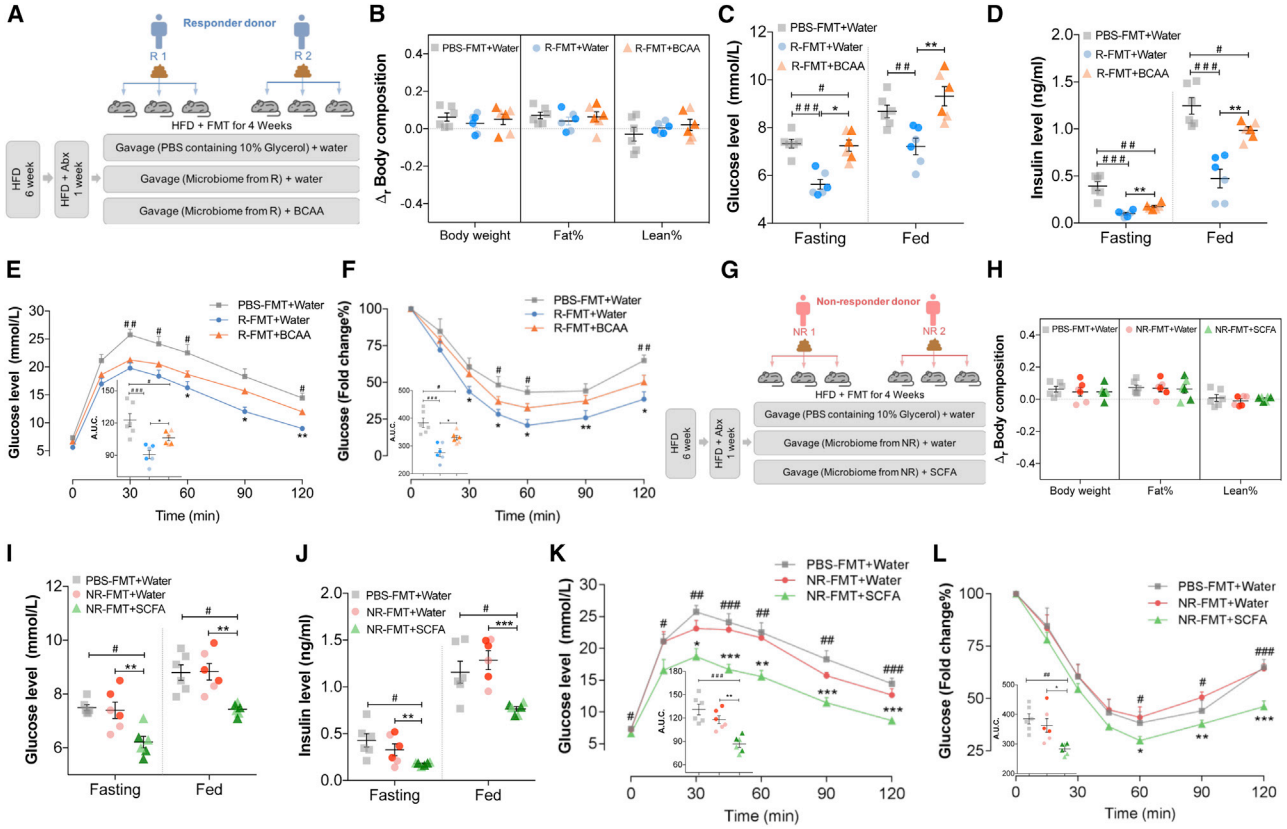

Figure 6. Supplementation with BCAAs Dampens the Metabolic Benefits of Microbiota from Responders while Replenishment with SCFAs Partially Restores the Effects of Microbiota from Non-responders on Insulin Sensitivity

(A–F) Supplementation of BCAAs in mice receiving FMT from responder donors. (A) Schematic diagrams of the study design. (B) Relative changes of body compositions. (C) Glucose and (D) insulin levels at both fasting and fed status. (E) Glucose tolerance test and (F) insulin tolerance test with AUC. *$p < 0.05$, **$p < 0.01$ between R-FMT + water and R-FMT + BCAAs; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ when compared with PBS-FMT + water.

(G–L) Supplementation of SCFAs in mice receiving FMT from non-responder donors. (G) Schematic diagrams of the study design. (H) Relative changes of body compositions. (I) Glucose and (J) insulin levels at both fasting and fed status. (K) Glucose tolerance test and (L) insulin tolerance test with AUC. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ between NR-FMT + water and NR-FMT + SCFAs; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ when compared with PBS-FMT + water.

Data were expressed as mean ± SEM (n = 6 mice/group). Significance was calculated by ANOVA followed by Turkey's multiple comparison tests. Dots with different colors represent individual mice receiving FMT from different human donors.

In this well-controlled interventional study conducted in medication-naïve individuals with prediabetes, we showed that gut microbiota was an important mediator conferring the effect of exercise on glucose metabolism and insulin sensitivity. The high interpersonal variability in the adaptive changes upon exercise intervention was attributed to divergent functional alterations of gut microbiota, leading to production of distinct sets of microbial metabolites. These findings were further strengthened by animal studies showing that exercise-induced differential changes in glucose homeostasis, insulin sensitivity, and metabolites in responders and non-responders can be transferred into mice by FMT.

Despite the overall metabolic benefits of exercise intervention, ~30% of our prediabetic participants responded poorly to exercise in terms of improvement in glycemic control and insulin sensitivity, a portion comparable to previous studies with similar designs (Böhm et al., 2016). Although heterogeneous degrees of adaptive changes in response to exercise intervention have been observed in many clinical studies, the underlying pathophysiology remains elusive. In this regard, our present study uncovered a maladaptation of the gut microbiota as an important pathomechanism for exercise resistance to glycemic control and insulin sensitization. This notion was supported by our findings that (1) there was a clear segregation in compositional and functional changes of gut microbiota between responders and non-responders in response to exercise intervention, accompanied by distinct alterations of microbial metabolites; and (2) colonization of mice with microbiota from non-responders led to no change in glycemic control and insulin sensitivity, in contrast to the substantial improvement in mice gavaged with microbiota from responders. Notably, mice transplanted with microbiota from responders and non-responders exhibited a similar degree of body weight reduction, despite divergent changes in glucose metabolism and insulin sensitivity. These data were in line with our clinical observation that exercise-associated weight loss in all participants was homogenous and modest (~2%), which did not reach the threshold required for improvement of metabolic functions in overweight and/or obese individuals with

insulin resistance (Magkos et al., 2016). These findings, together with the fact that the correlations between alterations of microbial species (such as *Alistipes shaii* and *Streptococcusmitis group*) and improvement of insulin sensitivity remain significant after adjustment for body weight and adiposity, suggest that gut microbiota mediates the effect of exercise on glucose metabolism independent of body weight and fat mass reduction.

As a promising probiotic, multiple health-promoting effects of *Akkermansia muciniphila* have been widely reported in both cross-sectional studies (Cani and de Vos, 2017) and clinical trials (Depommier et al., 2019). Interestingly, the relative abundance of *A. muciniphila* was found to be decreased after exercise training but remained consistently higher in responders throughout the study period (Figure 2E; Table S2). Despite its metabolic benefits, such a reduction was consistent with the observation in a calorie restriction trial in obese adults (Dao et al., 2016), raising the possibility that as a synergistic ecosystem, decreased relative abundance of *A. muciniphila* might be an adaptive response to exercise intervention, which in turn contributes to the expansion of other beneficial species and ultimately acts in concert to promote metabolic health. However, at current stage we cannot exclude the possibility that the paradoxical reduction was due to the differences in methodologies employed for metagenomic analysis (Nielsen et al., 2014) or the lack of absolute quantification with microbial load, which has been reported to influence the observed alterations in microbiota (Vandeputte et al., 2017).

Despite a modest alteration at taxonomic levels, significant changes at functional levels were identified through the integration of metagenomics and metabolomics. Consistently, several reports have found that a mild alteration in microbial composition is sufficient to drive significant functional changes (Bercik et al., 2011; Li et al., 2009). Our findings further reinforce the notion that the functional capacity of gut microbiota can be significantly altered without major shifts in its community structure, and that changes in host phenotype are dependent on the metabolic capacity and metabolites of the microbiome, instead of the composition per se. More importantly, we identified shifts in microbial fermentation preference as an underlying cause for the divergent response of glycemic control and insulin sensitivity to exercise intervention.

SCFAs are a major class of microbial metabolites that play a critical role in host metabolism and immunity through both local and systemic actions on multiple targets (Koh et al., 2016). Intriguingly, elevated abundance of *Eubacterium hallii* and *Coprococcus comes* selectively in the microbiome of responders were mirrored by enhanced capacity for biosynthesis of SCFAs from both carbohydrate and amino acids in responders, which was decreased in non-responders after exercise intervention. Several animal studies have consistently demonstrated the metabolic benefits of butyrate or propionate treatment in energy expenditure and glucose homeostasis (De Vadder et al., 2014; Lin et al., 2012). In humans, propionate supplementation for 7 weeks reduced fasting glucose and increased insulin secretion during glucose tolerance test (Venter et al., 1990). Mechanistically, SCFAs (especially propionate) have been shown to induce the secretion of Peptide YY and glucagon-like peptide-1 (GLP-1), two enteroendocrine hormones critical for energy balance and glucose homeostasis (Psichas et al., 2015). Consistently, two recent interventional trials suggest that the metabolic benefits of dietary fibers on insulin resistance and T2D are attributed to increased microbial production of SCFAs and elevated GLP-1 secretion (Upadhyaya et al., 2016; Zhao et al., 2018). Additionally, the glucose-lowering effect of the anti-diabetic drug metformin is also mediated in part by increased microbial biosynthesis of butyrate and propionate (Wu et al., 2017). Therefore, fermentation into SCFAs may represent a common pathway whereby dietary, pharmacological, and exercise interventions exert metabolic benefits through modulation of gut microbiota.

In both rodents and humans, responders were characterized by reduced level of BCAAs, a well-established mediator of insulin resistance mainly by activation of the mammalian target of rapamycin complex-1 signaling pathway (Yoon, 2016). High levels of BCAAs, together with AAAs, are reported to be independent biomarkers for insulin resistance and diabetes (Guasch-Ferré et al., 2016). Conversely, improved insulin sensitivity resulting from Mediterranean diet intervention (Ruiz-Canela et al., 2018) and bariatric surgery (Lips et al., 2014) in obese and insulin resistant individuals was accompanied by a decrease of BCAAs. Enhanced microbial biosynthesis of BCAAs by *Prevotella copri* was suggested to be an important contributor to its elevated circulating levels in insulin resistant individuals (Pedersen et al., 2016). As one of the preferred amino acid substrates of colonic bacteria, genes involved in BCAAs catabolism are also harbored by a cluster of microbial species (Kazakov et al., 2009). In this connection, our findings showed that exercise-induced decrease of BCAAs preferentially in responders was attributed to a combined effect on reduced replication rate of *Prevotella copri* and promotion of genes involved in the degradation of BCAAs.

In contrast to the increase of beneficial microbial fermentation products in responders, a cluster of metabolically detrimental metabolites, including glutamate, AAAs, proline, and p-cresol, all of which are reported to be elevated in insulin resistant individuals (Guasch-Ferré et al., 2016; Liu et al., 2017; Nakamura et al., 2014), were accumulated only in non-responders after exercise intervention. Particularly, glutamate is able to induce obesity and insulin resistance in rodents possibly by impairing lipolysis (Bunyan et al., 1976). Reduced abundance of *Bacteroides thetaiotaomicron* (a bacterium responsible for the catabolism of glutamate), together with an elevated glutamate in the circulation has been reported recently in obese Chinese, which can be reversed by sleeve gastrectomy accompanied with an alleviation of hyperglycemia and insulin resistance (Liu et al., 2017). Moreover, several microbial species, such as *Bacteroides* species can convert glutamate into GABA, which is able to increase glucose tolerance and insulin sensitivity by suppressing metabolic endotoxemia and protecting pancreatic β cells (Purwana et al., 2014; Tian et al., 2011). Consistently, our data showed that elevated glutamate in non-responders was accompanied by a decreased level of GABA after exercise intervention, suggesting that impaired microbial conversion of glutamate into GABA may underlie the failure of non-responders to exercise-induced improvement in glucose homeostasis and insulin sensitivity.

Although there was no obvious difference in baseline microbial structures between responders and non-responders, we were able to establish a model based on the microbiome signatures before exercise to accurately predict the exercise outcomes with respect to glycemic control and insulin sensitivity, raising the possibility of screening for individuals with high likelihood

of exercise resistance using gut microbiota, so that personalized adjustments can be implemented in time to maximize the efficacy of exercise intervention. Interestingly, personal microbiome signatures have recently shown to be a reliable predictor for the high inter-individual variability in postprandial glucose after identical meals (Zeevi et al., 2015), and also for the efficacy of barley kernel-based bread intervention on glycemic control (Kovatcheva-Datchary et al., 2015). Taken together, these findings uncover the diversity of gut microbiota as a key determinant for the variability of glycemic control after dietary and exercise intervention. However, how exercise imposes such a differential impact on the composition and function of gut microbiota remains unclear and warrants further investigation. We speculate that exercise may amplify subtle difference of gut microbiota at baseline by remodeling the intestinal microenvironment (such as inflammatory and oxidative status and local immunity) critical for microbial growth and interaction, which ultimately lead to a divergent response of glycemic control to exercise intervention.

In conclusion, our study uncovers gut microbiota and its metabolism as key molecular transducers to the heterogeneous adaption to exercise intervention on glucose metabolism and insulin sensitivity. This finding, together with our demonstration of the predictive value of baseline microbial signatures for individualized responsiveness to exercise, may facilitate clinical implementation of personalized lifestyle intervention for diabetes management.

Limitations of Study

The main limitation of the current study is a relatively small sample size and rigid inclusion criteria for our study participants, which constrains the applicability of this result (Richter et al., 2010). Though limited only to Chinese males, these results demonstrate the adequacy of this non-invasive proxy measurement in the prediction of exercise responsiveness. Considering the regional and ethnic variations in gut microbiota (Deschasaux et al., 2018; He et al., 2018), the wide applicability of our findings and prediction model needs further validation in larger and more diverse populations.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cmet.2019.11.001.

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AUTHOR CONTRIBUTIONS

Y.L. and Yao Wang designed the study, carried out the research, and interpreted the results. K.L. contributed to the design of the clinical study and edited the manuscript. C.C. and Z.X. helped with the recruitment of study participants. Y.N. performed bioinformatics analysis and interpreted the data. Yao Wang and Yu Wang performed metabolomics analysis. D.Y. and J.G. helped with the animal studies. M.T. designed and carried out the exercise intervention and wrote the manuscript. G.P. designed and supervised the bioinformatics analysis and edited the manuscript. Y.L. and A.X. wrote the manuscript. A.X. conceived and supervised the study and wrote and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Human Blood and Fecal Samples	This paper	N/A
Chemicals, Peptides, and Recombinant Proteins		
Ampicillin Sodium Salt	Affymetrix USB biochemicals	Cat# 11259
Metronidazole	Chem Cruz	Cat# sc-204805
Neomycin Sulfate	Chem Cruz	Cat# sc-3573
Vancomycin Hydrochloride	Chem Cruz	Cat# sc-224363
Isoleucine	Sigma-Aldrich	Cat# W527602
Leucine	Sigma-Aldrich	Cat# L8000
Valine	Sigma-Aldrich	Cat# V0500
Sodium Propionate	Sigma-Aldrich	Cat# P1880
Sodium Butyrate	Sigma-Aldrich	Cat# 303410
Critical Commercial Assays		
Human Insulin ELISA Kit	Immunodiagnostics	Cat# 31380
Human Adiponectin ELISA Kit	Immunodiagnostics	Cat# 31010
Human FGF21 ELISA Kit	Immunodiagnostics	Cat# 31180
Human hsCRP ELISA Kit	Immunodiagnostics	Cat# 31220
Human Leptin ELISA Kit	BioVendor	Cat# RD191001100
Deposited Data		
Metagenome Sequencing Data of Fecal Samples from Human Subjects and Mice	NCBI	NCBI Sequencing Read Archive under BioProject ID PRJNA454826
Experimental Models: Organisms/Strains		
C57BL/6J	The Jackson Laboratory	Stock No: 000664
Software and Algorithms		
GraphPad Prism 7.0	Graphpad	https://www.graphpad.com/
R Software	R Core Team	https://www.r-project.org/
Other		
High-Fat Diet	Research diet	D12079B

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents may be obtained from the Lead Contact, Aimin Xu (amxu@hku.hk).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study Participants

This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 15-370) and was in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained from all participants. Complete clinical trial registration is deposited at ClinicalTrials.gov (NCT03240978). Detailed inclusion and exclusion criteria were as follows:

Inclusion criteria were: (i) non-smoking and male Chinese aged between 20 and 60 years; (ii) weight stable (<5% weight change over last 3 months) and overweight/obese as defined by Asian criteria BMI>23 kg/m^2; (iii) pre-diabetes as defined by impaired glucose tolerance (7.8 mmol/L $\leq$ 2-h blood glucose $\leq$ 11.0 mmol/L after a 75-g oral glucose challenge) and/or impaired fasting glucose (5.6 mmol/L $\leq$ fasting blood glucose $\leq$ 6.9 mmol/L) following the American Diabetes Association practice guidelines; (iv) absence of any systemic, metabolic and cardiovascular diseases, as well as infections within the previous month; and (v) absence of any diet or medication that might interfere with glucose homoeostasis and gut microbiota, especially antibiotics and probiotics.

Exclusion criteria were: (i) acute illness or current evidence of acute or chronic inflammatory or infective diseases; (ii) any neurological, musculoskeletal or cardio-respiratory conditions, which will put them at risk during exercise or inhibit their ability to adapt to an exercise program; (iii) participation in regular exercise and/or diet program more than 2 times per week in the latest 3 months prior to recruitment; and (iv) mental illness rendering them unable to understand the nature, scope, and possible consequences of the study.

Mouse Model

All the animal experiments were approved by the Committee on the Use of Live Animals for Teaching and Research of the University of Hong Kong (CULATR No. 4361-17). Healthy C57BL/6J male mice (8-weeks old) were housed in groups of three mice per cage, with free access to food and water under a strict 12-h light/dark cycle at a controlled temperature (23°C±2°C).

METHOD DETAILS

Subject Recruitment and General Design

Overweight/obese subjects were recruited from our local community through flyers and advertisement. Oral glucose tolerance test (OGTT) was used to screen for potential participants who met the inclusion criterion of prediabetes (Figure S1). Eligible subjects were randomly assigned to exercise or sedentary group with a computer-generated randomization list prepared by an independent statistician blinded from study design. Aside from exercise training, all participants recruited were instructed to continue their normal routine and not make any changes to their habitual physical activity and diet. One-to-one interview with a validated questionnaire including nutritional intakes and physical activity was conducted every month to assess their adherence. Fecal samples were collected at baseline and 12 weeks after exercise training while fasting plasma samples were collected every 4 weeks. All examinations were conducted at 48-72 h from the final exercise session to control for the acute effects of exercise.

High-Intensity Exercise Training Protocol

The 12-week exercise program consisted of three sessions per week on non-consecutive days at the Active Health Clinic, Centre for Sports and Exercise, The University of Hong Kong, supervised by certified exercise specialists in a one-to-one manner. Compliance in the exercise session was highly encouraged and participants were required to take part in at least 85% of all the exercise sessions for inclusion into the analysis for responsiveness. The exercise program consisted of a combination of aerobic and strength training, which was selected for its superior effectiveness in the alleviation of insulin resistance (Sigal et al., 2007). The 70-min high-intensity combined aerobic and resistance interval training sessions consisted of a 10 min warm-up, followed by rotating participants through the three 10-min stations of high-intensity treadmill intervals, high-intensity resistance and calisthenics exercises intervals, and high-intensity stationary bike intervals, with 3-4 min recovery between stations. Each training session ended up with 10-15 min of cool-down and stretching exercises. Participants wore a wireless heart rate telemetry sensor (Polar H7 heart rate sensor, Polar Electro Oy, Kempele, Finland) throughout the exercise sessions to monitor their heart rate (HR) and to ensure that they were working at the appropriate intensity level. The intensity was adjusted according to the real-time HR telemetry and the subjects were encouraged to work at 80-95% HR_{max}. The treadmill interval station consisted of 3-4 bouts of 2 min running at 85-95% max aerobic capacity (VO_{2max}) separated by 30-45s intervals of active recovery at 50% VO_2max, during which speed and incline were changed to adjust intensity in accordance with individualized progression. The stationary bike station comprised 4-5 bouts of 45-60s cycling efforts at 90-95% work peak interspersed with 60-75s active recovery at 30% work peak, during which resistance and cadence were adjusted according to individualized progression. The station of resistance and calisthenics exercises intervals was made up of 2-3 sets of several types of high intensity exercises such as squats, kettlebell swings, planks and burpees, with 30s rest between each set. The intensity of this resistance and calisthenics intervals was progressed during the 12-week training to keep the exercises challenging and also to provide adequate work to rest ratio to stimulate high intensity interval training-based resistance intervals.

Collection of Dietary and Clinical Data

Dietary data were collected by means of food frequency questionnaire administered by a nutritionist. The daily food categories and nutrient intakes were calculated by dietary software based on the China Food Composition Database, which are regularly updated with commonly consumed foods and changes in nutrient compositions. After overnight fasting for approximately 10-12 h, blood samples were collected and stored at -80°C until analysis. 75-g OGTT was conducted after 10-12 h overnight fasting at baseline and after 12-week training. Blood samples were taken for the determination of plasma glucose and insulin levels at 0, 60 and 120 min after taking the 75 g glucose solution (TRELAN-G75, Ajinomoto Pharmaceutical Co. Ltd., Tokyo, Japan). Glucose, serum lipid profiles, including triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol, were determined with standard laboratory techniques on a Hitachi 717 Analyzer (Roche Diagnostics, Germany). Serum insulin, adiponectin, FGF21, leptin and hsCRP levels were measured with ELISA kits. Insulin resistance index (HOMA-IR) (Wallace et al., 2004) was calculated using homeostasis model assessment methods, defined as fasting insulin (μU/mL)×fasting glucose (mmol/L)/22.5, and Matsuda index (DeFronzo and Matsuda, 2010) was calculated based on the results of OGTT, defined as $10000/(G_0 \times I_0 \times G_{mean} \times I_{mean})^{1/2}$, where G and I represents plasma glucose (mmol/L) and insulin (μU/mL), and "0" and "mean" indicate fasting and mean value during the OGTT, respectively. Body composition was assessed by whole-body dual-energy x-ray absorptiometry (DXA) scans (Explorer S/N 91075, Hologic Inc., Waltham, MA, USA). Strength and cardiovascular physical

assessments were conducted at both baseline and within one week after the 12-week intervention. Maximal voluntary muscle strength (chest press and leg press) was assessed using the 1-RM method (Keiser A-300, Keiser Corp., Fresno, CA, USA) following the manufacturer's instructions. An integrative cardiorespiratory fitness test using the Balke treadmill protocol was carried out on a motor driven, electronically controlled treadmill (TrackMaster, Full Vision Inc., Newton, KS, USA) to assess the maximal oxygen uptakes before and after exercise intervention.

Definition of Responders and Non-responders

The distribution of exercise responsiveness in our cohort exhibits a two-sided shape, ranging from high responders to adverse responders with respect to changes in insulin sensitivity (data not shown). According to previous interventional surveys, the inter-individual variability in response was evaluated by technical error (TE), a parameter that captures the totality of the variance among laboratories or laboratory technicians and the normal day-to-day biological variation of a trait. It is defined as the within-subject standard deviation as derived from repeated measures (or assays) over a given period of time (Hopkins, 2000). A change greater than 2 times the TE means that there is a high probability that this response is a true physiological adaptation rather than a technical and/or biological variability. Therefore, non-response to exercise intervention was defined as a failure to demonstrate a decrease of HOMA-IR (levels at 12-week against those at 0-week) that was greater than 2-fold TE from zero (Álvarez et al., 2017).

Fecal DNA Extraction and Sequencing

Fecal samples were either frozen immediately at -80°C or briefly stored by participants in -20°C freezers before being transported to our laboratory within 12 hours on ice. Fecal genomic DNA from both human subjects and mice receiving fecal microbial transplantation from human donors was extracted as described (Qin et al., 2012). All samples were sequenced on the Illumina HiSeq 4000 platform (Illumina, San Diego, California, USA; Paired-end; insert size, 350 bp; read length, 150 bp) by BGI (Hong Kong S.A.R., China).

Targeted Metabolomics Profiling

Targeted metabolomics profiling of fecal and plasma samples of both human and mice were performed by Metabo-Profile (Shanghai, China). The fecal and plasma samples were prepared as described previously (Zhao et al., 2017). Briefly, 100 μl plasma samples were extracted with cold methanol for protein precipitation before lyophilization. For fecal samples, 10 mg of lyophilized feces were homogenized with 1M NaOH and methanol respectively and the resulting supernatants were combined together. Re-dissolved plasma samples (1M NaOH mixed with methanol and pyridine) and aqueous fecal samples were subjected to derivatization with methyl chloroformate (MCF, HPLC grade, Sigma-Aldrich, Stockholm, Sweden) on a MultiPurpose Sample PrepStation 2 (MPS2, Gerstel, Germany), followed by mixing with internal standards. Subsequently, the derivatized samples in the chloroform phase were collected and randomly analyzed by a gas chromatography coupled to time-of-flight mass spectrometer (GC/TOFMS) (Pegasus HT, Leco Corp, USA) equipped with a (5%-phenyl)-methylpolysiloxane capillary column (Rxi-5MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent) as described (Zhao et al., 2017). All the standards were obtained from Sigma-Aldrich (Stockholm, Sweden). The quality control samples were prepared following the same procedures as the test samples and were injected every 14 test samples to ensure reproducibility. Raw data from GC/TOFMS analysis were exported to ChromaTOF software (v4.50, Leco Co., CA, USA) and subjected to preprocessing as described (Zhao et al., 2017). Individual compound identification was performed by comparing both MS similarity and Kovats RI distance with reference standards in the alkyl chloroformate derivate library, with a similarity score cutoff of over 70%. Multivariate analysis of fecal metabolomics profiles and visualization were performed by MetaboAnalyst 4.0. Metabolites falling into AAAs (including L-tyrosine, L-phenylalanine, and L-tryptophan), SAAs (including methionine and homocysteine), BCAAs (including L-isoleucine, L-leucine, and L-valine), and SCFAs (including acetate, butyrate, and propionate) were shown both individually and collectively as a category.

Fecal Microbial Transplantation in Mice

500 mg of fresh stools obtained from donors before and after 12-week exercise intervention were suspended in 5 mL of PBS buffer containing 0.5 g/L cysteine as reducing agent. Two donors from exercise responders and non-responders respectively were randomly selected from each subgroup to perform fecal microbial transplantation. Stool samples from each individual were not pooled, and fecal slurry from each donor was transferred into 3 conventional antibiotics-treated mice with an antibiotic cocktail (ampicillin 1g/L, metronidazole 1g/L, neomycin 1g/L and vancomycin 0.5 g/L) as previously described (Hoyles et al., 2018). Mice were randomized to receive fecal slurry from the same responder donors or non-responder donors collected at both baseline and after exercise training. After a 4-day wash-out period to eliminate antibiotics, mice were gavaged once daily for three consecutive days in the first week of colonization, and during the remaining three weeks, fecal slurries were introduced every other day to reinforce colonization. For BCAAs replenishment, they were added in drinking water (1.5 mg/kg.bw per day, weight ratio, isoleucine: leucine: valine= 0.8:1.5:1) during the 4-week FMT period. Mice receiving FMT from responders after exercise with sterile tap water and mice receiving sterile PBS were included as controls. For supplementation with SCFAs, butyrate at 40 mM and propionate at 25.9 mM were added in drinking water during the 4-week FMT period. Mice receiving FMT from non-responders after exercise with pH and sodium-matched water and mice receiving sterile PBS were included as controls. All the mice were fed with high fat diet (40% kcal fat) for 6 weeks to induce obesity before and during the 4-week period of colonization. Stool samples were collected before gavage and at the end of the experiment, and immediately stored at -80°C until further analysis. Body composition was assessed by the Minispec LF90 body composition analyzer (Bruker, Billerica, MA) every week. Glucose and insulin levels at both fasting

and fed status, intraperitoneal and oral glucose tolerance (GTT), as well as insulin tolerance tests (ITT) were performed after 4 weeks of fecal transplantation as previously described (Hui et al., 2015). For GTT, mice were fasted for 14 h (from 19:00 PM to 9:00 AM), and for ITT, mice were fasted for 6 h (from 9:00 AM to 15:00 PM).

Hyperinuslinemic-Euglycemic Clamp

Hyperinsulinemic-euglycemic clamp was performed as described previously (Hui et al., 2017). Briefly, mice were catheterized for 4 days before the experiment and fasted for 6 h before given a 5 μCi bolus of [3-^{3}H] glucose at *t*=-90 min at the day of experiment. [3-^{3}H] glucose was infused at 0.05 μCi/min for 90 min. Basal glucose production was determined from blood samples at *t*=-10 and 0 min. The clamp began at *t*=0 with a continuous infusion of human insulin at 10 mU/kg/min (Humulin R, Eli Lilly) after a bolus. [3-^{3}H] glucose infusion was increased to 0.1μCi/min for the remaining part of the experiment and euglycemia was maintained by measuring blood glucose every 10 min from *t*=0 min. A 10μCi bolus of 2[^{14}C] deoxyglucose (2[^{14}C] DG) was given at *t*=75 min and blood samples were taken every 10 min from *t*=90 min to 120 min to determine [3-^{3}H] glucose and 2[^{14}C] DG levels. Finally, at 120 min, mice were sacrificed, and the soleus muscle, epididymal adipose tissue and liver were isolated and subjected to radioactivity analysis by scintillation counter (Beckman Coulter). In all experiments, the accumulation of 2[^{14}C] DG-6-phosphate was normalized to tissue weight. Hepatic glucose production and tissue glucose uptake were calculated as described (Kraegen et al., 1985; Steele et al., 1956).

Indirect Calorimetry

Whole-body oxygen consumption of mice was measured using the comprehensive laboratory animal monitoring system (CLAMS, Columbus Instrument) as described (Huang et al., 2017). Briefly, mice were housed singly in CLAMS cages and acclimated for 48 h before data were recorded every 18 or 22 min for another 48 h with temperature at 24°C and light on between 07:00 AM and 19:00 PM.

QUANTIFICATION AND STATISTICAL ANALYSIS

Metagenomics Analysis

Quality Control and Taxonomy Profiling

The average sequencing throughput for each sample was around 35 million reads. Human-derived reads were removed using bwa *mem* against human reference genome (ucsc.hg19), while adaptors, low quality reads, bases or PCR duplicates were filtered as previously described (Li et al., 2016). After quality control and filtering, 29 million reads per sample on average were remained and used in downstream analyses. MetaPhlAn2 was employed for community taxonomy profiling at different taxonomic levels (Truong et al., 2015). R package vegan was used to calculate the alpha diversity (Shannon index) in each sample based on the relative abundance of each genus. To deduce the community diversity between samples, we used the UniFrac distance (unweighted and weighted) calculated by Phyloseq (McMurdie and Holmes, 2013). Taxonomic variation at community level was further calculated from fold changes between microbial relative abundance at 12-week against those at 0-week. Before deriving fold changes, the zero values were first additively smoothed by the minimal non-zero abundance among all observed measurements. The log-transformed fold change profiles were then used to calculate Spearman correlation-based dissimilarity measures, which were further used in Non-metric Multidimensional Scaling Analysis to illustrate the differential variation between responders and non-responders. Metagenomic data from mice receiving fecal microbiota transplantation were processed in the same way (except that mice-derived reads were removed by bwa *mem* against mouse reference genome ucsc.mm10) for better consistency during comparison between human and mice taxonomic profiles.

Co-abundance Network Analysis

Co-abundance network analysis was conducted for samples before and after 12-week exercise respectively, using pairwise Spearman correlations between species present in at least 60% of samples and only the significant correlations (P<0.05) larger than 0.6 or smaller than -0.6 were used for network construction. The two networks built (before and after exercise intervention, respectively) were merged together to facilitate direct comparison and was further visualized in R with *igraph* package.

De Novo Assembly and Functional Annotation

The paired end reads were assembled using IDBA-UD (Peng et al., 2012) with k-mer size ranging from 20 to 150 bp. Contigs less than 300 bps were discarded from further analysis. The proportion of total mappable reads (to the corresponding assembly at the threshold of 95% identity) was over 72% (82% on average) for all the samples. MetaGeneMark (Zhu et al., 2010) was adopted to predict the CoDing Sequence regions in the assembled metagenome contigs using default parameters. The functional annotation to Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology (KO) was performed through the combinatorial use of DIAMOND (Buchfink et al., 2015) and KOBAS 2.0 *annotate* program (Xie et al., 2011). Bray-Curtis dissimilarity was used to evaluate functional diversity between samples. Pathway abundances were estimated by summing up the abundances of all genes present in the corresponding pathways (KEGG accessed in Aug 2017). Differentially altered KEGG pathways were identified using Wilcoxon signed rank test.

Strain-Level Analysis and Replication Rates

The stain-level gene content profiles were obtained by applying MIDAS (Nayfach et al., 2016) to the filtered reads with parameters "run_midas.py genes –s very-sensitive –species_cov 1". For each of the species with higher than 50% prevalence (i.e. present in more than 20 samples), pairwise Jaccard distances between subjects were calculated before and after exercise intervention, based

on strain-level gene-content profiles. For highly prevalent species, bacterial replication rates were estimated using iRep algorithm (Brown et al., 2016) in default setting, with genome sequences from MIDAS database.

Statistical Analysis

All statistical analyses were performed with R software, unless otherwise stated. A power of 95% was obtained using pwr package for this study, on the basis of 20 individuals, with paired design, 5% significance, and an estimated effect size of 0.85 for exercise in improving glucose tolerance. Participants' baseline characteristics and outcomes were expressed as mean ± SEM. The normal distribution assumption was tested with Q-Q plots and variables that were non-normally distributed (BMI, fasting insulin, HOMA-IR, Matsuda Index, triglyceride and hsCRP) were log-transformed before further analysis. The group comparison of baseline measurements was conducted with independent Student's t-test. Change from baseline within each group or between groups was evaluated by repeated-measures ANOVA. Difference between groups after intervention was evaluated by ANCOVA model controlling for the baseline measurements. The metagenomics data were not normally distributed and unless otherwise indicated, two-tailed Wilcoxon rank-sum tests or Wilcoxon signed-rank tests were used throughout the human study, for unpaired and paired samples respectively and adjusted by Benjamini-Hochberg correction when multiple comparisons was applied. Multivariate analysis ADONIS test was performed using R *vegan* package for 1000 permutations. Spearman's correlations between changes in microbial species and improvements in clinical indices were calculated based on species present in at least 20% of samples. Benjamini-Hochberg procedure with a cutoff of 0.1 was applied to all Spearman's correlations. Partial correlations were used when adjusting for the effect of body weight and adiposity. The profiles of fecal microbial species (MetaPhlAn2-derived relative abundances) and metabolomics at baseline were used to build a random forest model for predicting exercise responsiveness, with R caret package. A total of 29 differentially abundant features between responders and non-responders at baseline ($P<0.1$, by Wilcoxon rank-sum test), including 14 species and 15 metabolites (Figure S6), were used for model construction, with 5-repeated 10-fold cross validation and up-sampling strategy to account for the imbalance in the two classes (Ananthakrishnan et al., 2017). The AUC of the ROC curve was used as the main indicator of model performance. The generalization of our random forest model was further tested in an independent validation cohort. For animal studies, sample size was estimated from previous studies and no statistical test had been used to predetermine the sample size. In animal studies, all analyses were performed by GraphPad Prism 7.0. Comparison between groups was performed using ANOVA followed by Turkey's multiple comparison tests or Student's t-test.

DATA AND CODE AVAILABILITY

Metagenomic sequencing data for all samples have been deposited in NCBI Sequencing Read Archive under BioProject ID: PRJNA454826.