Title

The Influence of Different Physical Activity Behaviours on the Gut Microbiota of Older Irish Adults

Running Title

Physical Activity Behaviours and Gut Microbiota in Older Irish Adults

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ABSTRACT

Objectives: A 24-hour day is made up of time spent in a range of physical activity (PA) behaviours, including sleep, sedentary time, standing, light-intensity PA (LIPA) and moderate-to-vigorous PA (MVPA), all of which may have the potential to alter an individual's health through various different pathways and mechanisms. This study aimed to explore the relationship between PA behaviours and the gut microbiome in older adults. **Design:** Cross-sectional study. **Settings and Participants:** Participants (n=100; age 67.76 years [3.02]; 44% female) from the Mitchelstown Cohort Rescreen (MCR) Study (2015-2017). **Methods:** Participants provided measures of gut microbiome composition (profiled by sequencing 16S rRNA gene amplicons), and objective measures of PA behaviours (by a 7-day wear protocol using an activPAL3 Micro). **Results:** Standing time was positively correlated with the abundance of butyrate-producing and anti-inflammatory bacteria, including *Ruminococcaceae*, *Lachnospiraceae* and *Bifidobacterium*, MVPA was positively associated with the abundance of *Lachnospiraceae* bacteria, while sedentary time was associated with lower abundance of *Ruminococcaceae* and higher abundance of *Streptococcus* spp*.* **Conclusion:** Physical activity behaviours appear to influence gut microbiota composition in older adults, with different PA behaviours having diverging effects on gut microbiota composition.

Key Words: gut microbiota; physical activity; sedentary behaviour; older adults

1 INTRODUCTION

Physical activity (PA), particularly moderate-to-vigorous PA (MVPA), is associated with a range of beneficial health effects, including a reduced risk of cardiometabolic disease and all-cause mortality (1-3). Indeed, the benefits of achieving 30 minutes of MVPA, at the expense of time of sedentary time, have been modelled to show more favourable inflammatory and lipoprotein particle profiles in adults (4, 5). In light of the poor adherence rates to the PA guidelines (6), researchers have turned their attention to other potentially more modifiable PA behaviours, namely light-intensity PA (LIPA). Greater amounts of daily LIPA have been shown to be beneficial for a range of cardiometabolic health markers (7). More recently, using a compositional data analysis approach, Powell et al. have shown the beneficial effects of increased LIPA on adiposity in older adults (8). Aside from LIPA and MVPA, other PA behaviours, such as sleep (9), sedentary time (10) and standing time (11) have all been shown to influence cardiometabolic health.

In recent years, research has begun to focus on human microbial communities, as an increasing number of studies have reported that the microbiota, especially the gut microbiota, plays an important role in human health and disease (12). Dysbiosis from normal composition in gut microbiota has been identified as a contributing factor in the development of a range of diseases (13). Bäckhed et al. reported that germ-free mice were protected from glucose intolerance and insulin resistance, compared to conventional mice under increased caloric intake (14), while Larsen et al. observed a significant difference in composition of the gut microbiota between individuals with and without type 2 diabetes mellitus (T2DM) (15). Differences in gut microbiome-encoded metabolic activity have been observed between individuals with T2DM and controls (16), and microbiota modulation of branched-chain amino acid metabolism has been suggested as a mechanistic link (16). Meanwhile, increasing evidence demonstrates the relationship between PA and gut microbiota. It has been reported that exercise induces a change in gut microbiota composition that is different from high fat dietary effects (17), while evidence suggests that exercise levels are associated with the diversity of gut microbiota in elite rugby players (18).

However, the impact of specific PA behaviours (e.g. LIPA) on the gut microbiota in older adults is not clear and warrants further investigation. Therefore, the aim of the current study was to examine the associations between habitual PA behaviours and the gut microbiota in older adults.

2 METHODS

2.1 Study participants

Participants were recruited to the Mitchelstown Cohort Rescreen (MCR) Study, which has been described previously (8). Briefly, the MCR Study was a follow-up study to the 2010 Cork and Kerry Diabetes and Heart Disease Study (19), in which 1,378 participants returned for a rescreen (of an initial 2,047 participants) between November 2015 and May 2017. All participants were aged between 55 and 74 years. All participants who attended the clinic on days three, four and five, of a five-day testing week, were offered an activPAL3 Micro (PAL Technologies, Glasgow, Scotland) to wear. Three hundred and ninety-nine participants participated in the monitor wear protocol. Additionally, of the 1,378 participants, 435 participants provided stool samples for microbiota analysis. Participants were included in the final analyses if they met the PA inclusion criteria (detailed below) and supplied a useable stool sample. Ethics committee approval conforming to the Declaration of Helsinki was obtained from the Clinical Research Ethics Committee of University College Cork (approval number: ECM-4-(nnn)-07/07/15). All participants provided written informed consent, including permission to use their data for research purposes. There was no patient or public involvement in any of the study process.

2.2 Measurements

2.2.1 Physical Activity Behaviours

Habitual PA behaviours were assessed by wearing the activiPAL3 Micro monitor for 24 hours per day, for seven consecutive days. Methodologies employed for processing activPAL data have previously been described (8). Briefly, sleep, sedentary time, standing time, LIPA and MVPA were derived from the activPAL3 Micro data. Sedentary time and standing time were calculated using the postural function of the monitor, through the proprietary software (activPAL v 7.2.32). Bed-hours were calculated by first identifying a time at which participants had not yet woken (05:00 was used). The first registered non-sedentary epoch after 05:00 was identified as rise time. The last registered non-sedentary epoch of the day, which was followed by a long uninterrupted sedentary period (>2 hours), was identified as bed-hours. Bed-hours were calculated as [time between 05:00 and the first non-sedentary epoch] + [time between last non-sedentary epoch and the following 05:00 time point]. Bed-hours were then subtracted from daily sedentary time, to give sedentary time during the day, with bed-hours serving as a surrogate measure of sleep. Light-intensity physical activity was calculated as 24 hours minus [sedentary time + standing time + MVPA]. Moderate-to-vigorous physical activity

was identified using a previously developed and validated count-to-activity thresholds (5,123 counts per 15 second epoch) (20). To be included in the final analyses, participants had to provide ≥4 days of valid activity data (≥10 hours of waking data/day) and include at least one week and weekend day. Monitor non-wear time was defined as a period of ≥60 minutes of consecutive zero counts (21), which was cross-referenced with any self-reported non-wear time.

2.2.2 Gut Microbiota

Participants were provided with a plastic tub to collect stool samples, which were stored at -80°C until extraction. Microbiota analysis was performed as previously described (22). The composition of the gut microbiome in faecal samples was determined by 16S rRNA gene sequencing. The V3-V4 region of the 16S rRNA gene was amplified and sequenced on Illumina MiSeq 2 × 250 bp using the following primers: 341F (5'- CCTACGGGNGGCWGCAG -3') and 805R (5'- GACTACHVGGGTATCTAATCC -3'). Flash was used to join overlapping paired reads and exclude reads that had more than 25% incorrect bases in the region of overlap. Quality control was carried out using Quantitative Insights Into Microbial Ecology (QIIME) 1.9.0 (23). Reads were then summarized to Operational Taxonomic Units (OTU), with a 97% identity threshold, and chimeric sequences removed using USEARCH64. In order to calculate alpha- (within-participants) and beta- (between-participants) diversity, the complete OTU count table was rarefied to 10,000 sequences. Alpha and beta diversity metrics were calculated for the rarefied tables using QIIME 1.9.0. The representative OTU sequences were assigned taxonomy by Mothur 1.36.1, using the RDP database and method (22).

2.2.3 Demographic and Covariate Information

Covariates, including age, sex, disease status (previous heart condition and diabetes), medication use (blood pressure, cholesterol lowering, diabetes medications and antibiotics) and lifestyle factors (smoking status and alcohol consumption) were obtained by each participant completing a clinical report form and a computer-assisted personal interview general health questionnaire. Height (cm) and weight (kg) were measured without footwear or heavy outer clothes using a portable stadiometer (Seca, Leicester, United Kingdom) and portable electronic scales (Tanita, Amsterdam, Netherlands) respectively. Body mass index (BMI) was calculated using weight and height squared (kg/m^2). In addition, participants also completed a standard validated Food Frequency Questionnaire (FFQ), which allowed for a Dietary Approaches to Stop Hypertension (DASH) diet quality score to be derived (24). Briefly, the DASH score is a composite score derived from

standard food groups with the FFQ. For each food group, consumption was divided into quintiles, and participants were classified according to their intake. An overall DASH score was calculated for each participant, with a lower score indicating poorer diet quality.

2.3 Statistical Analyses

Statistical analyses were carried out using the R statistical package (3.5.1). Normality was assessed using the Shapiro-Wilk test. Those variables that did not meet normality were analyzed using a Mann-Whitney Test. Statistical analyses were performed using the R statistical package (3.5.1). Normality of variables was assessed using the Shapiro-Wilk test. Multiple linear regression analyses were performed to assess the associations of PA behaviours with alpha diversity and core taxonomy, following adjustment for covariates. The association between PA behaviours and beta diversity (Principal Coordinates Analysis (PCoA)) were investigated by permutational multivariate analysis of variance, based on distance matrices using *adonis* in R *vegan* package, following adjustment for potential confounders. Canonical Correlation Analysis (CCA) was used to visualise the association between PA behaviours and gut microbiota. Statistical significance was initially set at an alpha level of 0.05. In multivariate analyses, p values were corrected with the Benjamini-Hochberg false discovery rate (FDR < 0.05).

2.4 Co-occurrence Network Analysis

Operational Taxonomic Units read counts were normalized by variance stabilizing transformation in the DESeq2 R package. Co-occurrence networks, based on the normalized OUT, were constructed using the weighted gene co-expression network analysis (WGCNA) R package (25). Briefly, parameters for the module identification, using dynamic tree cut, were set using deepSplit (=2) and minModuleSize (=10). The relationship between modules and metabolic trait was calculated using Spearman's Rank Correlation Coefficient. Visualization of the network was performed using the *igraph* R package.

3 RESULTS

3.1 Participant Characteristics

One hundred participants (median age=69.0 (3.0), 56% male) were included in the final analyses (**Table 1**), with an average BMI of 27.8 kg/m^2. On average, the participants spent 8.8 hours/day being sedentary, with a median daily MVPA of 26.1 minutes. All participant characteristics are presented in **Table 1**.

TABLE 1 HERE

3.2 Core gut microbiota composition of participants

The core microorganisms, which were present in at least 95% of the samples, were explored, and the composition of gut microbiota at different taxonomic levels were investigated. The core phyla identified in the current study were Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria (**Supplementary Figure 1**). Fecal microbial communities displayed a typical Western diversity profile, dominated by phyla Firmicutes (mean=75.47%, range=22.61–97.46%) and Bacteroidetes (mean =16.28%, range=0.10–50.77%), followed by Actinobacteria (mean=3.55%, range=0.13–30.39%) and Proteobacteria (mean=2.46%, range=0.02–41.53%). At the genus level, 19 core genera were detected in at least 95% of the samples (**Figure 1**). Unclassified Ruminococcaceae (mean=10.82%) was the most abundant phylotype across the 100 samples, ranging from 0.22-44.30%, followed by *Faecalibacterium* (mean=10.06%, range=0.10–35.94%), unclassified Lachnospiraceae (mean=8.22%, range=1.13–20.34%), *Blautia* (mean=7.21%, range=0.43–32.81%) and *Roseburia* (mean=6.28%, range=0.16–33.73%).

FIGURE 1 HERE

3.3 Gut microbiota and Physical Activity Behaviours

No PA behaviour was significantly associated with alpha diversity parameters (**Supplementary Table 1**). The relationships between each PA behaviour and microbiota beta diversity are presented in **Table 2**. The beta diversity graphically separates participants/microbiota datasets based on the relatedness of the constituent microbial taxa, which are assigned to sequence-based divisions or OTUs, that are similar to traditional species. After adjustment, sleep was significantly associated with unweighted Unifrac distance metrics (p=0.024, R^2=0.026), while MVPA was significantly associated with both the unweighted (p=0.007, R^2=0.031) and

weighted (p=0.044, R^2=0.024) Unifrac distance metrics (**Table 2**). This indicates interaction between these global microbiota parameters and these specific PA behaviours.

TABLE 2 HERE

The association between the gut microbiota and PA behaviours were further investigated by CCA. The variances of CCA1 and CCA2 were 14.17% and 9.94%, respectively, and collectively explained 24.11% of the total variance of gut microbiota. The top 10 genera, which significantly contributed to the gut microbiota variance, were *Bifidobacterium*, *Barnesiella*, *Collinsella*, *Bacteroides*, *Prevotella*, *Akkermansia*, *Alloprevotella*, *Citrobacter*, *Lactobacillus*, and unclassified Porphyromonadaceae. Some PA behaviours also significantly contributed to the variation of gut microbiota. Specifically, MVPA coincided with *Prevotella*, while sedentary time and sleep time had a similar direction to *Bacteroides.*

FIGURE 2 HERE

The relationships between individual PA behaviours and taxonomy were evaluated by multivariate linear regression. No significant associations were observed between PA behaviours and the levels of the core phyla (**Supplementary Table 1**). At the OTU level (**Table 3**), after correction for multiple testing using Benjamini-Hochberg, there were significant associations between the levels of 15 OTUs and different PA behaviours. These associations remained significant even after adjustment for dietary intake. Two OTUs classified as *Alistipes* and *Bacteroides* were positively associated with sleep time. Two OTUs classified as *Ruminococcus* and *unclassified Ruminococcaceae* were negatively associated with sedentary time, while one OTU classified as *Streptococcus* was positively associated with sedentary time. Five OTUs classified as *unclassified Ruminococcaceae*, *Clostridium_XI, Ruminococcus, Turicibacter* and *Bifidobacterium* were positively associated with standing, while four OTUs classified as *Anaerostipes*, *unclassified Lachnospiraceae*, *Alistipes* and *Bacteroides* were negatively associated with standing. One OTU classified as *Alistipes* was negatively associated with LIPA. Four OTUs classified as *unclassified Lachnospiraceae*, *Prevotella* and *Alistipes* were positively associated with MVPA, while one OTU classified as *Ruminococcus* was negatively associated with MVPA.

TABLE 3 HERE

3.4 Co-occurrence Network Analysis

The major genera that dominate the co-occurrence, are characterized by their genus-associated abilities to produce a wide range of short-chain fatty acids, including butyrate, acetate, lactate, propionate, formate, and succinate, as well as ethanol, hydrogen, and carbon dioxide. The MEcoral group was dominated by *Prevotella,* containing dominant genera such as *Bacteroides* and *Faecalibacterium*. This group positively correlated with MVPA. The MEcyan group, dominated by *Blautia*, was negatively correlated with LIPA. Finally, the MElightgreen group, which includes *Anaerostipes*, was negatively correlated with both LIPA and standing time, and positively correlated with both sleep and sedentary time.

FIGURE 3 HERE

4 DISCUSSION

Optimal health appears to be influenced by the major PA behaviours that adults engage in daily. For example. Increased time spent sedentary has been linked with adverse cardiometabolic health (26), while the protective role of PA is well understood (27). More recently, the important role that the gut microbiota plays in the development of health and the prevention of disease has been explored (28). Thus, if PA affects the gut microbiota, these alterations could have a health consequence that would be easy to overlook, or to understand from a mechanistic perspective. While previous research has shown that more intense PA can influence the gut microbiota (18, 29), this is the first study to examine the association between different PA behaviours and gut microbiota in older adults**.** It is clear from the current study, the relationship between PA behaviours and the gut microbiota is a complicated one, particularly when one considers the many different PA behaviours that individuals engage in on a daily basis. Although no significant relationship between the different PA behaviours and the alpha diversity of the gut microbiota were evident in the current study, associations were identified with different PA behaviours and both the beta diversity of the gut microbiota, and the relative abundance of specific microbes in the gut.

While consistent with previous finding pertaining to no significant differences in gut microbiota alpha diversity between active and sedentary females (29), the current study also found no association between PA behaviours and alpha diversity, which is a measure of the global number, evenness and relatedness of the gut community. However, more frequent, intense PA of a longer duration may be an influencing factor for differences in microbiota alpha diversity, since the alpha diversity of professional rugby players has been shown to be greater than that of their non-athlete healthy counterparts (18). Obviously, this more intense PA is not an activity that older adults frequently engage in. Previous research has indicated that exercise can induce changes in the proportions of major bacterial phyla (17), which the current study also reports. However, as far as we are aware, this is the first study to show that specific PA behaviours (e.g. standing) are associated with beta diversity and the abundance of specific microbes, representing more discrete changes in microbiota composition that alpha diversity indices will not detect.

Previous research indicates that exercise has a strong influence on the human microbiome composition, independent of diet (30). Specifically, the current study found that the abundance of the *Ruminococcaceae* family including genus *Ruminococcus* (which collectively account for many of the associations in **Table 3**)

correlates negatively with sedentary time and MVPA, but positively with standing time. The *Lachnospiraceae* family bacteria (three associations in **Table 3**) are also observed to be positively correlated with MVPA. These diet-responsive bacteria are commonly found in the intestines of mammals, and they display the ability to produce butyrate by degradation of cellulose and hemicellulose in plant material (31), which can be absorbed and used for energy by the host (32). Furthermore, butyrate has been well demonstrated to be able to reduce gut inflammation by influencing host gene expression and interfering with pro-inflammatory signals. These relationships with PA indices were robust after adjustment for dietary intake, indicating that they were not based on dietary habits specific to certain PA patterns.

The bile-tolerant microorganisms *Alistipes* and *Bacteroides* are positively associated with sleep, and negatively related with standing time. In addition, *Alistipes* abundance is also positively correlated with MVPA, and negatively correlated with LIPA. The different directional associations of *Alistipes* between LIPA and MVPA may be caused by the intensity of exercise. Genus *Alistipes* is found to be positively correlated with a higher step count among older community dwelling men (33). However, it is also reported that endurance exercise can cause dehydration, which could alter the composition of intestinal microbiota by increasing the abundance of *Alistipes* (34). The genus *Bacteroides* is dominant in the gut microbiota of individuals with diets rich in animal protein and saturated fats (35) and has been purported to be a diagnostic biomarker for coronary artery disease (36). *Prevotella* was also reported to be positively associated with MVPA, with this particular genus being dominant in carbohydrate-enriched diets (35). Recently, greater *Prevotella* abundance has been reported for cyclists who exercise >11 hours/week, compared to those who exercise less than this (37). It is thought that a greater abundance of *Prevotella* is associated with improvements in glucose metabolism (37), which could have important health benefits. The differences in *Prevotella* abundance and levels of MVPA is one of the strongest findings of the current study.

The current research also highlights that genus *Streptococcus* abundance is positively associated with sedentary time. It is known that microbes related to *Streptococcus* are involved in the development of multiple metabolic disorders, diabetes and colon cancer (38). For example, an increase in the abundance of *Streptococcus parasanguinis* has been reported in atherosclerotic cardiovascular patients (39). With the increased research focus on individual's sedentary behaviours, findings from the current study add to the growing literature of the detrimental effects of prolonged sedentary time, specifically relating to alterations in

the gut microbiome. *Bifidobacterium* was found to be positively associated with standing time. Bacteria belonging to the genus *Bifidobacterium* represent a large portion of the microbiota of healthy adults, plays an important role in both gut homeostasis and health, and is important for healthy aging (40). This genus has also been shown to have beneficial health effects in the modulation of the activity of the innate and adaptive immune systems (41). With ambiguity existing within the literature as to what extent should standing time be promoted as a potential health improvement behaviour (8), the potential for increased standing time to positively influence *Bifidobacterium* abundance (and thus the health benefits associated with greater *Bifidobacterium* abundance) is an intriguing finding from the current analyses. It may be that potential health benefits associated with this PA behaviour, need to be contrasted against any potential detrimental health effects, before it can be championed as a viable health improvement behaviour.

4.1 Strengths and Limitations

One of the main strengths of this study is the use of an activPAL3 Micro to quantify time spent in all waking PA behaviours of interest. A specifically developed and validated count-to-activity threshold measuring MVPA using the activPAL3 Micro was employed in this study (20). This enabled the accurate determination of sedentary time, standing time, LIPA and MVPA from a single monitor. The activPAL3 Micro was also used to estimate bed-hours, which acted as a surrogate measure of sleep. This allowed for associations between each of the PA behaviours and alpha/beta diversity to be assessed. Another strength of this study is that it provided novel and fundamental data on the association between all PA behaviours and gut microbiota in older adults. Adjustment for sex, diseases, medications, antibiotics, and lifestyle factors minimized the potential effect of other variables on the gut microbiota.

The limitations of this study also need to be recognised. Due to the cross-sectional nature of the study, causation cannot be determined. Another limitation is participants' reactivity to wearing the monitor, which could have resulted in altered habitual PA behaviours. However, previous evidence highlighted that no differences were observed between weeks one and week two in sedentary time, standing time, LIPA and MPVA for participants who wore an activPAL3 Micro for 14 consecutive days (8), suggesting that the potential of this reactivity to effect findings was minimal. Additionally, participants were not given any feedback about their PA behaviours while wearing the devices, further reducing the risk of reactivity. A surrogate measure of sleep (bed-hours) was employed in this study. While the manual approach to determining bedtime followed a strict

protocol, a participant was deemed to be asleep based on a lack of movement during a specific timeframe. Finally, the included sample is small (n=100), as partaking in the PA and gut microbiota portions of the study were optional, thus increasing the risk of potential selection bias.

5 CONCLUSIONS

Standing time, LIPA and MVPA were positively correlated with specific microbes with reported beneficial health effects such as butyrate-production bacteria, anti-inflammatory bacteria and glucose metabolism improvement bacteria, while negative associations were observed between sedentary time and these bacteria. Taken collectively, findings from the current study suggest that increased levels of PA behaviours of a low intensity (i.e. standing and LIPA) have the ability to improve specific gut microbiota which may have health improving properties, but that more intense PA (i.e. MVPA) is needed to alter the abundance of some gut microbiota (e.g. *Prevotella*). However, the relationship between PA behaviours and the gut microbiome is complex and diverse. While the current findings are encouraging, further longitudinal investigations across different age groups are required to unlock this complex relationship.

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All participants provided written informed consent, prior to taking part in the research study. Participants also consented to their data being used for future publications.

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8 TABLES AND FIGURES

Table 1. Descriptive characteristics of the study sample (n=100)

Characteristic	n=100
Age (years) [b]	69.0 ± 3.0
Gender (male) [c]	56%
BMI (kg/m^2) [b]	27.8 ± 4.0
CVD [c]	76%
Type 2 Diabetes Mellitus [c]	7%
DASH score [a]	24.0 ± 5.5
Current smoker [c]	9%
Current alcohol consumption [c]	65%
Sleep (minutes/day) [a]	529.8 ± 62.9
Sedentary time (minutes/day) [a]	475.5 ± 98.3
Standing (minutes/day) [a]	300.3 ± 99.0
LIPA (minutes/day) [b]	95.1 ± 35.6
MVPA (minutes/day) [b]	26.1 ± 23.9
Chao1 Index [a]	335.6 ± 83.8
Observed Species [a]	273.0 ± 67.2
Shannon Index [b]	5.4 ± 0.6
Simpson Index [b]	0.9 ± 0.04

[a] Variables meet normality and displayed as mean ± SD.

[b] Variables do not meet normality and displayed as median ± SD

[c] Category variables and displayed as (%)

Table 2. Permutational multivariate analyses of the relationship between beta diversity and different PA behaviours

Behaviour (minutes/day)	Weighted Unifrac		Unweighted Unifrac	
	R^2	*p* value	R^2	*p* value
Sleep	0.026	**0.024**	0.012	0.123
Sedentary Time	0.006	0.752	0.013	0.094
Standing	0.008	0.461	0.010	0.370
LIPA	0.014	0.174	0.012	0.172
MVPA	0.031	**0.007**	0.024	**0.044**

P value < 0.05 are in bold.

Permutational multivariate analysis adjusted for age, sex, disease status (CVD and T2DM), medication use (blood pressure, cholesterol lowering and diabetes medication and antibiotics), lifestyle factors (DASH score, smoking status and alcohol consumption).

Table 3. Multiple linear regression analyses of the relationship between OTUs and different PA behaviours

OTU ID	genus	Mean ± SD	Sleep		Sedentary Time		Standing		LIPA		MVPA	
			Coefficient	**FDR**	**Coefficient**	**FDR**	**Coefficient**	**FDR**	**Coefficient**	**FDR**	**Coefficient**	**FDR**
Firmicutes												
OTU_10	unclassified Ruminococcaceae	0.95 ± 1.7	0.002	0.558	-0.005	**0.048**	0.005	**0.048**	-0.004	0.558	-0.003	0.668
OTU_15	Clostridium_XI	1.24 ± 1.82	-0.006	0.056	-0.005	0.056	0.007	**0.002**	0.007	0.210	-0.010	0.210
OTU_17	Streptococcus	1.02 ± 2.11	-0.002	0.715	0.007	**0.036**	-0.004	0.229	-0.010	0.229	0.002	0.861
OTU_25	Ruminococcus	1.42 ± 2.02	0.001	0.891	-0.001	0.891	0.003	0.583	0.001	0.891	-0.026	**0.021**
OTU_35	Anaerostipes	1.21 ± 1.76	0.007	0.055	0.003	0.229	-0.006	**0.014**	-0.005	0.344	0.007	0.344
OTU_48	Ruminococcus	0.33 ± 1.23	-0.003	0.217	-0.004	**0.039**	0.004	**0.039**	0.004	0.245	0.011	0.078
OTU_57	unclassified Lachnospiraceae	0.34 ± 1.23	-0.002	0.748	-0.001	0.824	0.000	0.824	0.003	0.748	0.014	**0.043**
OTU_59	unclassified Firmicutes	0.19 ± 0.55	-0.001	0.744	-0.001	0.704	0.000	0.744	0.004	**0.046**	-0.001	0.770
OTU_76	Turicibacter	0.15 ± 0.36	0.000	0.737	-0.001	0.053	0.001	**0.036**	0.001	0.681	-0.003	0.146
OTU_80	unclassified Lachnospiraceae	0.32 ± 0.34	0.001	0.053	0.001	0.109	-0.001	**0.019**	-0.001	0.296	0.000	0.897
OTU_101	unclassified Lachnospiraceae	0.11 ± 0.38	0.001	0.507	0.000	0.507	0.000	0.507	-0.002	0.427	0.006	**<0.001**
Bacteroidetes												
OTU_131	Alistipes	0.17 ± 0.26	0.001	**0.043**	0.000	0.253	-0.001	**0.027**	-0.002	**0.044**	0.003	**0.027**
OTU_146	Bacteroides	0.17 ± 0.37	0.002	**0.009**	0.000	0.849	-0.001	**0.011**	0.000	0.849	0.001	0.627
OTU_502	Prevotella	0.38 ± 3.07	-0.006	0.613	0.000	0.963	-0.002	0.768	0.005	0.768	0.055	**<0.001**
Actinobacteria												
OTU_19	Bifidobacterium	0.5 ± 1.26	-0.004	0.234	-0.002	0.256	0.004	**0.046**	0.000	0.904	-0.008	0.256

FDR < 0.05 are in bold.

Permutational multivariate analysis adjusted for age, sex, disease status (previous heart condition and diabetes), medication use (blood pressure, cholesterol lowering and diabetes medication and antibiotics), lifestyle factors (DASH score, smoking status and alcohol consumption).

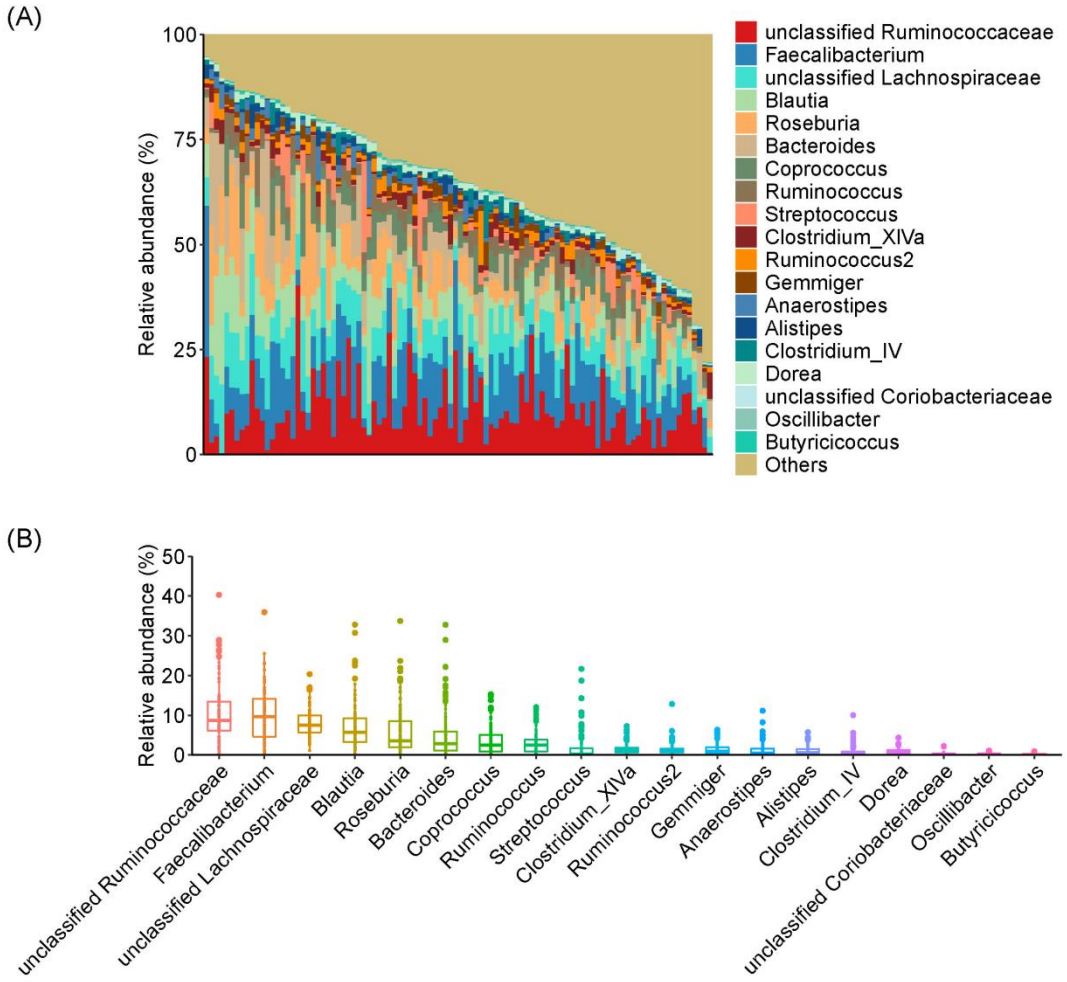


Figure. 1. Core genera composition of participants' gut microbiota. (A) Bar plot; (B) Box plot.

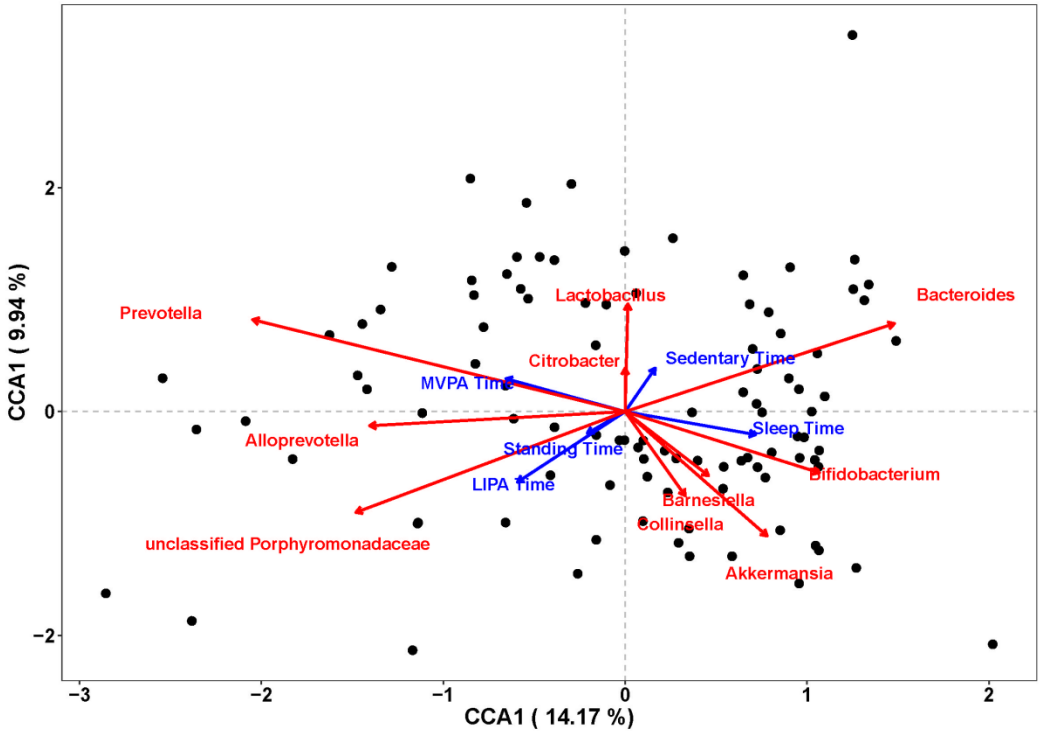


Figure 2. Microbial community variation in the MCR Study cohort. Top contributors of genus to the community variation as determined by CCA on scaled OTUs abundances (red arrows); PA behaviours contribute to microbiome community variation (blue arrows). Arrows scaled to contribution.

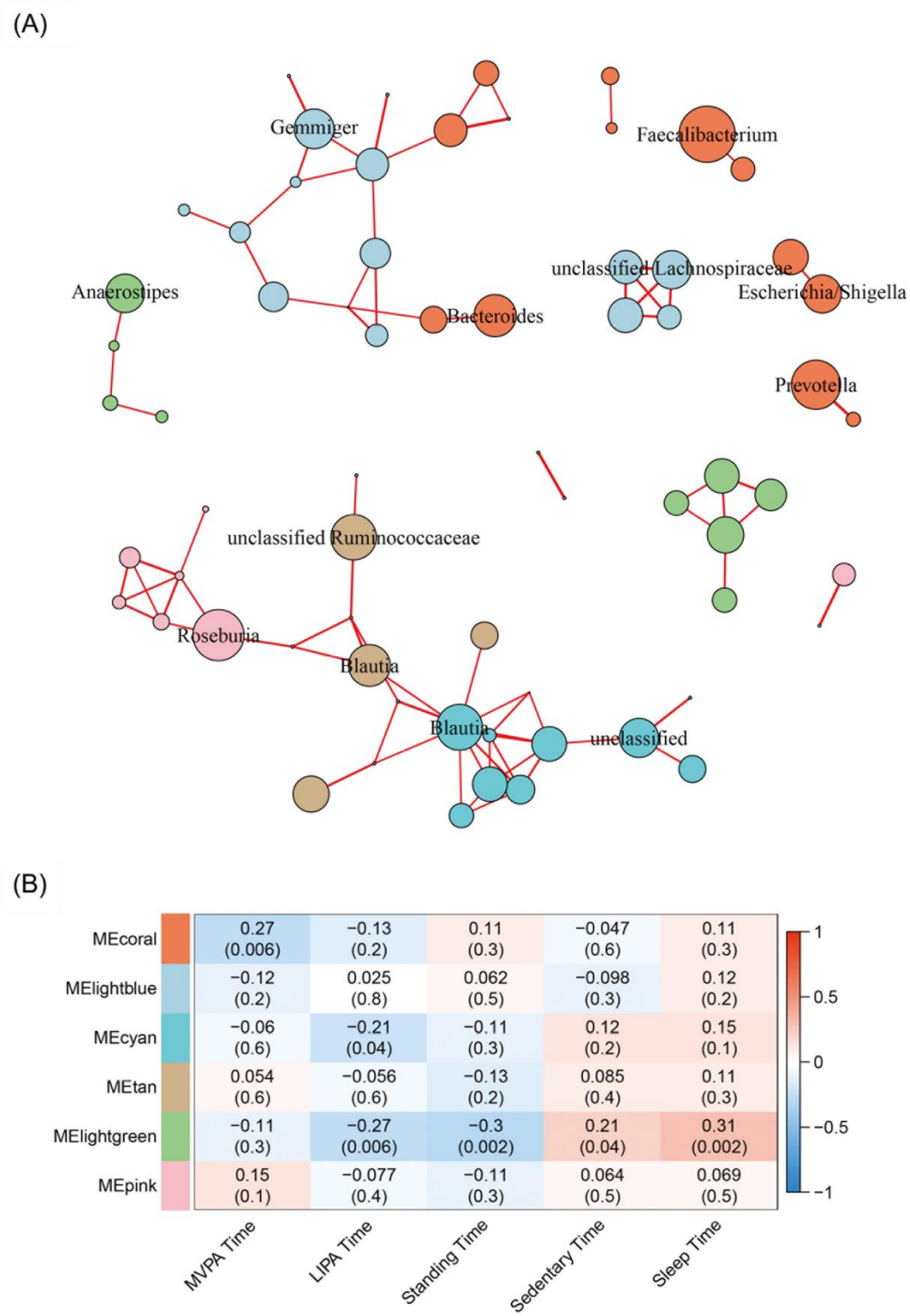


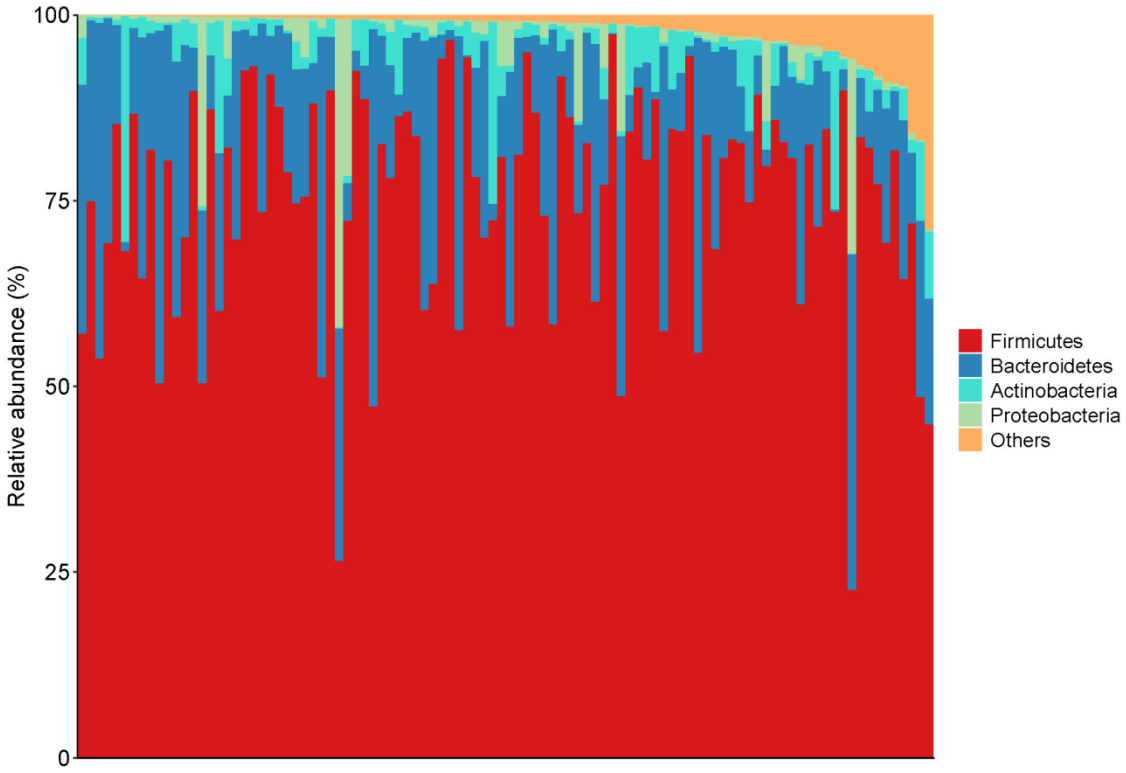
Figure. 3 OTUs Co-occurrence Network. OTUs (nodes) are coloured according to WGCNA module colours. Positive correlations are marked by blue edges correspond, and negative correlations are marked by red edges. Edges width and length are scaled to the correlation coefficient. Any resulting correlations with p value ≥ 0.05 and abs(r) < 0.6 were removed. Circle size indicates the normalized relative abundance of OTUs, and the OTUs with relative abundance > 1% are marked at genus level.

Supplementary Table 1. Multiple linear regression analyses of the relationship between alpha diversity parameters, abundant phyla and different PA behaviours

Alpha Diversity Parameters								
Behaviour (minutes/day)	**Chao1**		**Simpson**		**Shannon**		**Observed Species**	
	Coefficient	**FDR**	**Coefficient**	**FDR**	**Coefficient**	**FDR**	**Coefficient**	**FDR**
Sleep	0.088	0.875	0.000	0.126	0.002	0.321	0.077	0.814
Sedentary Time	-0.133	0.475	0.000	0.738	-0.001	0.636	-0.102	0.454
Standing	0.021	0.914	0.000	0.720	0.000	0.974	0.005	0.985
LIPA	0.315	0.475	0.000	0.504	0.000	0.974	0.313	0.454
MVPA	0.040	0.914	0.000	0.720	0.000	0.974	-0.005	0.985
Abundant Phyla								
Behaviour (minutes/day)	**Actinobacteria**		**Bacteroidetes**		**Firmicutes**		**Proteobacteria**	
	Coefficient	**FDR**	**Coefficient**	**FDR**	**Coefficient**	**FDR**	**Coefficient**	**FDR**
Sleep	-0.010	0.488	-0.039	0.146	0.057	0.086	-0.007	0.941
Sedentary Time	-0.004	0.488	0.000	0.946	-0.002	0.856	0.004	0.941
Standing	0.013	0.081	-0.001	0.946	-0.013	0.644	0.000	0.941
LIPA	-0.012	0.488	0.056	0.305	-0.028	0.742	-0.015	0.941
MVPA	-0.044	0.081	0.155	0.062	-0.094	0.536	0.002	0.941

FDR < 0.05 are in bold.

Permutational multivariate analysis adjusted for age, sex, disease status (previous heart condition and diabetes), medication use (blood pressure, cholesterol lowering and diabetes medication and antibiotics), lifestyle factors (DASH score, smoking status and alcohol consumption).



Supplementary Figure 1. Core phyla composition of participants' gut microbiota