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ORIGINAL RESEARCH

Altered gut microbiota richness in individuals with a history of lateral ankle sprain

Masafumi Terada [a], Masataka Uchida [b], Tadashi Suga [b] and Tadao Isaka [a]

[a]College of Sport and Health Science, Ritsumeikan University, Kusatsu, Shiga, Japan; [b]Research Organization of Science and Technology, Ritsumeikan University, Kusatsu, Shiga, Japan

ABSTRACT

This study aimed to examine differences in the intestinal microbiota diversity in individuals with and without a history of a lateral ankle sprain (LAS). Fifty male college student athletes with (n=32) and without (n=18) a LAS history participated in this study. Faecal samples were collected in the morning after awakening during an off-season, and faecal microbiota were characterized via bacteria 16S rRNA amplicon sequencing. Alpha-diversity metrics and ß-diversity indices were calculated to assess the gut microbiota diversity. The LAS-history group significantly had lower Chao1 (p=0.020) and abundance-based coverage estimators (p=0.035) indices compared to the control group. Gut microbiota composition was not significantly different between athletes with a LAS history and controls (R^2 =0.01, p 0.414). Athletes with a history of LASs had significantly higher proportions of *Bacteroides Fragilis* (p=0.024) and *Ruminococcus Gnavus* (p=0.021) compared with controls. The gut microbiota of athletes with a LAS history had less richness compared to controls, indicating potential associations between a LAS and the gut microbiota. This study highlights the potential link of a LAS to global health. This study may help raise awareness of strategies to prevent long-term health-related negative consequences in people suffering from LASs.

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Diversity; ankle injury; neuromechanical condition; brain

Introduction

A lateral ankle sprain (LAS) is an extremely prevalent musculoskeletal condition with a large financial burden (Doherty et al., 2014). Up to three-fourths of individuals with a LAS fail to fully recover from the ankle injury, re-sprain their ankle, and experience lifelong residual symptoms and ankle disability (Herzog et al., 2019). The long-term negative consequences of a LAS are evident as these patients present with decreased health-related quality of life and avoid physical and occupational activities that they remain capable of doing (Houston et al., 2015; Konradsen et al., 2002). Therefore, LASs could negatively impact long-term biopsychosocial health (Hertel & Corbett, 2019).

A large body of literature has documented numerous sensory-perceptual and motor-behavioural impairments following a LAS (Hertel & Corbett, 2019). One of the notable motor-behavioural impairments associated with a LAS is disrupted efferent signals from

CONTACT Masafumi Terada mterada@fc.ritsumei.ac.jp College of Sport and Health Science, Ritsumeikan University, 1-1-1 Noji-higashi, Kusatsu, Shiga 525-8577, Japan

the primary motor cortex (Kosik et al., 2016; Terada et al., 2020). Previous investigations have observed decreased excitability or increased inhibition of lower extremity muscles from the corticospinal tract in individuals with a history of LASs (Kosik et al., 2016; Terada et al., 2020). These individuals also have lower white matter microstructure of a major efferent tract of the cerebellum (Terada et al., 2018) and altered cortical activation of the supplementary motor area (Rosen et al., 2019). The collective findings from prior studies suggest that these motor-behavioural impairments associated LASs appear to play a prominent role in individual's health, and LASs should be treated as a global and not just a local injury (Hertel & Corbett, 2019).

It has become increasingly evident that there are the bidirectional interactions between the intestinal microbiota and the brain through neural, immune-related, endocrine, and metabolic signalling pathways (Sharon et al., 2016). For example, the intestinal microbiota is significantly altered following central nervous system (CNS) pathologies, including traumatic brain injury (George et al., 2021). A previous study has also reported that exposure to a stressful situation substantially changes the intestinal microbiota composition (Galley et al., 2014). Emerging evidence from previous studies provides the function link between brain and intestinal microbiota (Galley et al., 2014; George et al., 2021; Sharon et al., 2016).

An increased amount of evidence has demonstrated that the intestinal microbiome plays a key role in the promotion of overall health (Mailing et al., 2019; Sharon et al., 2016). Alterations in microbial diversity in the gastrointestinal tract have been identified in a growing number of conditions, including psychological and behaviour disorders (Jiang et al., 2015), traumatic brain injury (George et al., 2021; Wang et al., 2021), spinal cord injury (Gungor et al., 2016; Zhang et al., 2018), and osteoarthritis (Ulici et al., 2018). Specifically, previous investigations have reported a greater gut microbial diversity in patients with spinal cord injury (Gungor et al., 2016; Zhang et al., 2018) and an animal model with knee osteoarthritis (Ulici et al., 2018) compared with healthy controls. A decrease in gut microbial diversity has also been observed in an animal model following traumatic brain injury (Wang et al., 2021). Furthermore, it has been documented that negative psychological and emotional disturbances have been linked to the balance in the richness and composition of intestinal microbiome via the autonomic nervous system (ANS), the major route of central communication between the intestinal microbiota and the brain (Sharon et al., 2016). Houston et al. (2015) observed psychological deficits in individuals with chronic ankle instability (CAI) that develop following a LAS. Recently published systematic reviews reported increased levels of injury-related fear and decreased psychological health in individuals with recurrent ankle sprain and CAI (Bain et al., 2021; Suttmiller & McCann, 2021). Increased psychological stress and altered brain function due to LASs have the potential to negatively influence the intestinal microbiota. However, it remains unknown if the intestinal microbiota is altered in individuals with a LAS history. Therefore, the purpose of this study was to examine differences in the intestinal microbiota diversity in individuals with and without a previous history of LASs.

This is important to document because understanding the impact of a LAS on global health may inform future investigations on improving awareness of strategies to prevent long-term health-related negative consequences in individuals suffering from LASs. Proper care at the time of injury is critical to minimize or avoid long-term impairments following a LAS. However, most people perceive a LAS as an innocuous, minor injury that minimally influences general health and will heal with minimal treatment (Gribble et al.,

2016). This perception has led researchers to hypothesize that many individuals do not seek proper care at the time of injury (Miklovic et al., 2018). Unfortunately, a previous study has reported that a large proportion of people who sprain their ankle does not seek any types of care for the injury (McKay et al., 2001). Considering intestinal microbiota as an important element of health in a LAS is an innovative idea with significant scientific, medical, and socioeconomic consequences. Assessing associations between a LAS and the intestinal microbiota would constitute a significant step forward to improve awareness of strategies to prevent long-term negative consequences in people suffering from LASs.

Materials and methods

Study design

This study was conducted with a single-blinded, case–control design. A single investigator screened participants for inclusion criteria, and the investigator responsible for collecting faecal samples was blinded to group membership.

Participants

One hundred forty-two male participants were recruited from collegiate athletic teams (basketball, American football, and track & field). As this study focused on the effects of a history of a LAS, collegiate student athletes that have a previous history of musculoskeletal injuries other than a LAS (n = 92) were not included in this current study. Thirty-two athletes with a previous history of LASs were included in the case group (Table 1). Participants in the LAS-history group had a previous history of at least one significant LAS resulting in swelling, pain, and temporary loss of function for at least 1 day; otherwise, the participants were in good health. No participant with a previous history of LASs had acutely sprained his ankle in the previous 3 months. Eighteen participants entered the control group that reported to have no history of a LAS. All participants included in the analysis had no history of 1) diagnosed balance or vestibular disorders; 2) a concussion in the past 12 months; 3) any diagnosed

Table 1. Anthropometric and ankle injury characteristics for the lateral ankle sprain history (LAS) and control groups: mean (standard deviation).

Variable	LAS	Control	p-value
n	32	18	-
Age (year)	19.88 (1.01)	19.22 (1.00)	-
Height (cm)	177.06 (7.49)	175.52 (7.08)	0.481
Body Mass (kg)	76.76 (14.48)	79.53 (17.40)	0.550
Body Mass Index (kg/m^2)	24.48 (4.44)	25.60 (3.92)	0.380
The number of lateral ankle sprains	5.09 (5.96) (range: 1–25)	0.00	-
# of giving-way episodes in past 6 months	7.14 (16.24) (range: 0–72)	0.00	-
Distribution of # of giving-way episodes in past 6 months	More than twice = 12 once = 0 no episode = 19	More than twice = 0 once = 0 no episode = 10	-

gastrointestinal and cardiopulmonary disorders; 4) a history of any self-reported musculoskeletal injuries in the lower and upper extremities as well as trunk; 5) a previous history of fracture or surgery; and 6) any diagnosed neurovascular disorders. All participants read and signed an informed consent approved by the Ethical Committee on Human Research of the Ritsumeikan University Institutional Review Board.

Procedures

Faecal samples were collected from participants in the morning after awakening using commercial containers (FS-0008, TechnoSuruga Laboratory Co., Ltd., Shizuoka, Japan). To minimize the effects of training intensity and volume, we collected faecal samples during off-season. While we did not monitor participants' dietary intakes, all participants had meals offered from university. Samples were stored at −80°C until DNA extraction. Homogenization of the faecal samples (200 ml) was carried out using beads with 300 ml lysis buffer (No.10, Kurabo Industries Ltd., Osaka, Japan). Homogenization of the faecal samples (200 ml) was carried out using beads with 300 ml lysis buffer (No.10, Kurabo Industries Ltd., Osaka, Japan). The mixture was mechanically disrupted using a Cell destroyer PS1000 (Bio Medical Science, Tokyo, Japan). DNA extraction assays were extracted from faecal samples, and gut microbial DNA extraction assays were performed by Cosmo Bio Co., Ltd. (Tokyo, Japan) using QIAamp DNA Stool Mini Kit (Qiagen, Venlo, Netherlands) as described previously (Costea et al., 2017). The extracted DNA was determined using a NanoDrop Spectrophotometer ND-1000 (Thermo Fisher Scientific Inc., DE, USA). 16S rRNA sequencing was performed as described previously (Hosomi et al., 2017). The PCR and sequencing of the V3-V4 region (16S rDNA) was performed using the Illumina Miseq platform (Illumina, San Diego, CA, USA), as described previously (Hosomi et al., 2017). All sequence reads were analysed by the Cosmo Bio Co., Ltd. (Hokkaido, Japan). FASTQ files were obtained after Illumina pair-end 16S rRNA gene amplicon sequencing. Sequencing was performed using an Illumina MiSeq sequencer with 2 × 300 cycle MiSeq Reagent Kit v3. Sequences were grouped into operational taxonomic units (OTUs) with similarity ≥97% sequence identity and taxonomically classified to different levels (phylum, class, order, family, genus, and species) using Greengenes 16S rRNA gene database (version 13.8) within QIIME (version 1.8.0).

Statistical and bioinformatics analyses

To assess the gut microbiota diversity within each sample, we calculated α-diversity indices, such as the Chao I index, the abundance-based coverage estimators (ACE) index, the Shannon index, and the Simpson index, using QIIME from rarefied samples. Table 2 provides a definition of each microbiota diversity variable. To assess gut microbial communities, ß-diversity indices were calculated based on unweighted UniFrac and Euclidean distance matrix and visualized using non-metric multidimensional scaling (NMDS) and principal coordinate analysis (PCOA) with R (version 4.0.3).

Table 2. Descriptions of gut microbiota diversity analysis used in the current study.

Variable	Description	References
α-diversity	A summary of the structure of a bacteria community with respect to the number of different species and distribution of abundances of the species. The α-diversity focuses on species variation within a single community.	(Finotello et al., 2018)
The number of observed operational taxonomic units	The number of sequences that are observed for each operational taxonomic unit that is an operational definition used to classify groups of closely related species.	(Kim et al., 2017)
Chao1 index	A nonparametric measure for estimating the total number of different species (species richness) in a community. This measure considers the ratio of an observation with exactly one sequence in a sample to an observation with exactly two sequences in the sample.	(Chao, 1984)
Abundance-based coverage index	A nonparametric estimator of species richness. This measure divides observed frequencies into an abundant group with more than 10 species and a rare group with fewer than 10 species in a community.	(Chao & Lee, 1992)
Shannon index	A measure of species richness and evenness (the relative abundance of species). This measure is more influenced by species richness and the number of rare species.	(Lemos et al., 2011)
Simpson index	A measure of species richness and evenness. This measure puts more weight on species evenness and is sensitive to the number of common species.	(Simpson, 1949)
ß-diversity	A quantification of similarity or dissimilarity between communities	(Finotello et al., 2018)
Principal coordinates analysis	A multivariate statistical (metric multidimensional scaling) method that examines differences among the bacterial communities by measuring similarity or distance of between clusters of samples	(Gower, 2005)
Non-metric multidimensional scaling	A multivariate statistical technique that constructs spatial representation of the interrelationships among a set of data samples in multidimensional space.	(Young et al., 1995)

Statistical analyses were performed with R and SPSS 26.0 (SPSS, Inc. Chicago, IL, USA). Height, body mass, and body mass index were compared between participants with a LAS history and controls using independent t-tests. The number of observed OTUs and alpha diversity indices are presented as the means ± SD. Independent t-tests were utilized to compare differences in the gut microbial diversity and composition, as well as the relative abundance of the specie level between participants with and without a LAS history. To assess the magnitude of between-group differences in each variable, Cohen's *d* effect sizes using the means and pooled standard deviations were calculated. The strength of effect sizes was interpreted as weak ($d < 0.40$), moderate ($0.40 \leq d < 0.80$), or strong ($d \geq 0.80$; Cohen, 1992). A one-way analysis of similarity was performed to determine the differences in bacterial communities between groups with R. A *priori* alpha level was set at $p < 0.05$ for all statistical tests.

Results

Anthropometric characteristics were not different between participants with and without a history of LASs ($p > 0.05$, Table 1). A total of 5,718,427 raw reads were obtained by the 16S rDNA gene of bacteria and archaea from 50 faecal samples. After filtering low-quality reads, 5,243,148 clean reads were generated with a mean

Table 3. Intestinal microbiota variables for the lateral ankle sprain history and control groups.

Variables	Lateral Ankle Sprain	Control	P-Values	Cohen's *d* (95% Confidence Intervals)
The number of observed OTUs*	430.35 (451.40)	849.11 (283.98)	<0.001	−1.05 (−1.66, −0.43)
Chao1 index*	2227.62 (799.47)	2899.39 (1432.48)	0.020	−0.70 (−1.30, −0.11)
ACE index*	2234.14 (974.96)	3001.41 (1531.56)	0.035	−0.64 (−1.23, −0.04)
Shannon index	3.91 (0.67)	3.76 (0.55)	0.397	0.25 (−0.33, 0.83)
Simpson index	0.85 (0.09)	0.83 (0.07)	0.476	0.21 (−0.37, 0.79)
Bacteroides Fragilis* (Relative Abundant, %)	2.67 (4.26)	0.72 (1.51)	0.024	0.55 (−0.05, 1.13)
Ruminococcus Gnavus*(Relative Abundant, %)	1.45 (2.53)	0.35 (0.39)	0.021	0.54 (−0.06, 1.12)

Note: *There are statistically significant differences between groups ($p < 0.05$). Abbreviations: OTU = Operational Taxonomic Unit, ACE = Abundance-based Coverage Estimators

read length of 456.79 bp, and nearly 8.31% of raw data were filtered. The average number of high-quality reads per sample was 104,862 and ranged from 96,777.00 to 113,871.00 across all samples.

All 4,976,069.00 sequences were clustered into OTUs at the 97% similarity level. An average of 787.40 OTUs were identified from 50 faecal samples. Across all samples, 99.47% of sequences were assigned to a bacterial kingdom, whereas 0.53% of reads remained unclassified. A total of 16 bacterial phyla, 84 classes, 164 orders, 110 familia, 225 genera, and 300 species were detected from the samples. The dominant phyla among all participants were Firmicutes (mean relative abundance = 46.74%), and Bacteroidetes (42.57%), followed by Proteobacteria (8.21%), and Actinobacteria (2.08%). At the family level, *Bacteroidaceae* (36.20%) predominated, followed by *Veillonellaceae* (23.49%), *Lachnospiraceae* (11.83%), *Ruminococcaceae* (8.21%), and *Enterobacteriaceae* (4.85%). At the genus level, *Bacteroides* (36.19%) predominated, followed by *Veollonella* (11.60%), *Megasphaera* (4.53%), *Phascolarctobacterium* (2.31%), *Prevotella* (2.17%), and *Dialister* (2.02%).

Participants with a LAS history had significantly smaller number of observed OTUs ($p < 0.001$) compared to controls, with a strong effect size (Table 3). The overall gut microbiota composition of each group and each participant at each level is shown in Supplemental Files. Participants with a LAS history had significantly lower Chao 1 ($p = 0.020$) and ACE indices ($p = 0.035$) compared to controls, with moderate effect sizes (Table 3). There were no significant between-group differences in Shannon and Simpson indices ($p > 0.05$, Table 3). There were significantly higher proportions of *Bacteroides Fragilis* ($p = 0.024$) and *Ruminococcus Gnavus* ($p = 0.021$) at the specie level in athletes with a LAS history compared with controls (Table 3). There were no significant results for other proportions of each species ($p > 0.05$). A ß-diversity analysis revealed that gut microbiota composition was not different between athletes with a LAS history and controls ($R^2 = 0.01$, $p = 0.414$, Figure 1).

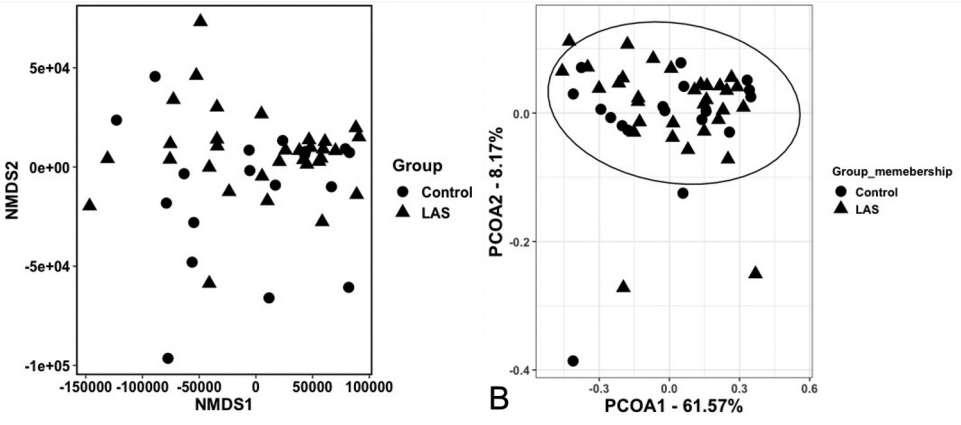

Figure 1. Diversity of the gut microbiota. **a)** Euclidean distance non-metric multidimensional scaling (NMDS) ($R^2 = 0.01$, $p = 0.53$, stress = 0.11), **b)** Euclidean distance principal coordinate analysis (PCOA) . Abbreviation: LAS = Lateral Ankle Sprain

Discussion

This study aimed to compare measures of gut microbiota diversity between participants with a LAS history and controls. We observed a smaller number of observed OTUs as well as less Chao 1 and ACE indices in participants with a LAS history compared to controls. The Chao 1 and ACE indices are measures of species richness that is the number of different species represented in a community (Kim et al., 2017). These results indicate that athletes with a previous history of LASs had fewer observed species and less species richness in the gut compared to controls. The moderate effect size values for the Chao 1 and ACE indices, with CIs that did not cross zero, suggests that clinically meaningful differences may be present. Participants with a LAS history appear to have altered intestinal microbiota richness, which may support the notion of potential global alterations related to a LAS (Hertel & Corbett, 2019). This current study provides valuable information to help our understanding of the impact of a LAS on global health.

While species richness in the gut was less in participants with a LAS history, there were no between-group differences in other α-diversity indices. The Simpson index reflects both species richness and evenness (the relative abundance of species) and provides more weights on species evenness, with a higher value indicating lower diversity of species in samples (Kim et al., 2017). The Shannon index accounts for both richness and evenness of the species present, with a higher value indicating higher diversity of the microbiota (Kim et al., 2017). Previous studies reported inconsistent relationships between α-diversity indices and disorders (Jiang et al., 2015; Ulici et al., 2018; Zhang et al., 2018). An animal model with knee osteoarthritis exhibited lower α-diversity indices compared to controls (Ulici et al., 2018). Researchers also observed lower α-diversity (the Simpson index) in patients with spinal cord injury compared to healthy controls (Zhang et al., 2018). Furthermore, a previous investigation demonstrated a decrease in α-diversity indices (Chao 1 and

Simpson index) following traumatic brain injury (Wang et al., 2021). However, a higher α-diversity (the Shannon index) was observed in the animal model with depression compared with controls (Jiang et al., 2015). Thus, species richness and evenness in the gut may relate differently to types of disorders.

In this current study, β-diversity indices based on the unweighted UniFrac and Euclidean distance were not different between the LAS-history and control groups, indicating that the microbiota composition of athletes with a LAS history may not vary from that of controls. Previous studies have reported that there were significant differences in the structure and composition of the intestinal microbiota community between individuals in a known disease state and healthy individuals (Breban et al., 2017; Gungor et al., 2016; Jiang et al., 2015; Zhang et al., 2018). In addition, Ulici et al. (2018) suggest that the altered structure and composition of gut microbiota may promote the development of osteoarthritis following a joint injury. However, data from this current study revealed that a LAS may not associate with the heterogeneity and composition of the gut microbiota.

While the exact neurophysiological mechanism of altered gut microbiota richness associated with a LAS remains unknown, it is hypothesized that maladaptive neuroplasticity following a LAS may influence species richness in the gut. An abundance of existing literature provides evidence of central alterations in nervous system in patients with a LAS and those with CAI such as deleterious changes in white matter microstructure (Terada et al., 2018) and a lower level of neurocognitive function associated with visual memory and attention (Rosen et al., 2021). Researchers have highlighted bi-directional interaction between the brain and the intestinal microbiota profile (Sharon et al., 2016). The bidirectional communication involves various communication pathways including the CNS, the ANS, the enteric nervous system, the neuroendocrine system, and the neuroimmune system (Sharon et al., 2016). A previous study observed changes in structural connectivity of the brain was associated with diet-dependent gut microbiota (Ong et al., 2018). Furthermore, a previous study using animal models with elimination of gut microbiota showed a decrease in neurocognitive performance in tasks that require intact spatial and working memory (Gareau, 2014). Emerging evidence suggests that the diversity of the gut microbiota may be decreased following neurotrauma, such as spinal cord (Gungor et al., 2016; Zhang et al., 2018) and traumatic brain injuries (Wang et al., 2021). Perhaps altered structural and functional adaptations in the brain may impact multiple systems that communicate with the gut microbiota, resulting in altered species richness in the gut in individuals with a LAS history. Therefore, altered species richness in the gut observed in this study might be unique neural consequences of a LAS.

Another potential theory that explains our observations is that increased psychological stress due to a LAS may influence species richness in the gut. A previous study reported psychological deficits in individual with LASs (Houston et al., 2015). Some of the athletes in the current study reported repetitive episodes of the ankle giving way and recurrent ankle sprains that persist for more than 1 year after the initial injury, possibly resulting in persistent stress. It is well documented that acute and chronic psychological stress can influence the activity of the ANS and the hypothalamic–pituitary–adrenal axis that regulate gut physiological functions (Sharon et al., 2016). Dysregulation of ANS and hypothalamic–pituitary–adrenal activities is associated with mental health and psychological disorders, which are known to affect the community structure of the gut microbiome (Wong et al., 2016). In addition, altered diaphragm contractility has been observed in

individuals with a LAS history (Terada et al., 2016). The motor control of the diaphragm involves the involuntary activity of brainstem (Corfield et al., 1998) that regulates ANS and hypothalamic–pituitary–adrenal activities. Decreased psychological health and altered diaphragm function associated with LAS may influence ANS and hypothalamic–pituitary–adrenal activities, potentially altering species richness in the gut. This is speculative and future examination of psychological patient-reported outcomes and diaphragm function in addition to the gut microbiota composition in individuals with a previous history of a LAS is needed to support this theory.

The potential crosstalk between joint and gut microbiota, termed as the gut-joint axis, has been hypothesized by researchers (Arora et al., 2021; Hao et al., 2021; De Sire et al., 2020). It has been established that some bacteria in the gut produced various molecules, which influence joint metabolism, including proinflammatory metabolites (Arora et al., 2021; Biver et al., 2019; Guss et al., 2019; Lan et al., 2021; Schott et al., 2018). In this study, there were significantly higher proportions of *Bacteroides Fragilis* and *Ruminococcus Gnavus* in athletes with a history of LASs compared with the controls. Previous studies have shown that the relative abundance of *Bacteroides Fragilis* and *Ruminococcus Gnavus* increased in inflammatory diseases such as spondylarthritis (Breban et al., 2017). After a LAS, the injured tissues accompany inflammatory responses. In this study, we did not include athletes who acutely sprained their ankles in the previous 3 months. Twelve out of 32 athletes with a history of LASs reported at least two episodes of giving-way, perceived instability, and/or residual symptoms that could be associated with chronic inflammation, potentially influencing relative abundance of *Bacteroides Fragilis* and *Ruminococcus Gnavus*. Many individuals, who have experienced an acute LAS, go on to suffer residual impairments (5% to 53%) such as chronic pain, persistent swelling, and perceived ankle instability (Van Rijn et al., 2008). An increase in the relative abundance of these bacteria might be a potential marker that can be used to identify patients with a LAS who have incomplete recovery from the injury. However, we acknowledge that the explanations are speculative, and the utility of gut microbiota remains uncertain. Clearly, future work that examines associations between inflammatory makers and gut microbiota in this ankle injury cohort is necessary to support these speculations.

Clinical implications

Our results may provide a new insight into the current management of a LAS and pose a possible future direction. Patients with an acute LAS present with a unique combination of pathomechanical, sensory-perceptual, and motor-behavioural impairments (Hertel & Corbett, 2019). Thus, rather than administering the same management approach for all patients with a LAS, personalized, impairment-based intervention following a LAS is recommended to manage the injury (Donovan & Hertel, 2012; Hertel & Corbett, 2019). Gut microbiome can be incorporated as a component of personalized, impairment-based management of a LAS. Gut microbiota profiles have an emerging role as a biomarker for disease prognosis and individual's response to therapeutic intervention (Kashyap et al., 2017; Schupack et al., 2022). For example, an increase in the relative abundance of specific bacteria, such as *Bacteroides Fragilis* and *Ruminococcus Gnavus*, might be a potential biomarker to optimize detection of

inflammation and incomplete recovery from the injury. Gut microbiota has been established as targeted biomarkers for certain diseases (Erickson et al., 2012; Qin et al., 2012; Schupack et al., 2022). Assessing alterations in gut microbiota profiles may be a potentially novel approach to manage LASs, as well as be valuable in secondary prevention of LASs and an increase in long-term recovery rate of LASs. The findings provide a focused area for future research to examine the predictive and diagnostic quality of gut microbiota diversity in patients with LASs and determine if assessing gut microbiota diversity can improve the current care given for a LAS.

Based on our findings, gut microbiota is a potential target for future clinical interventions for a LAS. Gut microbiota can be modifiable with therapeutic interventions, including diet, probiotic supplementation, lifestyle change, exercises, and faecal microbiota transplantation (Arora et al., 2021; D'Amato et al., 2020; Du et al., 2021; Gubert et al., 2020; Lew et al., 2019; Li et al., 2021; Tillisch et al., 2013). Recently, intervention strategies for a LAS have focused on addressing changes in CNS plasticity and neurocognitive function to improve patient outcomes (Bruce et al., 2020; Mohammadi et al., 2021). Human and animal studies have reported that diet-based intervention and faecal microbiota transplantation have been effective in improving CNS function and restoring the balance of gut-brain axis (D'Amato et al., 2020; Du et al., 2021; Lew et al., 2019; Tillisch et al., 2013). Modulation of gut microbiota diversity through clinical interventions may become a future novel approach for the treatment of a LAS to restore CNS function and control inflammatory response to injury. It remains unknown what clinical interventions for patients with a LAS would be most effective in modulating gut microbiota and if modulation of gut microbiota can improve patient outcomes. Clinical intervention trials are required to evaluate microbiota-based interventions as an effective as an adjunct with current managements for a LAS.

Limitations

The present study has several caveats for further investigations into understanding associations between LASs and the gut microbiota. The diversity of the gut microbiota are highly variable because many factors, including physiological, psychological, genetic, dietary, cultural, and environmental determinants, influence the structure and composition of the gut microbiota (Mailing et al., 2019). In addition, the cross-sectional nature of this study limits the ability to draw a causal link between a LAS and the identified alterations in the gut microbiota. These factors must be considered in future prospective design studies to clarify the cause-and-effect between gut microbiota and LASs. While all participants in the current study had the opportunity to have meals offered from university, we did not control and monitor participants' dietary intakes. Therefore, it is possible that inter-individual variability in dietary behaviour influences relative abundance of specific bacteria observed in the current study. With 32 participants with a history of LASs and 18 controls, there was a potential for a statistical error. Although a priori sample size calculation was not conducted in this study because it was exploratory in nature, post hoc power analyses showed that we had moderate to strong observed power to detect between-group differences (observed powers = 0.55–0.99). Furthermore, the Cohen's *d* effect sizes with a calculation of 95% CIs strengthens our results by emphasizing the magnitude of differences between groups. However, non-significant findings were associated with low statistical power (observed powers = 0.21 − 0.22), increasing the risk of a type II error. The effect sizes

reported were low for non-significant between-group differences with associated 95% CIs crossing zero, indicating that these differences may not be clinically significant. In cases of moderate effect sizes with the 95% CIs that crossed zero, these relationships may be associated with a statistical error and strengthened with an expanded sample size.

Conclusion

The intestinal microbiota of athletes with a previous history of LASs was less rich compared to controls, indicating potential associations between a LAS and the gut microbiota diversity. Therefore, a LAS is a kind of neuromechanical condition impacting multiple body systems. However, there were no differences in structural and composition of the gut bacteria species between groups. Continued work is needed to determine a causal link between a LAS and the altered richness of the intestinal microbiota as well as improve the understanding of the underlying neuroimmunological mechanism of altered gut microbiota diversity among individuals with a history of LASs.

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Disclosure statement

No potential conflict of interests was reported by the authors.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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ORCID

Masafumi Terada http://orcid.org/0000-0002-0534-4772
Tadashi Suga http://orcid.org/0000-0002-0313-9660
Tadao Isaka http://orcid.org/0000-0002-8190-3281

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