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# 'It takes a village': deciphering the role of the gut microbiome in the health and performance of military personnel

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Received 1 May 2024

Accepted 3 July 2024

## ABSTRACT

The human gut microbiome can be impacted by a range of environmental and lifestyle factors including diet, antibiotics, physical fitness and acute and chronic stressors. There is also evidence to suggest that specific compositional and/or functional features of the gut microbiome are mediators of aspects of health and performance including disease susceptibility, cognitive and physical states and the immune response. Therefore, understanding microbe-to-microbe and nutrient-to-microbe interactions in the gut and how they interact with host biology (eg, via the gut-brain axis) could enable better design of interventions aimed at modulating the gut microbiome to improve the health and performance of the military. Accordingly, this review summarises a thematic session hosted at the 6th International Conference on Soldier Physical Performance which provided an overview of military-relevant research related to the gut microbiome. It articulates a timely opportunity to leverage this rapidly advancing area to improve personnel health and military performance.

## INTRODUCTION

We are all mostly microbe. The human body hosts trillions of micro-organisms including bacteria, viruses, fungi, phage and archaea, which constitute the human microbiota. There are an estimated 500–1000 bacterial species in the human body at any one time, with each species comprising potentially thousands of strains.<sup>1</sup> Therefore, the combined genetic material of these microbial ecosystems, known as the human microbiome, exceeds that of the human genome and represents an immense and modifiable reservoir of genetic diversity and functional biological potential.<sup>2</sup>

The gastrointestinal (GI) tract hosts a particularly diverse, dense and active microbial ecosystem, which has been the focus of research to date. Associations between the structure and activity of the gut microbiota and a range of health-related outcomes have been investigated. Subsequent mechanistic work has also begun to reveal bidirectional interactions between these microbial communities and various biological processes within their human hosts, which could underpin these associations. Furthermore, the composition and activity of the gut microbiota varies considerably between individuals, and is known to be impacted by a range of environmental and lifestyle factors. As such, there is growing recognition that the plasticity of these microbial ecosystems represents an opportunity to

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The composition and activity of the gut microbiome has been associated with a wide range of biological processes and disease states in humans, which appears to be underpinned by host-microbiome interaction across a number of mechanistic pathways.

## WHAT THIS STUDY ADDS

⇒ A complementary portfolio of microbiome research is being conducted across nations which seeks to leverage these insights in order to enhance the health and performance of military personnel.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Sophisticated tools and techniques (eg, cultureomics, multiomics) are rapidly emerging which could enable exploitation of the gut microbiome as a reservoir of novel biomarkers, antimicrobials and probiotics within defence, with currently unrealised potential as predictors and modulators of military health and performance.

target and modify a range of biological processes within their human hosts with a view to improving health.

While work in this area has centred on clinical cohorts and health-related outcomes, these developments also raise a number of considerations for defence. Military personnel are required to carry out a range of physically and cognitively demanding tasks, often at short notice and against a backdrop of stressful and challenging operational environments. This is particularly true when performance must be sustained throughout overseas deployments, which often feature changes in time zone, high cognitive load, physical exertion, sleep deprivation, exposure to endemic pathogens and climatic extremes. Therefore, understanding the role of the gut microbiome in sustaining and enhancing the health and performance of military personnel in these scenarios could present a number of opportunities. However, the contribution of the gut microbiome in this regard is currently understudied and underappreciated.

At the 6th International Congress on Soldier Physical Performance (ICSPP), a collaborative, international community of researchers spanning



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**To cite:** Templeman I, Parish E, Rimmer J, et al. *BMJ Mil Health* Epub ahead of print: [please include Day Month Year]. doi:10.1136/military-2024-002746

defence and academia hosted a thematic session describing military-relevant research related to the human gut microbiome, which was the first time this area of research had been presented to this audience. The outcomes from a complementary portfolio of research across nations were presented in relation to three potential application areas:

1. Prevention of travellers' diarrhoea (TD).
2. Modulation of the stress response via the microbiota–gut–brain axis (MGBA).
3. Optimisation of physical and cognitive performance.

This review summarises this session and articulates a timely opportunity to leverage this rapidly advancing area to improve personnel health and optimise military performance.

### Prevention of TD

TD is one of the most prevalent health challenges facing military personnel deployed overseas. US data suggests 60% of troops returning from overseas operations experienced at least one diarrhoeal episode during the deployment.<sup>3</sup> In addition, of that 60%, 30% reported two episodes and 46% reported three or more episodes. Similarly, UK data collected regarding Operation HERRICK in Afghanistan (2002–2014) suggested that approximately 40% of deployed personnel experienced at least one episode of diarrhoeal disease over 6 months.<sup>4</sup> Based on self-reported data, each episode resulted in 2.8 days off duty on average, with a further 4 days of underperformance. When scaled to a whole-force perspective, this was estimated to amount to 18 943 lost duty days on Operation HERRICK 10 (April to October 2009), with a further 27 313 days of underperformance.

Strategies to overcome the deleterious effects of TD on performance have previously been explored using a *Campylobacter jejuni* human challenge model.<sup>5</sup> This study evaluated the efficacy of rifaximin as chemoprophylaxis for campylobacteriosis in a sample of 28 healthy adults. The study demonstrated that there was no significant reduction in the incidence of diarrhoeal disease with rifaximin. However, unpublished data from the study revealed that for one subject, the onset of diarrhoea resulted in a 10-fold increase in response time during psychomotor vigilance testing. This equated to a 2.7 s delay, which could lead to fatalities in operational scenarios that rely on quick reactions, such as the detection of improvised explosive devices. Accordingly, complimentary research in the USA and the UK has sought to better understand the causes of TD among deployed military personnel, including the potential role of the gut microbiome. These activities are currently being driven by the Air Force Research Laboratory and Naval Medical Research Center in the USA as well as the Department for Military Medicine and the Defence Science and Technology Laboratory in the UK.

While the incidence of diarrhoeal disease on deployment has reduced over the past decade through improvements in education and infrastructure, the high numbers of personnel routinely affected still represents an enduring challenge to operational effectiveness.<sup>6</sup> However, data from deployed military personnel and civilian travellers suggests that, while many cases of TD could be attributed to known enteric pathogens, the causative agents were unknown in over 40% of cases.<sup>7</sup> This raises the possibility that changes in the composition and activity of the gut microbiota could be implicated in the development of some TD cases. This is also supported by widely observed postinfectious irritable bowel syndrome following pathogen clearance and return from deployment.<sup>8</sup>

A pilot study therefore investigated whether the composition of the gut microbiome before and after deployment overseas was associated with self-reported diarrhoeal disease.<sup>3</sup> This established that while the gut microbiome remained largely stable from pre to post deployment regardless of diarrhoeal disease, the differential abundance of certain bacterial taxa prior to deployment appeared to predict TD susceptibility. The abundance of specific genus, including *Weissella*, *Butyrivibrio*, *Leptolinea*, *Corynebacterium* and *Jeotgallibaca*, and family, *Ruminococcaceae*, *Erysipelotrichaceae* and *Enterobacteriaceae*, were found to differ significantly in personnel reporting an episode of TD.

Building on this finding, stool samples obtained during the *C. jejuni* challenge described previously were further analysed.<sup>5,9</sup> The composition of stool before and after exposure to a known enteric pathogen provided a valuable resource for exploring these resilience and susceptibility characteristics. This established that prior to the challenge, some uncharacterised bacterial genera were more abundant in those who did not develop campylobacteriosis, while *Bacteroides* abundance was reduced. It was therefore proposed that such differences could modify the availability of key substrates for *C. jejuni* and in doing so make colonisation by the pathogenic strain less likely.

It has also been observed that Nepalese-born Gurkha soldiers exhibit lower rates of TD than British-born soldiers during overseas deployment.<sup>7</sup> Consequently, metagenomic sequencing was performed on stool samples from the two groups to determine whether this could be underpinned by differences in the gut microbiome.<sup>10</sup> Distinct clusters emerged for the two populations based on principal coordinate analysis, with differences in diversity indices and the abundance of several bacterial genera and species. However, the extent to which these microbial signatures could be directly influencing susceptibility to TD remains unclear.

Collectively, these findings suggest that the composition of the gut microbiome is associated with resilience and susceptibility to TD. The identification of microbial characteristics that appear to predict the risk of developing TD could therefore provide an informed basis for disease management and guide the development of preventative treatments. However, the mechanisms underlying these associations need to be established in order to inform this, which should be considered in the approach to future studies.

### Modulation of the stress response via the MGBA

The existence of a bidirectional communication system operating between the gut and the brain, the gut–brain axis, is well known.<sup>11</sup> Several pathways are considered within this, including stimulation of the vagus nerve, changes in endocrine signalling, modulation of the immune system and changes to the hypothalamic–pituitary–adrenal (HPA) axis. The microbial communities hosted in the GI tract are now also considered to be part of this network, which is referred to as the MGBA. The microbes themselves generate metabolites which can influence the axis in various ways, meaning that changes in the composition and activity of the gut microbiome can modify host–microbe dialogue and drive changes in host biology. This encompasses local effects on GI function such as gut motility, secretion, barrier permeability and mucosal immunity, in addition to more complex effects elsewhere on pain perception, hunger, mood, cognition and behaviour.

Such effects are alluded to by an unpublished study undertaken in the US Navy, which collected stool samples from

submariners before, during and after a 3-month deployment. When paired with indices from a Profile of Mood States Questionnaire, gut community structure was associated with self-reported fatigue. Furthermore, sequencing of these samples revealed that this community remained relatively stable within individuals over the course of their deployment. This therefore raises the possibility that a predeployment intervention targeting the gut microbiome could provide a level of enduring protection against fatigue on operations.

Similar associations have also been reported between the composition and activity of the gut microbiome and mental health. To establish whether these microbial characteristics could be causally involved in symptom development, faecal samples from patients with depression have been transplanted into a rodent model, which resulted in the emergence of behaviours and neurobiology reminiscent of a depressive phenotype.<sup>12</sup> This faecal microbiota transplantation approach is widely used to assess causality and mechanisms and has also recently been used to demonstrate the transfer of anxiety-related phenotypes.<sup>13 14</sup>

These studies contribute to a growing body of evidence that points to a potential causal role of the gut microbiome in modulating brain function, cognition and mental health. This raises the possibility that this understanding can be mined and the underpinning pathways targeted to improve various outcomes, thereby giving rise to the concept of a psychobiotic.<sup>15</sup> Applying this approach to modulate the stress response is appealing both within defence and society more widely, owing to the pervasive nature of many stressors (eg, sociopolitical instability, perceived threat). In such instances, it is typically the biological and psychological response that elicits harm, as opposed to the stressor itself, so understanding the role of the MGBA in this context could present several opportunities. Exploring this notion has been the principal focus of a programme led by researchers at APC Microbiome Ireland.

An important knowledge gap in this regard pertains to the MGBA response to acute stress.<sup>16</sup> To address this, mice were exposed to 15 min of acute stress to demonstrate that the gut microbiota plays an important role in modulating acute serotonergic responses at both levels of the gut–brain axis.<sup>17</sup> Related to this, the gut microbiome has also been implicated in the control of tryptophan metabolism, which is a precursor for a variety of neuroactive compounds including serotonin, an important neurotransmitter and therapeutic target for antidepressant and anxiolytic drugs.<sup>18</sup> Researchers have also shown that corticosterone secretion is significantly increased in germ-free mice when exposed to stressors and that administration of a specific probiotic can attenuate corticosterone secretion.<sup>19 20</sup> Such findings illustrate the emerging role of the gut microbiome in regulating various dimensions of the biological response to stress.

To explore the translational relevance of these findings, 1 month of *Bifidobacterium longum* treatment was shown to modulate the stress response in healthy human volunteers.<sup>21</sup> Relative to a placebo, participants given the *B. longum* treatment reported lower levels of perceived stress, exhibited a blunted cortisol response to an acute stressor, had an improved profile of resting brain activity and made fewer errors in a memory task linked to hippocampal function. Due to the density of glucocorticoid receptors within the hippocampus, the authors propose that modulation of the HPA axis by the gut microbiome could be influencing cognitive function via this pathway, with hippocampal expression of brain-derived neurotrophic factor having previously been shown to change

in response to *Bifidobacterium* administration. Further to this, another study has considered the efficacy of a psychobiotic diet, which is similar to a Mediterranean diet but with increased consumption of fermented foods.<sup>22</sup> Over 4 weeks, those consuming a psychobiotic diet reported lower levels of perceived stress compared with baseline. Moreover, the reduction in perceived stress was found to increase with greater adherence to the intervention. In this instance, accompanying changes in urinary markers of tryptophan metabolism were also apparent, which echoes the findings of several animal studies discussed previously.<sup>12 17</sup> Accordingly, the enrichment of tryptophan metabolites across a number of microbial pathways and their subsequent uptake could represent another viable mechanism.

Although further exploration and validation remains necessary, these findings allude to the broad range of benefits that could be achieved by modulation of the gut microbiome. The defence operating environment is characterised by a complex range of stressors, and as such, exploring the efficacy of microbiome modulation as a means of producing a beneficial stress response in these scenarios is a key focus.

### Optimisation of physical and cognitive performance

As outlined in previous sections, distinct microbiome profiles are consistently reported in patients when compared with healthy controls. This includes patients with disorders such as depression, where the transplantation of microbial communities results in the onset of donor symptom profiles.<sup>23</sup> Consequently, the extent to which microbial signatures of enhanced performance can be identified and exploited is also an area of interest within defence.

For instance, Barton *et al*<sup>24</sup> compared the gut microbiome of elite Irish rugby players against a cohort of healthy controls. Principal coordinate analysis revealed differential clustering between the two groups, suggesting that elite athletes did exhibit distinct microbial profiles. Furthermore, the athlete samples were found to be enriched in short-chain fatty acids (SCFAs) and across 29 of the 34 metabolic pathways classified, which could collectively confer a number of health and performance benefits. However, based on these data, it was not possible to delineate whether the gut microbiome contributed to their elite status or whether it was an indirect consequence that reflected the unique lifestyles of athletes.

Building on such observations, an extensive review of the evidence regarding the relationship between the gut microbiome and physical performance has been undertaken.<sup>25</sup> Drawing from this, probiotic interventions in animal models have been suggested to increase grip strength and time to exhaustion, while disturbance of the gut microbiome with antibiotics has been linked with attenuated muscle growth in response to resistance training. Similar effects have also been observed in human trials, with probiotic supplementation resulting in a reduction in perceived muscle soreness following exercise, in addition to an increase in time to exhaustion during running. A number of different mechanisms are proposed to underpin these observations, including altered protein metabolism, SCFA production, enhanced lactate turnover, modulation of adenosine monophosphate-activated protein kinase, mammalian target of rapamycin and insulin-like growth factor 1 signalling pathways. However, while these insights are promising, the performance outcomes employed are highly specific, and as such, the same efficacy may not be seen in more complex military tasks.



As an alternative approach, engineered probiotics are also being developed to explore potential applications in enhancing resiliency to fatigue and improving cognitive performance. One example undergoing development in the USA uses *Escherichia coli* Nissle 1917 that has been modified to upregulate the production and secretion of guanidinoacetic acid,<sup>26</sup> which is involved in creatine biosynthesis. Creatine is an important component of human energy metabolism and can help to maintain adenosine triphosphate concentrations in the brain and other tissues when subjected to high loads (eg, sleep deprivation, intense exercise). It has been associated with improved performance across a number of dimensions during sleep deprivation, which could be highly valuable in an operational context.

## MODULATION OF THE MICROBIOME

These applications allude to the range of opportunities that microbiome modulation could offer in terms of protecting and enhancing the health and performance of military personnel. Accordingly, various intervention strategies including fermented foods, natural bacterial isolates and microbial metabolites have been investigated for their potential utility in addressing defence challenges.<sup>27</sup> However, bridging the gaps in the evidence base represents a considerable undertaking that carries both scientific and financial challenges.

A highly productive approach to overcoming these challenges within defence has been to use in vitro models of the human GI tract. This allows for the evaluation of candidate interventions and determines the subsequent impact on microbial community composition and function. These outcomes can then be mapped to the findings of predictive studies, as discussed throughout this review, in order to understand likely health and performance impacts and support the prioritisation of candidates for in vivo preclinical and human intervention studies.

One example of this approach is the evaluation of polyphenol and fibre supplementation by the US Army. In vitro approaches established that combined treatment modified the composition and activity of the gut microbiome, resulting in an increase in SCFAs and antioxidant capacity, while the production of proinflammatory metabolites was suppressed.<sup>28</sup> This is consistent with an improvement in gut health and barrier function, both of which have been shown to be negatively impacted by operational stressors.<sup>29–30</sup> Consequently, this combined treatment is currently being tested for its efficacy in managing the impairments seen on rapid ascent to high altitude.

## CONCLUSION

Emerging evidence presented at ICSP supports microbiome modulation as a promising new tool for defence stakeholders. This progress is the product of collective efforts across multiple services, research organisations and nations, with the thematic session providing a valuable opportunity to bring the community together. As such, a key message from all contributing authors was the necessity for collaboration and coherence across defence, and with academia and industry more widely, in driving the area forward to meet the needs of military personnel.

**Contributors** IT: minuted the conference session and drafted and revised the manuscript. EP and SH: convened and chaired the conference session and drafted and revised the manuscript. JR, GC, TT, MSG and JWS: provided scientific content for the conference session and supported manuscript preparation and revision.

**Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** GC has received honoraria from Janssen, Probi and Apsen as an invited speaker, is in receipt of research funding from Pharmavite, Reckitt, Tate and Lyle, Nestle and Fonterra and has received payments as a consultant from Yakult, Zentiva and Heel Pharmaceuticals. This support neither influenced nor constrained the content of this article. All other authors have no competing interests to declare.

**Patient consent for publication** Not applicable.

**Ethics approval** Not applicable.

**Provenance and peer review** Not commissioned; externally peer reviewed.

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