

# Theoretical Engineering of the Gut Micro biome for the Purpose of Creating Superior Soldiers

Garrett R Schmidt-McCormack<sup>1,2</sup>, Kristina M Feye<sup>2</sup>, Sreemoyee Acharya<sup>2</sup>, Gregory SA Mlynarczyk<sup>1,2</sup>, Stephen J Anderson<sup>1,2</sup>, Patricia Izbicki<sup>1,3</sup>, Emir Malovic<sup>1,2</sup>, Luna KC<sup>2</sup>, Joe S Smith<sup>5</sup>, Matthew A Jefferson<sup>1,4</sup>, Aron Nakama<sup>1,6</sup>, Kasandra Diaz Santana<sup>1</sup>, Naveen C Kondru<sup>2</sup>, Michael D Kleinhenz<sup>4</sup>, James G Tipton<sup>2</sup>, Shivani Choudhary<sup>2</sup>, Robyn D Kokemuller<sup>1,2</sup>, Sireesha Manne<sup>2</sup>, Marson R Putra<sup>1,2</sup>, Nyzil Massey<sup>2</sup>, Denusha Shrestha<sup>2</sup>, Diou Luo<sup>2</sup>, Shaunik Sharma<sup>2</sup>, Pongrat Jaisil<sup>1,2</sup>, Carrie A Berg<sup>2</sup> and Steve A Carlson<sup>2\*</sup>

<sup>1</sup>Neuroscience Interdepartmental Program, Iowa State University, Ames, IA, USA

<sup>2</sup>Department of Biomedical Sciences, Iowa State University College of Veterinary Medicine, Ames, IA, USA

<sup>3</sup>Department of Psychology, Iowa State University College of Liberal Arts and Sciences, Ames, IA, USA

<sup>4</sup>Department of Kinesiology, Iowa State University College of Veterinary Medicine, Ames, IA, USA

<sup>5</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University College of Veterinary Medicine, Ames, IA, USA

<sup>6</sup>Department of Genetics, Developmental and Cellular Biology, Iowa State University College of Veterinary Medicine, Ames, IA, USA

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### \*For Correspondence

Steve A Carlson, Department of Biomedical Sciences, Iowa State University College of Veterinary Medicine, Ames, IA, USA, Tel: 515-294-0912.

**E-mail:** stevec@iastate.edu

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### ABSTRACT

The purpose of this review is to highlight research raising the possibility of exploiting the host-microbiome gut axis for military purposes. Through optimizing the gut-microbiome environment it is possible to enhance nutritional access to indigestible material, provide local and systemic analgesia, enhance psychological robustness to battlefield stress, produce endogenous steroids, reduce muscle fatigue, and promote peripheral wound healing. However, this approach is still in its early stages and thus has not been explored to its full potential. The challenges that are currently preventing the practical use of gut-bacteria include the following: inconsistency of clinical outcomes, transient effects requiring continuous supplementation, the type of regimen selected, the initiation and cessation of regimen, and the broader clinical studies needed to validate this research. This review is intended to shed light on the numerous and varied positive impacts such an approach could have for the military if further developed.

## INTRODUCTION

Exploiting the host-microbiome interactions for favorable physiological conditions is an emerging focus in research medicine. Changes in the gut microbiome have dramatic effects upon essentially all systems of the body such as the cardiovascular <sup>[1]</sup>, respiratory <sup>[2,3]</sup>, integument <sup>[4]</sup>, musculoskeletal <sup>[5]</sup>, neurologic <sup>[6]</sup>, endocrine <sup>[7]</sup>, renal <sup>[8,9]</sup>, reproductive <sup>[10,11]</sup> and immune <sup>[12]</sup> systems. Additionally, a dis-biosis in the gut microbiome has been implemented in a number of important diseases <sup>[13]</sup>. It appears that there is a significant level of interconnectedness between host physiology and microbial populations that may provide far-reaching opportunities to enhance general physiology.

The advent of fecal transplants for the treatment of *Clostridium difficile* <sup>[14]</sup> instigated the need to understand how the gut impacts overall health and physiology continuously expands <sup>[15]</sup>. While still controversial, the utilization of prebiotics, postbiotics and probiotics as nutraceuticals shows promise for beneficial modulation of the host microbiome <sup>[16]</sup>. Although these effects may be transient, evidence points to a persistent positive change of the health of the host through the manipulation of the bacteria in the gut microenvironment, as long as the regimen is followed.

Given recent discoveries concerning the ability of gut microbes to modulate host metabolism and physical endurance, biomedical science may have a role to play in increasing the effectiveness and resilience of soldiers in combat situations. This unexplored facet of military medicine is in its early stages but offers long-term, low-cost increases in combat efficiency. We have identified five different branches of microbiome research in which the exploitation of and remodeling of a healthy microbiota can be used for the enhancement of military personnel. These opportunities include: (i) supplementing the microbiota with cellulolytic bacteria to enhance foraging for calories in remote locations; (ii) seeding the microbiota with bacteria actively secreting opioids; (iii) assuaging combat stress with anti-anxiety bacteria; and (iv) using steroid-producing bacteria to enhance overall physical strength; (v) modulating the microbiota to promote wound healing. It is through our understanding of model microbial ecology and application of genetic engineering in the gut that we hope to exploit and optimize these conditions.

The first of these strategies will address food scarcity, a potential problem for soldiers in resource-depleted locations. Humans cannot regularly harvest significant energy from  $\beta$ -glucans and cellulose-based material; whereas ruminant animals have commensal bacteria with the biochemical catabolic pathways to accomplish this task. Interestingly, cellulolytic bacteria such as Firmicutes like *Ruminococcus bicirculans*<sup>[17]</sup> and *Ruminococcus champanellensis*<sup>[18]</sup> can colonize the human gut and aid in the digestion of plant material. Further it may be possible to exploit a greater repertoire of catabolic carbohydrate pathways through the bioengineering of resident microorganisms to artificially introduce cellulolytic capabilities to the duodenum, which is the major site of carbohydrate absorption in humans. Couple this potential with the ability to introduce pathways capable of detoxification of poisonous plants results in an extraordinarily attractive opportunity for the military. By being able to utilize these otherwise indigestible carbohydrates, soldiers in nutritionally sparse locations can survive on cellulose-based materials like branches and grasses without the fear of being poisoned.

A second example of microbiota research relevant to the military is the modulation of nociception via the gut microbiome. Typically, opioids and non-steroidal anti-inflammatory drugs are prescribed for pain management by military doctors. Recent evidence has emerged that through controlling the inflammatory response in the gut, it is possible to have both local and systemic attenuation of nociception. There are two potential possibilities to activate the endogenous opioid pathways through the modulation of the microbiota: (a) gut bacteria that release NK3 receptor antagonists<sup>[19]</sup>; and, (b) seeding of *Lactobacillus paracasei* that minimizes the inflammatory pain response<sup>[19,20]</sup>.

A third proposed military application of research associated with the optimization of the gut microbiome is its ability to reduce psychological stress and anxiety. A reduction in the physiological response to stress requires a suppression of corticosteroid release. The modulation of the gut microbiota to include microorganisms such as *Lactobacillus* spp.<sup>[21]</sup> has been observed to reduce corticosteroid release in mice. Furthermore, anxiolytic metabolites such as the precursors to catecholamine and benzodiazepine agonists as well as numerous neurotransmitters have been identified as products of microbiota<sup>[22]</sup>. These findings suggest the potential for seeding microorganisms to alter the gut microbiota of soldiers to reduce fear-based responses in battle, increase the threshold for physiological stress, and enhance mental well-being.

The fourth prospective military application for the modulation of the gut microbiome is the production of anabolic steroids and increased stamina<sup>[23]</sup>. The ability of bacteria to naturally or synthetically produce anabolic steroids has been previously investigated and reviewed<sup>[24]</sup>. *Escherichia coli* have been engineered since the 1980s for production of anabolic steroids and human growth hormone<sup>[25]</sup> and are a potential tool to enhance physical strength through seeding the gut microbiota. In addition, targeting and enhancing gut microbiota has been proposed to enhance the rehabilitation of stressed muscle and improve stamina<sup>[26]</sup>. Thus, there are two suggestive mechanisms for targeting soldiers for enhanced strength and stamina: directly engineering and seeding *Escherichia coli* to produce anabolic steroids and the promotion of a healthy gut-microbiome axis to promote stamina.

The final target for the modulation of the microbiota regards promoting an attenuated inflammatory response to wounding. Guiding the inflammatory response to promote tissue repair in the periphery via the gut commensal microbiota could reduce healing time for soldiers wounded in the field. Research suggests that immunomodulatory control of the inflammatory response in the periphery can be accomplished in the gut, specifically linking dominant *Lactobacillus* sub species populations in the reduction of surgical healing time.

Together, these five potential strategies bring forth the prospect of optimizing the gut microbiome a possible interest for military research and development. While currently out of reach, the permanent modulation of the host microbiome would be the ultimate objective. The following review is a discussion of the possibility of both short- and long-term modifications of the microbiota for the creation of "super soldiers".

## CELLULOLYTIC GUT BACTERIA

A serious issue faced by soldiers is being cut off from supply lines and procuring food. This problem could be resolved by deriving nutrition from cellulose sources that are abundant in many regions if soldiers somehow had the capabilities of doing so. Humans are inefficient at utilizing cellulose due to the inability to cleave  $\beta$ -1,4-glucan bonds. Additionally, there is concern about accidental toxicity resulting in death and illness as detoxification pathways are different between humans and herbivores.

The bioengineering of gut microorganisms and subsequent colonization in favorable locations within the alimentary tract can potentially solve the aforementioned issues.

The colon of the human gastrointestinal tract houses a number of cellulolytic bacteria but cellulose-derived energy is significantly less than that of ruminant and non-ruminant herbivores. Specifically, herbivores acquire 70% of total energy from their commensal microbiota and the breakdown of cellulolytic substrates while humans only obtain 10% of their total energy from commensal microbiota, mainly through savaging of carbohydrate sources in the cecum<sup>[27]</sup>. Humans possess Firmicutes, Bacteroidetes, and Actiobacteria that produce volatile short chain fatty acids (SCFA), such as butyrate which is a useable energy source for human enterocytes and is utilized via a minor metabolic pathway for energy salvaging<sup>[28]</sup>. Exploiting the anabolic potential of this pathway could be accomplished through supplementation with the native butyrate-producing bacteria, or with similar non-endogenous cellulolytic and butyrate-producing bacteria.

Further opportunities for energy harvesting would be through the breakage of the  $\beta$ -1,4 glucan bond in the duodenum. In doing so, we could potentially harvest monomers from this breakdown as a complete energy source<sup>[28]</sup>. Accounting for approximately 4% of all microbes occurring in the cecum of the gut, these microorganisms are improperly located for proper energy harvesting. If these pathways can be cloned into members of Lactobacillaceae located in the duodenum, there is a strong potential to enhance energy harvesting in otherwise wasted carbohydrate sources<sup>[29]</sup>. By utilizing a natural commensal of the foregut, it is possible to circumnavigate opportunistic opportunities associated with the introduction of locally foreign microbes into the gut. Interestingly, using similar technologies, it may even be possible to utilize gut bacteria that enable the digestion of toxic plants as presented recently for the desert woodrat that can ingest plants containing toxic levels of creosote<sup>[30]</sup>. This bifurcated approach would not only enhance carbohydrate catabolism but result in making toxins biologically inert.

Given that cellulolysis is an inherent part of the human gut, augmenting this process could be a strategy for utilizing otherwise indigestible fiber in soldiers lacking normally digestible food. This process could be accomplished by enhancing endogenous bacteria or by supplementation with exogenous cellulolytic bacteria that are known to be suited to the human gut.

## ENDORPHINOGENIC GUT BACTERIA

The interactions between the gut microbiome and the nervous system have been well documented and there is potential for exploiting this relationship to provide analgesia to soldiers. Severe, acute pain controlled by large, episodic doses of opioids like morphine is an unlikely goal using the proposed approach. However, long-term analgesia resulting from a reduction in the inflammatory response or through local production of nociceptive antagonists is proposed to target chronic pain. There are two proposed mechanisms to accomplish these goals: (a) the bioengineering of gut bacteria that release neurokinin (NK) receptor antagonists; and, (b) the seeding of beneficial gut microorganisms that minimize the inflammatory pain response<sup>[31]</sup>.

The gut microbiome-mediated antagonism of receptors responsible for the local and systemic pain response is an interesting possibility. A paradigm for this strategy is the inhibition of the pro-analgesia NK receptors found in the gastrointestinal tract and dorsal root ganglion which are activated by tachykinins such as substance P, neurokinin A and neurokinin B<sup>[32]</sup>. While there are no gut bacteria that naturally release NK receptor antagonists, it should be possible to engineer an *E. coli* that secretes a peptide capable of specifically blocking the NK1 receptor, which is the target of most opioids. Thus local and systemic pain management may be possible if engineered antagonists can be properly absorbed. Although this idea is as of yet unproven, there are three established considerations that justify this theory: the NK1 receptor is a G protein-coupled receptor<sup>[33]</sup>; this family of receptors is highly druggable<sup>[34]</sup>; and, there is a genetic system that creates *E. coli* capable of releasing recombinant peptides amenable to high-throughput screening for the desired effect<sup>[35]</sup>. Therefore, it is conceivable to create an *E. coli* capable of seeding the gut and releasing an NK1 receptor antagonist. Since NK1 receptors are also present in the dorsal horn of the spinal cord<sup>[36]</sup>, this peptide could have systemic analgesic effects<sup>[37]</sup>. This effect will depend on the bioavailability and pharmacokinetics of the NK1 receptor antagonist.

There are populations of bacteria capable of residing in the gut whose populations can be augmented to benefit soldiers for pain management in the field. Transient supplementation of the probiotic *Bifidobacteria infantis*, strain 35624, increases the pain threshold in rats<sup>[38]</sup>. *Lactobacillus acidophilus* reduces visceral pain by quasi-epigenetically<sup>[39]</sup> increasing the expression of cannabinoid 2 receptor and  $\mu$ -opioid receptor expression in colonic epithelium, which is also the proposed mechanism for the analgesic effects of *Bifidobacteria infantis* strain 35624<sup>[38]</sup>. Furthermore, *Lactobacillus paracasei* directly has been linked to a reduction of inflammatory pain in the viscera of mice<sup>[31]</sup>. While the mechanisms underlying these effects are not completely clear, evidence continues to emerge that probiotic supplementation with these microorganisms has the potential to alter the enteric nervous system nociception. While acute and traumatic injuries may be outside of the scope of this treatment regimen, more mild injuries such as muscle or ligament damage have the potential to be managed using this approach.

Thus, it may be possible to antagonize nociceptive receptors and alter the threshold of pain for soldiers in combat situations. This would be accomplished through either bioengineering microorganisms to deliver analgesics directly to the gut or via the probiotic modulation of the gut microbiome. Either option, while speculative, is based on established approaches that simply need to be explored further for this endeavor to reach its full potential.

## NEURO-MODIFYING GUT BACTERIA

Soldiers are exposed to many physiological and psychological stressors that impair performance on the battlefield and make assimilation back to civilian life challenging. Recent advancements in the understanding of the gut-brain axis and neuromodulating commensal microorganisms underscore the potential for the amelioration of combat stress, anxiety, and fear<sup>[40]</sup>. Not only is this useful for combat situations but for post-deployment as well. Thus, a military application of this understanding is the manipulation of the gut-brain axis for enhanced psychological functioning.

Thoroughly characterized pathways relaying bidirectional communication interconnect the gut and a number of other systems, e.g. the autonomic and enteric nervous systems, as well as the neuroendocrine system. Neuroactive metabolites produced by resident gut bacteria may affect brain function by entering the systemic circulation or through interacting with the Vegas X nerve directly<sup>[41]</sup> acting as neurotransmitters and neuromodulators resulting in reduced anxious and depressive behaviors<sup>[42]</sup>. For example, *Lactobacillus* and *Bifidobacterium* spp. produce gamma-aminobutyric acid, an inhibitory neurotransmitter in the brain implicated in the pathophysiology of anxiety and depression<sup>[43]</sup>. *Escherichia* spp. *Bacillus* spp. and *Saccharomyces* spp. produce norepinephrine, a monoamine neurotransmitter involved in the stress response system as well as anxiety and depressive disorders<sup>[42]</sup>. *Candida*, *Streptococcus*, *Escherichia* and *Enterococcus* spp. produce 5-HT, a neurotransmitter whose synaptic reuptake is blocked by certain antidepressant and anti-anxiety medications<sup>[42]</sup>. Finally, *Bacillus* and *Lactobacillus* spp. produce acetylcholine<sup>[43]</sup> that is involved in stress response, anxiety, and depression in the brain<sup>[44,45]</sup>.

Further, the gut microbiome also affects hormone production. Administration of *Lactobacillus* and *Bifidobacterium* spp. (reduced hypothalamic-pituitary axis) stress response in rats by modulating levels of plasma adrenocorticotropic hormone, corticosteroids, and corticotrophin-releasing hormone<sup>[46]</sup>. *Bifidobacterium infantis* probiotic therapy in rats has been associated with reversal in behavior problems, norepinephrine levels in the brain, and decreased activity neural processes<sup>[47]</sup>. This effect has been characterized successfully in human clinical trials as well<sup>[48]</sup>.

Overall, the colonization of gut microbiota plays a role in neurochemistry as well as the neuroendocrine system. By using these bacteria in soldiers as psychobiotics, there are opportunities to benefit the mental health of service personnel on and off the field.

## MUSCLE ENHANCING GUT BACTERIA

Enhancing muscle strength and recovery is of concern for combat situations, and center to that potential is the gut microbiome. Two approaches are suggested, the first being the endogenous production of anabolic steroids and human growth hormone. The second being control of the inflammatory responses associated with muscle fatigue and weakness. Through using this approach, it may be possible to enhance soldier strength and stamina.

Steroids and human growth hormones have been exploited for years to enhance muscle physiology. Given the negative effects of these hormones when used in therapeutic or supra-therapeutic doses<sup>[49]</sup>, sustained low doses may avoid these consequences while remaining efficacious<sup>[50]</sup>. Interestingly, it may be possible to engineer a common gut commensal, *Escherichia coli*, to produce anabolic steroids as well as human growth hormone<sup>[25]</sup>. These hormones are known to enhance physiological functioning, strength, and power that will be beneficial to soldiers in combat. The ability of gut bacteria to naturally or synthetically produce anabolic steroids has been previously investigated and reviewed<sup>[24,51]</sup>.

Chronic muscle fatigue is a common problem in any physically exerting situation and combat is no exception. Much of what is known about muscle atrophy is directly tied to a breakdown of the gut barrier, an increase in inflammation, and a resulting change in metabolic energy partitioning associated with prolonged physiological stress<sup>[52]</sup>. Enhancing the anti-inflammatory effects of a healthy microbiota has been proposed as a solution to muscle fatigue and strain<sup>[26]</sup>. Dis-biosis associated with prolonged inflammation coincides with sustained physical exertion exacerbates muscle weakness due to an increase in fatty acid catabolism in muscle<sup>[26]</sup>. Therefore, with the re-establishment of a healthy, anti-inflammatory microbiome, it may be possible to abrogate the effects of continued physiological stress that instigates muscle fatigue.

Research into this phenomenon has just begun. While this avenue as a treatment option has not yet been explored, it has the potential to augment soldier strength and stamina if properly applied. Whether by seeding steroid-producing microorganisms in the gut or by modulating the inflammatory response that causes muscle fatigue, this approach could physically enhance soldiers and solve many issues associated with the physical stress associated with combat.

## GUT BACTERIA THAT PROMOTE WOUND HEALING

Microbiomes exist on extraintestinal surfaces, which are exemplified by the unique and well-documented cadre of microbes present on the skin. While our understanding of the interconnectedness of all of these microbial communities is in its infancy, evidence is emerging that is suggestive of a link a change commensal microbiota in the gut with the outcome of wound repair in the periphery<sup>[53,54]</sup>.

The importance of this relationship seems to converge both in the agonism of specific pathways of the innate immune system

and the modulation of the inflammatory response through a healthy balance of commensal microorganisms<sup>[53]</sup>. *Lactobacillus* spp. in the gut have directly been tied to an increase in ERK activation in the periphery, which has been linked to beneficial changes in immune cell migration during chronic wound healing to the extremities<sup>[54]</sup>. Conversely, the gut pathogens *Klebsiella pneumoniae* and *Escherichia coli* are capable of unbalancing the immune response resulting in impaired wound healing when their gut populations are overgrown<sup>[54]</sup>. Thus, through reducing the total inflammation of the gastrointestinal tract, it is possible to tip the inflammation towards a beneficial state that may promote wound healing in soldiers.

## CONCLUSION

A number of the discoveries relating to the host-gut microbiome axis potentially allow us to exploit its benefits for military medicine. While manipulating the gut microbiome for optimizing host health is still controversial, evidence has emerged to breathe legitimacy into the tactic as reviewed here; however, there is some unanswered questions making the executing this approach difficult. Challenges include the type of regimen selected, the initiation, length and cessation of regimen, inconsistency of clinical outcomes, and the broader clinical studies needed to validate this research. As our understanding of the host gut-microbiome axis expands, resolution to the aforementioned problems is likely and the potential for exploiting it for the benefit of the military is only going to become more attractive.

One of the most pressing issues for successfully manipulating the gut microbiome is the type of regimen selected and kind of “biotic” used. Manipulating the microbiome can occur via both colonization and enhancement. Regarding colonization, it is likely that a formulation can be produced that will be beneficial to the host system<sup>[55]</sup>, although enhancement is a much more feasible route in the short term. Microencapsulation of either ideal resident or bioengineered strains of microorganisms has the potential to increase delivery of viable microbes to the gut and enhance their efficacy while overcoming some of the survival and colonization limitations of probiotics<sup>[56]</sup>.

The other option is the enhancement of the gut-microbiome axis through targeting gut epithelial integrity and enhancing the sustainment of beneficial microbial populations. Evidence suggests that some probiotics exacerbate and even initiate inflammation resulting in a reduction in gut endothelial integrity. Therefore, it appears that the selected regimen should be designed to circumvent the potential deleterious effects of probiotics. Prebiotics are not necessarily the answer either<sup>[57]</sup>. It seems that post biotics have promise and some evidence suggests that they positively assist in the modulation of gut integrity and beneficial gut microorganisms<sup>[58,59]</sup>. Thus, we propose a selective seeding of the gut as well as a post biotic supplement to effectively use both possible routes of control. Importantly, the regimen must be combinatorial and must be started ahead of combat related duties to attenuate any inflammation that may abrogate the effects and mechanisms suggested in this paper<sup>[59]</sup>.

It should be noted that there are potential negative impacts for the engineering described herein. Altering the gut microbiome can possibly allow the expansion of pathogenic bacteria that were being inhibited by gut bacteria unintentionally, excluded by the introduction of new bacteria. Additionally it is possible that an immunologic response could be directed at compounds secreted by the new members of the gut microbiome in the engineered scenario. Thus these caveats need to be addressed.

In summary, our review has proposed five potential strategies to modulate physiology and improve the performance of the combat soldiers. The first proposed application is seeding the gut microbiome with cellulolytic bacteria to use energy from traditionally non-digestible plants in remote areas. The second is to generate analgesia by supplementing the gut microbiome with bioengineered bacteria actively releasing NK receptor antagonists, or with gut microbes minimizing the inflammatory pain response. The third strategy is reducing combat-induced anxiety of soldiers via modulation of neural activities by bacteria producing anxiolytic metabolites. The fourth application is the microbial production of anabolic agents that improve strength and performance of the soldiers in the battlefield, as well as to decrease inflammatory responses linked to muscle fatigue. Finally, modifying the gut microbiome could alter the inflammatory response promoting wound healing in soldiers.

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