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Gut microbiota in professional and amateur athletes: the impact of physical activity on microbiota - what do we know? A literature review

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Abstract

Introduction and purpose: The gut microbiota, a diverse community of microorganisms inhabiting our intestines, is pivotal for human health. This intricate microbial ecosystem influences various aspects of health, including digestion, metabolism, immune function and even mental well-being. Comprehending connections between physical activity and gut microbiota is vital for public health advocacy, particularly amidst the rise of sedentary lifestyle. The nature of modern lifestyles contributes to decreased physical activity, exacerbating the health consequences associated with a disrupted gut microbiota. In

conditions such as type 2 diabetes, hypertension, and obesity, alterations in the composition and function of the gut microbiota have been observed. These changes, often referred to as dysbiosis, are characterized by a decrease in microbial diversity and shifts in the relative abundance of certain bacterial taxa. The aim of this study is to provide a comprehensive overview of the current state of knowledge regarding the impact of professional and amateur physical activity on gut microbiota.

Description of the state of knowledge: Recent research reveals a significant link between physical activity and the gut microbiota, hinting at its potential health implications. Studies indicate that exercise may promote greater microbial diversity in the gut, contributing to improved metabolic balance and reduced inflammation.

Summary: Exercise can modulate the composition and diversity of gut microbes, promoting a more beneficial profile associated with enhanced metabolic health and immune function. Ongoing research in this area could guide the development of exercise plans that enhance not just physical fitness but also gut health and overall well-being.

Keywords: physical activity; physical exertion; professional athletes; gut microbiota; intestinal microbiota.

Introduction

Physical activity encompasses any bodily movement resulting in energy expenditure, ranging from structured exercise to daily activities like walking or gardening.[1] Physical activity exerts multifaceted effects on human health, encompassing both physiological and psychological domains.[2] The value of incorporating regular physical activity into daily routines is widely acknowledged and backed by substantial evidence. Moreover, there's a growing understanding of the mutual relationship between the human body and beneficial bacteria, which number in the billions.[3] There's increasing emphasis on learning to cooperate with these microbes, with research highlighting how physical exertion affects their composition and function. Particularly, there's a growing interest in the role of symbiotic microbes in the gut, underlining the importance of understanding how physical activity influences their dynamics and the overall health of the host organism. This is especially

important considering that imbalances in the gut microbiota are associated with various civilization-related diseases.[4]

The articles forming the basis of our review were identified through a literature review using the keywords "physical activity", "physical exertion" and "professional athletes" in conjunction with the phrases "gut microbiota", "intestinal microbiota" in literature databases on PubMed and Google Scholar platforms. In selecting the bibliography, we prioritized originality, scholarly value, research ethics involving human subjects and the publication date of the manuscripts.

Gut microbiota

The gut microbiota refers to the diverse community of microorganisms (bacteria, archaea and eukarya) inhabiting the gastrointestinal tract (GI) of humans.[5] It has evolved alongside the host for millennia, establishing a complex and mutually beneficial relationship.[6]

The composition of the gut microbiota in humans has changed over evolutionary time. According to Moles L and Otaegui D factors such as changes in diet, lifestyle, environment and interactions with other species have influenced this variability. Shifts in dietary patterns from hunter-gatherer to agricultural societies have impacted the microbial composition in the gut.[7] Similarly, Rodríguez JM and colleagues underscore that geographical location, urbanization and the introduction of antibiotics may have significantly influenced the evolution of the gut microbiota composition in humans.[8]

After birth, the human gut quickly becomes home to a variety of microbes, with factors like gestational age, delivery method (vaginal or assisted), feeding (breast milk or formula), hygiene and antibiotic use playing key roles in this colonization process.[9] Initially, facultative anaerobes pave the way for the establishment of strict anaerobes like *Bacteroides*, *Clostridium*, and *Bifidobacterium* spp. In newborns, the gut microbiota is characterized by low diversity, with Proteobacteria and Actinobacteria dominating, but diversity increases over time, with Firmicutes and Bacteroidetes becoming more prevalent. By the end of the first year, infants develop a unique microbial profile, gradually resembling that of adults by 2–5 years old.[10] Hence, the first 3 years of life are crucial for dietary interventions aimed at enhancing child growth and development, as this period is when the gut microbiota, crucial for health

and neurodevelopment, is established. Any changes during this time can significantly impact the host's health and development.[11,12],

Estimates suggest that nowadays there are over 10^{14} microorganisms inhabiting the GI tract, comprising approximately ten times more bacterial cells than human cells and containing over 100 times the genomic content (microbiome) compared to the human genome.[13] A recent update proposes that the balance between human and bacterial cells in the body is more equal, nearing a 1:1 ratio.[14] Because of the substantial population of bacterial cells, the host and its resident microorganisms are commonly labeled as a 'superorganism'.[15]

The role of gut microbiota

In recent years, there has been significant interest in the gut microbiota within the scientific community. The gut microbiota maintains a harmonious partnership with the gut mucosa, playing pivotal roles in metabolism, immunity, and gut protection within a healthy individual. Considered akin to an organ in itself, this microbiota thrives on nutrients sourced from the host's diet and the shedding of epithelial cells.[16]

The gut microbiota primarily derives its nutrients from dietary carbohydrates, which undergo fermentation by colonic organisms like *Bacteroides*, *Roseburia*, *Bifidobacterium*, *Fecalibacterium*, and *Enterobacteria*. This fermentation process yields short-chain fatty acids (SCFAs) such as butyrate, propionate, and acetate, serving as significant energy sources for the host.[17] SCFAs interact with the G protein-coupled receptor Gpr41 and the enteroendocrine hormone PYY, contributing to host energy balance. Butyrate also plays a role in preventing the accumulation of toxic metabolic by-products like D-lactate.[18,19] *Bacteroides*, particularly *Bacteroides thetaiotaomicron*, express a variety of enzymes involved in carbohydrate metabolism, exemplified by its genome coding for over 260 hydrolases, surpassing the human genome's count. Additionally, gut bacteria like *Oxalobacter formigenes*, *Lactobacillus* species, and *Bifidobacterium* species help counteract oxalate synthesis in the intestine, thus reducing the risk of kidney stone formation.[20]

The gut microbiota also plays a crucial role in synthesizing vitamin K and several components of vitamin B. Additionally, certain bacteria from the *Bacteroides* genus have the ability to produce conjugated linoleic acid (CLA), which is recognized for its various health-promoting properties, including anti-diabetic, anti-atherogenic, anti-obesogenic, hypolipidemic and immunomodulatory effects.[21,22] According to Chung H et al. the gut microbiota collaborates with both the innate and adaptive immune systems to modulate gut immunity.

This involves various components and cell types, such as gut-associated lymphoid tissues (GALT), effector and regulatory T cells, IgA-producing B cells, Group 3 innate lymphoid cells, and resident macrophages and dendritic cells in the lamina propria. The gut microbiota's influence on the development of a healthy GALT is indicated by the altered formation of Peyer's patches and isolated lymphoid follicles, characterized by an abundance of IgE+ B cells rather than the typical IgA+ B cells.[23] In terms of effector T cell responses, the intestine primarily exhibits Th2 responses instead of Th1 responses, which are normally mediated by Th1 and Th17 cells. Gut commensals are believed to activate IL1 β through TLR-MyD88 signaling, consequently promoting the development of IL17.[24]

Hooper LV and colleagues report that *Bacteroides thetaiotaomicron* enhances lipid metabolism by increasing the efficiency of lipid hydrolysis. This is achieved through the upregulation of colipase expression, which is essential for lipid digestion by pancreatic lipase, thereby mitigating the inhibition of lipoprotein lipase activity in adipocytes.[25]

Disturbances in the composition of the microbiota in diseases

Disruptions in the composition of gut flora have been linked to a wide range of human diseases, including inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), metabolic conditions such as obesity and diabetes, allergic diseases, and even neurodevelopmental disorders.[26] Spor et al. and Perry S et al. suggest that in inflammatory bowel disease (IBD), the dysbiosis of the overall gut microbial community is more significant than the influence of individual bacterial species as causal factors. Researchers note an imbalance between various components of the microbiota in inflammatory bowel disease (IBD). They highlight the following changes: decreases: Bacteroidetes, Lachnospiraceae, *Clostridium leptum*, *Clostridium coccoides*, *Faecalibacterium prasnitzii*, Firmicutes/Bacteroidetes ratio, Bifidobacteria and increases: Actinobacteria and Proteobacteria.[27,28] According to Dicksved et al., patients with Crohn's disease exhibit lower diversity in their gut microbiota compared to healthy individuals. Specifically, they observed an increase in *Bacteroides ovatus* and *Bacteroides vulgatus*, along with a decrease in *Bacteroides uniformis*. [29] In type 2 diabetes, Clemente JC reported the following alterations in gut microbiota composition: a decrease in Firmicutes, particularly Clostridia, an increase in Bacteroides-Prevotella compared to Clostridia coccoides-Eubacterium rectale, an increase in Betaproteobacteria and an elevated Bacteroidetes/Firmicutes ratio. These shifts in gut

microbiota have been associated with elevated plasma glucose concentrations.[4] According to Ley RE et al., Pflughoeft KJ with Versalovic J and Turnbaugh PJ et al., significant changes in the gut microbiota, particularly a decrease in Bacteroidetes and Methanobrevibacter smithii, along with an increase in Lactobacillus and a decrease in the Firmicutes/Bacteroidetes ratio, are associated with increasing obesity.[30,31,32] Round JL et al. suggest that early colonization with a more diverse microbiota might prevent allergies.[33]

Physical activity and the gut microbiota – what do we know?

The connection between physical activity and the gut microbiota is a rapidly evolving area of research. Studies have shown that exercise can modulate the composition and diversity of gut microbes, promoting a more beneficial profile associated with enhanced metabolic health and immune function. Mechanisms underlying this interaction include changes in intestinal motility, gut permeability and the production of metabolites such as short-chain fatty acids (SCFAs), which exert anti-inflammatory effects and contribute to host energy metabolism.[34,35] Furthermore, exercise-induced alterations in systemic factors like cortisol levels and inflammatory cytokines may indirectly influence the gut environment, shaping microbial communities.[35]

The gut microbiome influences the motivation for exercise

In a study led by Dohnalová Let al., researchers investigated the connection between the gut microbiome and exercise performance in mice. They found that the microbiome plays a crucial role in enhancing exercise performance by influencing dopamine signaling in the brain. Specifically, they discovered that gut bacteria produce endocannabinoid metabolites, which stimulate sensory neurons expressing TRPV1 receptors in the gut. This stimulation leads to increased dopamine levels in the ventral striatum during exercise, enhancing the rewarding properties of physical activity. The researchers observed that activating this pathway improved running performance in mice. Conversely, depleting the microbiome, inhibiting peripheral endocannabinoid receptors, ablating spinal afferent neurons, or blocking dopamine receptors all resulted in a decrease in exercise capacity.

These findings suggest that the gut microbiome influences the motivation for exercise through interoceptive circuits, providing a microbiome-dependent explanation for individual differences in exercise performance. Furthermore, the study proposes that molecules mimicking interoceptive signals from the gut to the brain could potentially enhance the motivation for exercise.[36]

The connection between physical activity and the gut microbiota in physically active individuals

Numerous studies have examined variances in gut microbiota composition between physically active individuals (including athletes) and various populations.

The influence of physical activity on the gut microbiota in amateur athletes

In their study, Bressa et al. investigated the gut microbiota composition of sedentary and active premenopausal women. They enrolled 40 participants who met specific inclusion criteria, including being premenopausal women aged 18 to 40 years with a body mass index (BMI) between 20 and 25 kg/m². Sedentary participants were defined as those who did not meet the minimal exercise guidelines established by the World Health Organization, while active participants engaged in at least 3 hours of physical exercise per week. Exclusion criteria comprised any pathology, previous gastrointestinal surgery, recent antibiotic use, smoking, prebiotic or probiotic consumption, vegetarian or vegan diet, pregnancy, or lactation. All participants were Caucasian, and ethical approval was obtained from the Ramón y Cajal Hospital Ethics Committee. Stool samples were collected from all participants for microbiota analysis. Physical activity levels were objectively assessed using accelerometers, while dietary patterns were evaluated through a food frequency questionnaire. Body composition was measured using dual-energy X-ray absorptiometry (DEXA). DNA extraction from stool samples was followed by sequencing and bioinformatic analysis to characterize the gut microbiota composition. The study unveiled significant disparities in body composition parameters between sedentary and active women, particularly in adiposity and muscle parameters. Although both groups adhered to balanced diets, the active group consumed significantly more fiber, fruits, and vegetables compared to the sedentary group. Significant discrepancies in physical activity levels were observed between the two groups, with the active cohort engaging in more moderate physical activity and exhibiting higher energy expenditure. Sedentary behavior patterns also differed, with sedentary individuals spending more time in prolonged bouts of inactivity.

Microbiota analysis demonstrated no significant differences in alpha diversity between sedentary and active groups. However, beta-diversity analysis unveiled distinct microbial community structures between the cohorts. While no significant variations were observed at the phylum level, certain bacterial families and genera differed significantly between sedentary and active women. *Bifidobacterium*, *Faecalibacterium prausnitzii*, *Roseburia*

hominis, and Akkermansia muciniphila were found in higher abundance in active women compared to sedentary women, suggesting potential associations between physical activity levels and specific microbial taxa.

Overall, this study provides valuable insights into the intricate relationship between physical activity, dietary habits, body composition and gut microbiota composition in premenopausal women. The findings underscore the potential impact of lifestyle factors, such as regular physical activity, on shaping microbial communities and, consequently, overall health.[37]

Estaki et al. conducted a comprehensive study to explore the impact of physical exercise on the composition of the gut microbiota, particularly focusing on its association with cardiorespiratory fitness (CRF). The research involved a cohort of healthy individuals, carefully selected to exclude any contraindications to vigorous exercise. CRF was assessed using peak oxygen uptake (VO_{2peak}), a widely accepted measure of cardiovascular fitness, determined through a maximal exercise test on a cycle ergometer. Participants were categorized into low, average, or high fitness groups based on established norms adjusted for age and sex. Stool samples were collected from participants following standardized protocols and stored for subsequent analysis. The samples underwent high-throughput sequencing to characterize the microbial communities present. Bioinformatic analyses were then employed to assess alpha and beta diversity, taxonomic composition, and predicted metagenomic functions of the gut microbiota.

The results revealed a significant positive correlation between CRF and microbial diversity, specifically taxonomic richness. Individuals with higher CRF demonstrated greater microbial diversity in their gut microbiota. Moreover, the microbial profiles of physically fit individuals were found to favor the production of butyrate, a short-chain fatty acid associated with improved gut health. This was supported by measured increases in fecal butyrate production among participants with higher CRF.

Furthermore, the study identified specific bacterial taxa associated with increased CRF, including key butyrate-producing genera such as Clostridiales, Roseburia, Lachnospiraceae, and Erysipelotrichaceae. These findings underscore the potential role of physical exercise in shaping the composition and function of the gut microbiota, with implications for overall host health. Overall, the research provides valuable insights into the interplay between physical activity, cardiorespiratory fitness, and the gut microbiota composition. The findings suggest that regular exercise may exert beneficial effects on gut microbial diversity and function,

highlighting the importance of lifestyle factors in modulating microbiome-host interactions and potentially mitigating dysbiosis-associated diseases.[38]

The gut microbiota of professional athletes

Recent research in the field of microbiology has increasingly focused on understanding the complex interplay between physical activity and gut microbiota composition, particularly among professional athletes. One key finding comes from the study of Clarke et al., where the gut microbiota of professional male rugby players (n = 40) was compared to healthy non-athlete subjects (n = 46). It was discovered that the athletes had higher microbial diversity, particularly an increased abundance of the genus *Akkermansia*, associated with metabolic health.[39] Additionally, Barton et al. conducted a study utilizing whole metagenome shotgun sequencing, revealing that athletes exhibited metabolic pathways and fecal metabolites indicative of enhanced fitness and overall health compared to sedentary controls. Specifically, athletes had an enriched profile of short-chain fatty acids (SCFAs), which are known for their numerous health benefits and association with a lean phenotype. Moreover, athletes exhibited higher levels of the metabolite trimethylamine N-oxide (TMAO), which has been linked to cardiovascular health and may reflect the impact of exercise on metabolic function and gut microbiota composition.[40]

Petersen et al. conducted a study to explore the gut microbiotas of professional and amateur competitive cyclists and their relationship with exercise duration and intensity. They found a positive correlation between exercise duration (>11 hours per week) and the abundance of *Prevotella*, a genus associated with plant-rich diets. Additionally, *Methanobrevibacter smithii*, involved in methane production and energy metabolism, was upregulated in some professional cyclists. These findings suggest that exercise type and intensity may influence the composition and functionality of the gut microbiota in athletes. The study provides valuable insights into the diversity of microorganisms in athletes' gut microbiomes at both the metagenomic and metatranscriptomic levels. Despite limitations such as the lack of in-depth dietary analysis and a matching non-cyclist cohort, the data offer significant contributions to understanding the gut communities of cyclists. Moreover, the study highlights potential avenues for future research, including the investigation of dietary influences alongside exercise and the prevalence of specific microbial species in different athlete populations.[41]

The study conducted by Jang et al. aimed to compare the fecal microbiota characteristics of bodybuilders and distance runners, alongside sedentary controls, and investigate the relationship between microbiota, body composition, and nutrition. The research involved 45 male participants aged 19–28 years, comprising healthy sedentary men (as controls; $n = 15$), bodybuilders ($n = 15$), and elite distance runners ($n = 15$). Body composition was assessed using DXA, physical activity level with IPAQ, and dietary intake was analyzed using a computerized nutritional evaluation program. Fecal samples were collected and subjected to DNA extraction, followed by sequencing for gut microbial diversity analysis through a bioinformatics cloud platform. The results revealed that exercise type was associated with athlete diet patterns. Bodybuilders followed a high-protein, high-fat, low-carbohydrate, and low-fiber diet, while distance runners adhered to a low-carbohydrate, low-fiber diet. Bodybuilders exhibited lower levels of beneficial bacteria and reduced microbial diversity compared to controls. Additionally, aerobic or resistance exercise training with an unbalanced intake of macronutrients and low dietary fiber led to similar gut microbiota diversity. Notably, daily protein intake was negatively correlated with gut microbiota diversity in distance runners. These findings suggest that high-protein diets may negatively impact gut microbiota diversity in athletes, particularly those in resistance sports following high-protein, low-carbohydrate diets.[42]

In their cross-sectional study, Mörkl et al. investigated the gut microbiota among 106 female participants, comprising individuals with anorexia nervosa (AN) ($n = 18$), athletes ($n = 20$), and those with varying weight statuses: individuals with normal weight ($n = 26$), overweight ($n = 22$) and obesity ($n = 20$). Stool samples were collected from each participant for 16S rRNA gene analysis using the Quantitative Insights Into Microbial Ecology (QIIME) software. Alongside microbiota analysis, the researchers conducted diverse assessments including anthropometric evaluations, ultrasound measurements of subcutaneous adipose tissue, bioimpedance analysis, depression inventories, and dietary intake evaluations. Their findings revealed significantly lower alpha diversity in AN patients and obese individuals compared to other groups, whereas athletes exhibited the highest diversity. Interestingly, factors such as total fat mass, serum lipids, C-reactive protein (CRP), depression scales, and smoking status were inversely associated with microbiota diversity, suggesting a potential lifestyle-microbiota relationship. Moreover, Coriobacteriaceae emerged as the only enriched phylotype in AN patients relative to other groups. This study underscores the presence of intestinal

dysbiosis in AN and provides insights into gut microbiome characteristics across different BMI and physical activity groups.[43]

The investigation conducted by O'Donovan CM et al. involved the procurement of fecal and urine samples from 37 elite Irish athletes of international caliber. Employing shotgun metagenomic sequencing, fecal samples were subjected to comprehensive microbial analysis, while both fecal and urine samples underwent metabolomic profiling. The study delineated discernible disparities in the compositional and functional attributes of the gut microbiota among athletes belonging to various sports classification groups (SCGs). Particularly noteworthy were the distinct microbial signatures observed in athletes engaged in sports characterized by high dynamic components, as well as those involved in activities encompassing both dynamic and static elements. Through taxonomic profiling, heightened abundance of *Bifidobacterium animalis*, *Lactobacillus acidophilus*, *Prevotella intermedia*, and *Faecalibacterium prausnitzii* was discerned in athletes exhibiting pronounced dynamic activity. Conversely, athletes participating in disciplines combining dynamic and static components manifested augmented levels of *Bacteroides caccae*. Metabolomic analysis further elucidated differential profiles of microbial and host-derived metabolites among SCGs, with significant variations in the concentrations of key metabolites such as cis-aconitate, succinic acid, lactate, and creatinine. These findings underscore the putative influence of training modalities or intensities on the gut microbiota, thereby prompting further inquiry into the mechanistic underpinnings thereof. Notably, microbial diversity exhibited no significant disparities across sport classifications. [44]

The study, conducted by Scheiman J et al., aimed to investigate the influence of physical activity on the composition of gut microbiota, focusing particularly on the genus *Veillonella* and its potential association with exercise performance. Through extensive sampling and sequencing of stool samples collected from both professional and non-professional athletes before and after exercise, significant changes in the abundance of specific microbial genera, notably *Veillonella*, were identified. This included a significant increase in *Veillonella* abundance in marathon runners post-marathon, suggesting a potential link between this bacterial genus and athletic performance. Furthermore, the researchers isolated a strain of *Veillonella atypica* from stool samples and demonstrated its ability to improve exercise performance in mice. This was evidenced by a significant improvement in treadmill run time following inoculation with *Veillonella atypica*. Shotgun metagenomic analysis in elite athletes showed an enrichment of genes involved in metabolizing lactate into propionate post-exercise.

Mass spectrometry results further supported these findings by demonstrating the production of acetate and propionate by *Veillonella* strains isolated from athletes.

The study also confirmed that systemic lactate can cross the epithelial barrier into the gut lumen, where it may be metabolized by *Veillonella*. Animal experiments with *Veillonella* colonization and intracolonic propionate infusion replicated the enhanced exercise phenotype observed in athletes. These results suggest that *Veillonella* colonization may promote performance by metabolizing systemic lactate into propionate, which then enters circulation and enhances athletic performance. The findings suggest a symbiotic relationship between *Veillonella* and physically active hosts, with the high-lactate environment of athletes providing a selective advantage for colonization by lactate-metabolizing bacteria. Further exploration of these mechanisms could have significant implications for human health and performance enhancement strategies.[45]

The gut microbiota in the context of aging

The study conducted by Strasser B and colleagues sheds light on the intricate relationship between aging, physical activity and the gut microbiota. Aging is often accompanied by an increased susceptibility to chronic diseases, which can disrupt the delicate balance of gut flora, leading to dysbiosis. However, the researchers highlight the significant role of lifestyle factors, particularly regular physical exercise and a balanced diet, in positively influencing skeletal muscle and immune aging across all age groups. Their review emphasizes the physiological effects of aging on the gut microbiota, immune system, and skeletal muscle, emphasizing the crucial link between physical activity and gut microbiota composition in older adults. They underscore the importance of immune protection through physical activity and specific exercise interventions during aging, alongside the impact of exercise and protein intake on skeletal muscle performance in older individuals. Importantly, the findings suggest that physical activity, especially endurance exercise, serves as a potent strategy for promoting healthy aging and combating cellular immunosenescence and inflammaging. Resistance training also emerges as vital for preserving functional capacity and muscle performance in older adults. Furthermore, the study highlights the potential role of physical activity interventions in modulating the gut microbiota, promoting the synthesis of metabolically active mediators such as butyrate and other short-chain fatty acids. Although evidence indicates beneficial effects of aerobic exercise on gut microbiota composition, more research is warranted to explore the effects of different exercise modalities, frequencies and intensities, especially in the elderly population.[46]

Summary

Overall, the findings suggest that physically active individuals, especially athletes, harbor a gut microbiota profile enriched with health-promoting bacterial species and metabolic pathways. These insights highlight the potential role of exercise in shaping the gut microbiome and its implications for overall health and athletic performance. Understanding the intricate interplay between physical activity and the gut microbiota holds great promise for elucidating novel pathways linking lifestyle factors to health outcomes. Continued research in this field may inform strategies for optimizing exercise regimens to promote not only physical fitness but also gut health and overall well-being.

Author's contribution

Conceptualization, Sebastian Szopa and Julia Szymonik ; methodology, Sebastian Szopa; software, Sebastian Szopa; check, Julia Szymonik and Sebastian Szopa; formal analysis, Sebastian Szopa; investigation, Julia Szymonik; resources, Julia Szymonik; data curation, Sebastian Szopa; writing - rough preparation, Sebastian Szopa and Julia Szymonik; writing - review and editing, Julia Szymonik; visualization, Sebastian Szopa; supervision, Sebastian Szopa; project administration, Julia Szymonik; All authors have read and agreed with the published version of the manuscript.

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Conflict of Interest Statement

All authors declare that they have no conflicts of interest.

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