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# The gut glycome-microbiota axis: a missing key to optimizing endurance fitness

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## Abstract

Endurance athletes offer unique physiology and metabolism compared to sedentary individuals. Athletes training at high intensities for long periods are at risk for impaired intestinal barrier function, gastrointestinal and respiratory disturbances, and an imbalance of the microbiota-glycome axis. The gut glycome profoundly affects the assembly and function of the gut microbiome, immune and mitochondrial function, and the gut barrier homeostasis. At the same time, microbes' composition and function regulate the *O*-glycome, mucus secretion, intestinal barrier integrity, and immune response. During endurance exercise, microbial glycans foraging might enhance fuel bioavailability for the mitochondria while improving aerobic work competencies, sparing glycogen usage, and increasing performance. Understanding how interactions between the gut microbiome and host glycome affect gastrointestinal physiology, immune modulation, and the ability to harness energy in response to exercise offers the opportunity to shape lifelong gut, immune, and metabolic health and enhance athletic performance. Dietary glycans, pro-, and postbiotics supplementation have been shown to improve intestinal mucus production and barrier function, offering new opportunities for enhancing the nutritional plans for athletes.

Keywords: gut microbiota, energy, endurance, dietary glycans, leaky gut, glycome, mucins, stress

## Introduction

Endurance exercise can be defined as prolonged cardiovascular efforts —such as running, cross-country skiing, cycling, aerobic exercise, or swimming —performed for an extended period <sup>1–3</sup>. Endurance exercise performance primarily depends on physiological adaptations to sustain the metabolic and thermoregulatory demands of such activity <sup>4</sup>, including coordinated muscle contractions, fatty acid oxidation, increased use of glycogen stores, mitochondrial biogenesis, increased reactive species (ROS) production, and electrolyte rebalance <sup>5</sup>. The development of fatigue limits sports performance, so the athletes with the most outstanding fatigue resistance often succeed <sup>6</sup>.

It is estimated that 30-50% of endurance athletes suffer from gastrointestinal complaints, including diarrhea, vomiting, nausea, and ischemia <sup>7</sup>, in which individuals show temporary gut microbial imbalances, impaired mucus integrity, intestinal barrier damage, and inflammatory responses, the so-called, leaky gut. Moreover, the excessive strain on the respiratory system (relative to its capacity) leads to exercise-induced hypoxemia <sup>8</sup>. Therefore, excessive and prolonged high-intensity exercise may impart digestive and respiratory problems, reduced protection from many diseases <sup>9</sup>, and worse sports performance.

New evidence has shown that the gut microbiome plays a role in athletic performance <sup>10–12</sup>. The human gut microbiome contains more than 100 trillion microorganisms and up to 10 million non-redundant genes <sup>13</sup>. It spans over 2,000 bacterial species <sup>14,15</sup> and has a density of ~ 40 – 300 billion microorganisms in the colon <sup>16</sup>.

The athlete's gut microbiome differs from non-athletes in composition and function<sup>10,11,17</sup>. Ultimately microbiota composition and function impact host athletic performance directly<sup>11,12,18–20</sup>, and indirectly through myokines and other cytokines, modulating the activation of the hypothalamic-pituitary-adrenal (HPA) axis and metabolic pathways<sup>12,21,22</sup> (Figure 1). For example, changes in microbial composition and increased diversity correlate with improved performance and cardiorespiratory fitness (as predicted by  $\text{VO}_2 \text{ max}$  or the  $\text{VO}_2 \text{ peak}$ ) in marathon runners regardless of sex, age, body mass index, and diet<sup>23</sup>. Increases in the Firmicutes-Bacteroidetes ratio<sup>24</sup> or depletion in *Eubacterium* spp enhance the cardiovascular capacity of athletes, as assessed by the maximum oxygen consumption<sup>25</sup>. Disruption of intestinal microbiota following antibiotic treatment can cause skeletal muscle endurance impairment<sup>26</sup>. Similar work demonstrated significant reductions in short-chain fatty acids (SCFAs), gut microbiota, and endurance capacity after antibiotic treatment<sup>27</sup>. Infusion of the SCFA acetate resulted in the restoration of endurance capacity. Deeper characterization of the links between athletic performance and gut microbiome revealed that just one bacterium (*Veillonella atypica*) was required to enhance the athletic outputs in treadmill mice experiments by producing propionate from exercise-induced lactate<sup>12,19</sup>. Enriching the genus *Bacteroides uniformis* and its preferred substrate,  $\alpha$ -cyclodextrin, enhanced endurance exercise performance in mice and humans, possibly by facilitating endogenous glucose production in the liver during exercise<sup>11</sup>. Lastly, exercise performance was enhanced in mice by augmenting dopamine signaling during physical activity, illustrating that gut-derived interoceptive circuits influence the rewarding properties of exercise<sup>28</sup>.

Complementary to these results, compelling new evidence suggests that intestinal glycome - the ensemble of glycans - may be a critical factor in overall enteric health and energy production, serving as a potential missing factor in improving athletic performance. Using horses as an endurance exercise model and along with shotgun sequencing, our team showed better athletic performance in subjects who harbored highly diverse gut microbiomes with a greater capacity to cleave host glycosaminoglycans from the gut mucus layer<sup>29</sup>, mainly composed of mucin 2 (MUC2). This higher ability to use host gut mucins could offer complimentary or unique metabolic pathways that enhance fuel bioavailability for the mitochondria while improving aerobic work competencies and sparing glycogen usage. This was the case even after factors such as diet were accounted for. Mucins are critical for pathogen recognition, immune activation, selection of commensals to repress pathogens, and, even more importantly, building protective barriers<sup>30</sup>. Most of these mucin functions are governed via the *O*-glycome - the ensemble of glycans and their *O*-glycosylation<sup>31</sup>. Changes in the expression and glycosylation of mucins are associated with dysbiosis, immune dysregulation, and disease such as diabetes<sup>32,33</sup>. An ever-expanding field suggests that MUC-2 glycosylation and *O*-GlcNAcylation regulation of cell nucleocytoplasmic and mitochondrial proteins play a role in energy metabolism, intestinal barrier, and immune function<sup>33,34</sup>, representing a significant challenge endurance exercise biology today.

There is a mutualistic relationship between the gut glycome, gut microbiota, and derived metabolites. The gut microbiota is required to synthesize large gel-forming mucins completely, including encapsulation, glycosylation, changes in fucosylation and sialidation patterns, and thickness<sup>35–37</sup>. Being both an environmental niche and a food source, this high diversity of gut mucins significantly impacts the gut microbiota composition, diversity, and stability, which can, in turn, stimulate mucosal expression and, together, form the basis of the host-microbial symbiosis<sup>37,38</sup>. Most bacteria use dietary fiber and starch as nutrient sources<sup>34</sup>; however, many *Bacteroides* spp and *Akkermansia muciniphila* contain an extensive

repertoire of enzymes that metabolize the host mucus layer<sup>32,39</sup>. In turn, the fermentation of these indigestible mucus layer glycans produces SCFAs<sup>38</sup>, which confer numerous well-known health benefits for the host, including energy production, immune regulation, mucosa protection, and remodeling, as well as modulation of mitochondrial function<sup>40</sup>, the limiting factor for fatigue onset and athletic performance. Overall, there is an urge to delineate the key role that glycome-microbiota interaction might have in athletes.

Given the increasing interest in exercise and the growing evidence of gastrointestinal complaints in endurance athletes, this review will provide insights into the crosstalk of the gut microbiome, the host glycome landscape, and its potential impact on exercise performance. Glycans, pre-, and post-biotics have a high potential to be applied in the dietary plans of athletes to prevent intestinal problems, while improving athletic performance in endurance events<sup>5</sup>. Therefore, we argue that the next phase in the gut microbiome and athlete research should be focused on the design of nutritional strategies that target the microorganisms able to regulate the *O*-glycome or the microbiota composition and function to maintain gastrointestinal mucosal health, reduce the prevalence of gastrointestinal symptoms and enhance athletic performance.

### **Gastrointestinal symptoms in endurance athletes: the mucosal glycome damage matters**

Over the last decades, the increased participation of the general population in endurance events has raised concerns regarding the impact prolonged and intense exercise has on immune and gastrointestinal health, which can lead to worse performance<sup>41</sup> and recovery<sup>42</sup>. For example, 30-50% of endurance athletes<sup>5,43</sup> and 96% of ultramarathon runners experience gastrointestinal distress, in which nausea and vomiting are the main reasons for withdrawal from the competition<sup>44,45</sup>. Although most symptoms are mild and do not cause long-term health effects, epigastric pain, heartburn, nausea, vomiting, abdominal pain, bloody diarrhea episodes, and dehydration are typical responses in endurance athletes. Oesophagitis, hemorrhagic gastritis, gastric ulcer, gastrointestinal bleeding, and ischaemic bowel may involve severe medical complications in endurance athletes<sup>5</sup>.

Vigorous endurance training ( $\geq 60$  min and  $\geq 70\%$  of maximum work capacity) increases sympathetic nervous system activity and redistributes blood flow from the splanchnic organs to the working skeletal muscles and peripheral circulation. This results in gut hypoxia and hypoperfusion, reduced intestinal motility and absorption capacity, damage of the specialized antimicrobial protein-secreting cells (Paneth cells), the mucus-producing cells (such as goblet cells), and increased loosening of tight junction protein structure<sup>46</sup>. The deterioration of the gastrointestinal mucosal barrier enables the translocation of bacteria and lipopolysaccharides (LPS) outside the gastrointestinal tract, triggering immune and inflammatory responses often resulting in increased intestinal permeability<sup>1</sup> and, eventually, endotoxemia<sup>47-49</sup>. Moreover, it induces oxidative stress via ROS production<sup>50</sup> and changes the oxygen concentration. A rat model showed that colonic ischemia destroyed intestinal MUC2, damaging the protective intestinal mucus layer<sup>51</sup> (Figure 2). Repairing the mucus layer is a time and energy-consuming process, and increased bacterial invasion of the lamina propria can trigger acute colitis<sup>52</sup>. Furthermore, emotional stress experienced by many endurance athletes can also decrease the intestinal mucus layer<sup>34</sup> and increase intestinal permeability<sup>1</sup>. Repeated bouts of stress decrease MUC2 synthesis and the number of goblet cells via notch signaling suppression in rats<sup>53</sup>. More recently, Silva and colleagues revealed that stress strongly affects the *O*-glycosylation of mucins, resulting in the flattening and loss of cohesive properties of the mucus layer<sup>34</sup>.

On the other hand, moderate and regular physical activity improves gastrointestinal symptoms in subjects with irritable bowel syndrome in humans<sup>54</sup>. In mice, moderate exercise has been associated with a lesser degree of intestinal permeability, preservation of mucus layer thickness, and lower rates of bacterial translocation along with up-regulated anti-microbial protein production and gene expression in small intestinal tissue ( $\alpha$ -defensin,  $\beta$ -defensin, Reg IIIb, and Reg IIIc)<sup>55</sup>. Therefore, variations in physical training, such as intensity, volume, recovery, training time, and fluid restriction during exercise, are determinant factors that may contribute to intestinal mucus layer impairment (*e.g.*, reduction in mucus synthesis, secretion, thickness, or viscosity)<sup>46</sup>.

The consequences of gut disturbances in endurance athletes may not be limited to the gastrointestinal system. Recent evidence suggests that gastrointestinal damage may be associated with respiratory comorbidities partly due to the bidirectional inter-organ communication of the gut-lung axis<sup>56-59</sup>. Even if the mechanisms bridging gut and respiratory mucins are poorly understood, respiratory disorders and exercise-induced hypoxemia often cause morbidity in top-level endurance athletes due to exertion in extreme conditions. Studies elucidating how the gut-lung axis communicates and the respective role of mucins are warranted.

### **The gut glycome composition and their primary functional roles in the gut**

One of the most critical host-produced glycans is mucin, which is continuously secreted from the goblet cells of the small intestine and colon or the surface mucous cells of the stomach<sup>60</sup>. Mucins are a large family of heavily *O*-glycosylated proteins (from MUC1 to MUC20) which cover all mucosal surfaces and constitute the major macromolecules in most body fluids<sup>61,62</sup>. The prominent gut mucin in the gastrointestinal tract is the gel-forming glycoprotein MUC2<sup>63</sup>, which is comprised mainly of glycans, > 80% by weight, which is made up of > 100 structures containing combinations of at least five mono-saccharides: galactose moieties (Gal), *N*-acetylgalactosamine (GalNAc), *N*-acetylglucosamine (GlcNAc), sialic acid (Sia), sulfate, and fucose (Fuc)<sup>64</sup>. Sialic acids protect underlying glycan chains from enzymatic degradation<sup>65</sup>.

The expression and composition profile of mucins vary among host tissues and within the gastrointestinal tract, which displays the highest and most diverse levels of mucin expression in the body<sup>36</sup>. Mucins clear, contain, feed, prevent the proteolytic activity of bacteria and digestive enzymes, and continuously replenish the gut microbiome<sup>64</sup>. Precisely, the MUC2 of the stratified inner mucus prevents microbes from reaching and translocating to the intestinal epithelium<sup>66</sup>. In contrast, the MUC2 of the outer mucus layer provides a natural habitat and carbon source for the commensal bacteria adapted to low levels of oxygen<sup>36,67</sup>.

Most of the functions of MUC2 are governed by the glycosylation of the glycome, which plays a crucial role in adaptive and innate immune responses beyond acting as just a barrier between the intestinal luminal antigens and microbes<sup>31</sup>. *O*-glycosylation is the main modification and type of glycosylation that affects mucins<sup>30</sup>. *O*-glycosylation hides the protein core of the mucins and protects it from endogenous protease degradation, helps with pathogen recognition and trapping, and activates the innate and adaptive immunity allowing tolerance throughout toll-like receptor activation, cytokines, and antimicrobial functions<sup>31</sup>. *O*-glycosylation differs along the different gastrointestinal tract regions and individuals (genetic factors partly determine them<sup>37</sup>), depending on glycosyltransferase expression, health or disease status, internal and external cues, microbiota composition, and function<sup>68</sup>. Several studies suggest that the mucus layer also has direct immunological effects due to their

glycans, which can bind directly to immune cells through the lectin-like proteins found in the latter<sup>60</sup>.

Unlike complex glycosylations, *O*-GalNAcylation, which consists of adding a single *N*-acetylglucosamine unit to a serine or threonine hydroxyl group of cytoplasmic, nuclear, and mitochondrial target proteins, can also influence the mucin function during exercise<sup>69</sup>. This *O*-GalNAcylation is a dynamic process by the enzymes *O*-GlcNAc transferase (OGT) and the *O*-GlcNAcase (OGA)<sup>70</sup>. The *O*-GalNAc glycosylation is controlled by nutrient and stress-sensing<sup>70,71</sup>, both important during endurance exercise. In fact, *O*-GalNAcylation shifts related to differential modulation of the carbohydrate metabolism and mitochondrial function via OGT catalyzation and subsequent AMPK activation<sup>70,72</sup>. *O*-GlcNAcylation also regulates protein synthesis/degradation balance via key signaling pathways such as Akt/GSK3 $\beta$ , Akt/mTOR, Myogenin/Atrogin-1, Myogenin/Mef2D, Mrf4, and PGC-1 $\alpha$  in the skeletal muscle, all of which might influence the response to exercise<sup>73</sup>.

We, therefore, posit that changes in glycosylation and *O*-GlcNAcylation via the gut microbiome might lead to differential modulation of carbohydrate metabolism and mitochondrial function and modulation of the gut response to exercise, illustrating how regulating the glycome can be a decisive factor in improving exercise performance. Reciprocally, the mucinome might modulate the gut microbiome composition and function, and it may be the gateway for researchers aiming to influence gut health.

### **The gut glycome-microbiota axis: two-way traffic**

As previously mentioned, there is a mutualistic relationship between gut mucins and the microbiota, especially with luminal and mucosal-associated microbiota. First, the mucin glycosylation profile can influence the composition of mucus-associated bacteria by protecting against pathogens and selecting specific species<sup>68</sup>. On the other hand, the gut microbiota and its metabolites facilitate the creation of the mucus layer in the colon<sup>35,36</sup> by regulating *O*-glycosylation and *MUC2* expression in the colon goblet cells<sup>74</sup>. Using germ-free mice, Johansson and colleagues (2015)<sup>75</sup> showed that the gut microbiota is fundamental for forming a proper mucus layer in mice. First, the number of filled goblet cells in germ-free mice was lower. Second, the inner mucus layer in the colon was thinner and, therefore, penetrable to bacteria compared to conventional mice, and the glycosylation patterns differed between groups. Indeed, Firmicutes, which are highly abundant in the mucus layer, have been shown to specifically induce *MUC2* expression and secretion in proximal colon goblet cells, leading to their encapsulation<sup>74</sup>. Hanson and colleagues used germ-free vs. ex-germ-free mice to show that microbes cause changes in *O*-glycan structure and expression of pertinent glycosyltransferases and glycan-modifying enzymes<sup>30</sup>. Bacteria or microbial products such as LPS, flagellin, and lipoteichoic acid, among others, might target the *MUC2* promoter by activating nuclear factor (NF)- $\kappa$ B<sup>75</sup>. For example, an *in vitro* study in human mucin-secreting goblet cell line HT29-MTX revealed that LPS increased mucin *MUC5AC*, *MUC5B*, and cytokine mRNA expression<sup>76</sup>.

Interestingly, mucin synthesis is regulated at the transcriptional and epigenetic levels via the gut microbiota and its metabolites. Several studies have shown that after glycan fermentation, the SCFAs butyrate and propionate epigenetically regulate *MUC2* gene expression in the human goblet cell-like LS174T cells<sup>77</sup>, which help maintain intestinal barrier function. In line with these findings, an elegant study by Bergstrom and Xia<sup>31</sup> showed that the SCFAs resulting from *O*-glycans fermentation drove Foxp3-derived regulatory T cell (Treg) production and regulated intestinal O<sub>2</sub> levels and barrier function. Another study in

inflammatory bowel disease patients in Japan<sup>78</sup> found that ulcerative colitis patients had dysbiosis, fewer mucin *O*-glycans, and lower SCFA production, suggesting that these individuals harbored fewer bacteria that ferment mucosal glycans<sup>79</sup>. Ulcerative colitis subjects also showed altered MUC2 *O*-glycosylation, carrying smaller glycans and less complex glycans in the mucus layer, and also presented more inflammation and an active disease state<sup>78</sup>. *In vitro* and *in vivo* experiments in dysbiotic diarrheal pigs have also shown aberrant mucin *O*-glycosylation and a consequent inflammatory response that damaged mucosal barrier function<sup>80</sup>. Beyond the presence and activity of gut microbes, environmental factors such as pH, water content, gut motility, food consistency, and stool passages also induce regulation of host mucus layer glycan expression, increasing the diversification of mucin structures<sup>81</sup>. Finally, new evidence also suggests that exercise-associated stress can alter mucin synthesis or glycosylation patterns.

### **The gut glycome-microbiota-immune axis: tolerance and pathogen recognition**

Clear evidence of mucin-microbiota interactions has come from studies about the diverse monosaccharide components, glycosidic linkages, and modifications that act as a barrier to pathogen-induced damage or innate host defense strategies that protect from inflammatory interactions with commensal microbiota<sup>31,74</sup>.

In eubiosis, the intestinal epithelium can distinguish commensal and pathogenic microbiota by specific molecular patterns from microorganisms. In humans, the immune system develops over time to recognize pathogen-associated molecular patterns (PAMPs) such as LPS, which are glycans mainly found on the cell wall of Gram-negative bacteria<sup>82</sup>, and other cell surface glycan antibody epitopes. Both secreted and transmembrane mucins offer interaction and attachment sites to glycan-binding components of microorganisms and help to regulate and tolerate the enormous diversity of the human microbiota in the eubiosis<sup>83</sup>.

Gut microbiota immune tolerance and bacterial epithelial surface protection are also regulated by the secretory IgA (SIgA), a highly abundant antibody mainly located in the intestinal mucosa, especially in Peyer's patches<sup>79</sup>. SIgA is heavily glycosylated in which carbohydrates comprise 25% of the secretory component<sup>84</sup>. Recent studies have shown that their glycan structures are important microbiota-binding sites, thus illustrating their role in modulating and tolerating gut microorganisms<sup>85</sup>. Some commensals express highly strain-specific superantigens that induce SIgA responses, whereas IgA glycosylation enables the recognition of Gram-positive bacterial glycans<sup>86</sup>. Besides SIgA, intestinal IgA interacts directly with intestinal microbial glycans in which microbes can bind to *N* and *O*-linked glycans<sup>86</sup>, making it a key driver of commensal bacteria selection. Interestingly, endurance athletes have been shown to have higher amounts of sIgA secretion by favoring mucosal adaptation to exercise-induced tissue damage<sup>87</sup>. The remaining question is whether immune cell glycome modifications are complementary mechanisms of the holobiont to adapt to endurance exercise.

Disadvantageously, mucin-type *O*-glycans can also tip the balance in favor of susceptibility to microbial infections, virulence, and ensuing tissue damage<sup>31</sup>. Pathogens utilize mucin *O*-glycans as receptors to evade host immune responses and act as a food source<sup>30,62,82,88</sup>. Many pathogens have adapted to use the host glycan structures siglecs, a family of sialic acid-binding immunoglobulin-like receptors that regulate adaptive and innate immune responses through their glycan ligand recognition<sup>31</sup>. For example, *Neisseria meningitidis*, *Haemophilus influenzae*, *Campylobacter jejuni*, *Pseudomonas aeruginosa*, *Group B Streptococcus*, and *Escherichia coli* K1<sup>89,90</sup> utilize host sialic acid as a means for their survival and evasion of

host immune responses. *Group B Streptococcus* binds to siglecs to downregulate leukocyte bactericidal capacity, thus escaping host immune recognition<sup>89</sup>. *Bacteroides theta* uses the 9-*O*-acetyltransferase EstA to remove 9-OAc, allowing pathogenic *E. coli* strains to use their sialidases to liberate Sia for nutritional sources<sup>65</sup>.

Although the interaction between infection and glycan regulation isn't fully understood, it is known that pathogens not only reshape the host glycome on host cell surfaces and cause aberrant expression of host glycosyltransferases and glycosidases<sup>30</sup> but also create LPS sialylation. Interestingly, pathogens can add sialic acid units to their oligosaccharides and glycoproteins, allowing pathogens to evade host immune responses by disguising themselves as a host cell, otherwise called molecular mimicry. In other words, some bacteria can modify their capsule composition, promoting immune system evasion. This process also enables the pathogen to interact directly with sialic acid-specific siglecs found on the surface of host immune cells<sup>90</sup>. Complementary, other pathogens can degrade the mucus layer and penetrate or modify mucus pH, influencing its viscosity. This is possible thanks to mucin-degrading proteases, chemotaxis, and flagella, which allow pathogens to move inside the mucus, adhere to and produce infection<sup>68</sup>.

### **The gut glycome-microbiota axis: Host mucin foraging, a feast for all**

Interconnections between the gut microbiota and colonic mucus are not limited to the gut microbiota-glycome-host immune system connections and attachment site<sup>74</sup>. Mucins also serve as metabolic substrates, providing a nutritional advantage to microorganisms adapted to the mucosal environment and favoring their replication and colonization<sup>91</sup>. Only a limited number of bacterial species from the Gram-negative Bacteroidetes, Gram-positive Firmicutes, Actinobacteria, and Verrucomicrobia phyla can directly access mucin as a carbon source, utilizing the obtained energy to feed themselves and to produce SCFA<sup>92</sup>. These bacteria encode thousands of carbohydrate-active-enzymes (CAZymes) that are strain or subspecies-dependent<sup>93-95</sup> and associated transport systems, enabling complex polysaccharide chain uptake, depolymerization, and fermentation into CO<sub>2</sub> and host-absorbable SCFA, namely acetate, propionate, and butyrate<sup>96</sup>. These SCFAs diffuse back through the inner mucus layer and serve as an energy source for local epithelial cells and, to some extent, the body<sup>97</sup>. Given that an estimated 70% of caloric needs come from SCFAs in ruminants and 10% in humans, they are a critical fuel source for the body<sup>98</sup>. Once bacterial glycosidases have started to act on the mucin glycans, the host-specific glycan epitopes will disappear. The bacteria will continue to degrade the glycans, and finally, the protein backbone will be exposed and degraded, contributing to mucus degradation. Only small amounts of mucin appear in the feces showing that the commensal bacteria efficiently utilize host mucins<sup>97</sup>.

The primary enzymatic activity needed for MUC2 breakdown is glycosyl hydrolases (GHs), such as endo- $\beta$ -*N*-acetylgalactosaminidase, fucosidases, *N*-acetyl- $\beta$ -hexosaminidases,  $\beta$ -galactosidases, and sialidases. The first targets for these exoglycosidases are the terminal residues on the *O*-glycans, such as sialic acid, fucose, and glycosulfate. The proportion of these terminal glycan epitopes varies along the gastrointestinal tract, with a decreasing gradient of fucose and an increasing amount of sialic acid from the ileum to the rectum in humans<sup>99</sup>. Sialic acid, therefore, represents a much-coveted source of nutrients for the gut bacteria that inhabit the mucus niche in the large intestine<sup>67</sup>. This can result in the release of monosaccharides, such as *N*-acetylglucosamine, *N*-acetylgalactosamine, and galactose, used by the microbial degrader itself or by other resident microbes<sup>37</sup>.



The glycobiome of Clostridia, *Bacteroides* spp., *Barnesiella intestinihominis*, *Eubacterium* spp., *Bifidobacterium* spp., *Vibrio cholerae*, *Ruminococcus* spp., and *Akkermansia muciniphila* represents one of the largest predicted enzyme arsenals for mucin metabolism<sup>31,52</sup>. For example, *A. muciniphila*, a symbiotic bacterium of the mucus layer, utilizes mucin as its sole carbon, nitrogen, and energy source via a combination of different enzymatic activities, including proteases, sulphatases, and GHs<sup>100</sup>. However, Ravcheev and Theile (2017)<sup>52</sup> reported that genes for the cleavage of mucin glycans were found in 86% of the 397 genomes of gut microbes, which is significantly higher than a previous estimation. We expect those populations frequently found on the mucosal side (e.g., *Lachnospiraceae*, *Enterobacteriaceae*, *Bacteroides*, *Eubacterium rectale*, *Faecalibacterium prausnitzii*, *Eubacterium cylindroides*, *Clostridium histolyticum*, *Clostridium lituseburense*, and *A. muciniphila*) harbor the enzymatic machinery to forage mucins. *In vivo* complete degradation of mucins in the gut likely relies on the cooperative action of several hosts glycan-consuming microbial species. It is also possible that many uncharacterized bacteria, archaea, or other microorganisms can grow on host glycan by cross-feeding activities dependent on other glycan utilizers.

### **Microbial mucus layer foraging: extra energy and an athletic performance advantage**

Endurance exercise has been studied as a significant long-term modifier of the intestinal microbiome composition and function in animal and human studies<sup>12,17,101,102</sup>. Shotgun metagenomics analysis of the fecal samples shows that athletes had a different microbiome composition from a functional point of view, with the increased microbial representation of genes involved in carbohydrate and amino acid metabolism and SCFA production<sup>21</sup>. In another study, the average abundance of taxa involved in energy and carbohydrate metabolism, including *Prevotella* and *Methanobrevibacter smithii*, was significantly higher in professional cyclists compared to amateur cyclists and was correlated with the frequency of training<sup>103</sup>. Barton and colleagues (2018)<sup>101</sup> found induction of microbiome pathways involved in glycan biosynthesis and lipid metabolism in professional rugby athletes. The mucin-degrading commensal *A. muciniphila*, which regulates intestinal inflammation and barrier function, has also been reported to be more abundant in athletes<sup>22,102</sup>. In agreement with these findings, our team revealed in an Arabian horse endurance model that gut microbiomes with high abundances of Verrumicrobia, including the *A. muciniphila* and Actinobacteria taxa, showed an enrichment in CAZymes families that act in a concerted manner to cleave host glycans such as glycoproteins (e.g., mucin) into fermentable monosaccharides<sup>29</sup>. For instance, GH29, which encodes fucosidases, and GH20 families, which include hexosaminidase and Lacto-*N*-biosidase activities<sup>104</sup>, were enriched in fit individuals, coupled with the GH18, GH88, and GH95, and the polysaccharide lyases (PL) PL8, PL12, GH18, GH88, and GH95. Associated with this glycan enzymatic machinery, we also observed an enrichment of KEGG orthologous groups (KOs) related to glycan biosynthesis and metabolism (K09953, K18770, K12309, K12551, K01137, K03276, K12985, K14459). These findings suggest that in athletes mucin cross-feeding is essential to microbial community development<sup>104,105</sup>.

Such results are further supported by metatranscriptome data of bifidobacteria, known mucin degraders, which revealed pronounced transcription of GH gene families involved in mucin degradation<sup>106</sup>. Recent data also showed that athletes harbor higher abundances of *Bifidobacterium*<sup>107</sup>. Moreover, it is plausible that the forage and fermentation of *O*-glycans in athletes' guts not only influences the microbe-microbe interaction and the fitness of affected organisms by releasing molecules in the environment, such as acetate and lactate but, in turn, may affect the gut homeostasis and control inflammation and gut barrier function<sup>108</sup>. Barton

et al.<sup>101</sup> already speculated that the athlete's gut microbiome possesses a functional capacity primed for tissue repair and a more remarkable ability to harness energy (Figure 3). This assertion reflects the significant energy demands and tissue adaptation during intense exercise and elite sports<sup>21</sup>.

During exercise, it is estimated that most SCFA (60-70%) serve as an energy source for colonocytes<sup>98</sup>. The remaining SCFAs are sensed by G-coupled-protein-receptors, primarily GPCR41, GPCR42, and GPCR109A, located in enteroendocrine cells or can be transported to the bloodstream to hepatic, muscle cells and adipocytes where they can regulate host glucose and fat metabolism by stimulating the release of hormones such as glucagon-like peptide 1 (GPL-1)<sup>98</sup>, which are critical fuel sources for athletes<sup>109</sup>.

Although SCFAs production from host glycan catabolism might be an effective and alternative way to fuel host during extraneous activity, the authors caution against the need to understand the consequences of excessive mucus layer foraging during exercise. The insufficient host replacement of the complex glycans following the microbiota consumption rate might impair mucosa functioning. A thinner mucus layer due to microbial degradation has been associated with severe gastrointestinal problems, including LPS and bacteria translocation<sup>110</sup>, dysglycemia<sup>111</sup>, and increased susceptibility to overt pathogens, including *Citrobacter rodentium*<sup>112</sup>. Nonetheless, Jakobsson et al. (2015)<sup>110</sup> showed that a thinner mucus layer is still functional and does not directly lead to a loss of tolerance defense toward the microbiota, as evidenced by a lack of overt idiopathic inflammation. This shows the power of MUC2 O-glycans in protecting against aggressive communities in these extreme conditions, allowing a co-existence with the gut microbiota, often mutualistic, despite the potential of the microbes to cause harm<sup>31</sup>.

A symbiotic and coevolved relationship between the microbiome-encoded enzymatic machinery capable of digesting host mucins and the host's dynamic expression of mucin glycans seems to be established upon exercise. Considering the difficulty in obtaining these measurements *in vivo*, this assumption remains to be tested. These results raise the possibility that the makeup of the gut microbiome and its capacity to metabolize glycans may be a determiner of the effectiveness of exercise. In line with this, a recent study by Morita et al.<sup>11</sup>, shows that *Bacteroides uniformis*, a dominant component of the gut microbiota with an expanded glycolytic capability to utilize both dietary and endogenous glycans, enhances endurance exercise performance in mice and human males. Therefore, properly seeding the gut microbiota and mucosa early with those microorganisms might maximize athletic performance.

### **O-GlcNAcylation, energy metabolism, and exercise: a potential key for optimizing athletic performance**

To date, researchers still haven't agreed about the exact role O-GlcNAcylation plays in response to exercise. Looking closer, O-GlcNAcylation is essential for glucose and carbohydrate metabolism during exercise and skeletal muscle mitochondrial function<sup>72,73,113,114</sup>. In an elegant study, Shi and colleagues (2018)<sup>72</sup> analyzed muscle tissue from muscle-specific OGT knockout mice in metabolic cages. The OGT knockout mice showed increased whole-body energy expenditure, changes in muscle substrate use, and mitochondrial dysfunction compared to wild-type mice. The shifts in mitochondrial function included increased energy substrate oxidation and mitochondrial dysfunction, confirming that OGT plays an essential role in mitochondrial and overall energy metabolism and, therefore, fatigue resistance. OGT and OGA expressions differ depending on muscle type metabolism

during exercise and in response to reactive oxygen species (ROS) and heat shock proteins, altering carbohydrate metabolism and various signaling pathways such as PGC-1 $\alpha$  in the skeletal muscles<sup>73</sup>. Thus, exercise is a crucial regulator of the *O*-GlcNAc gene expression, signaling, and protein modification, all of which modulate energy metabolism<sup>69</sup>.

As previously mentioned, the *O*-GlcNAcylation of transcription factors and enzymes involved in oxidative stress signaling, glucose metabolism, gluconeogenesis, and mitochondrial function is needed to cope with stress and nutrient depletion during exercise, including transcriptional activator peroxisome proliferator-activated receptor one alpha (PGC-1 $\alpha$ ), AMPK, forkhead box 1 (FOXO1), and sirtin 1 (SIRT1)<sup>70,72,115</sup>. Precisely, OGT activates PGC-1 $\alpha$ , which promotes hepatic gluconeogenesis<sup>114</sup>, and regulates lipid and glucose metabolism via activating the transcription factors peroxisome proliferator-activated receptor (PPAR) $\alpha$ , PPAR $\delta$ , PPAR $\gamma$ , liver X receptor (LXR), and farnesoid X receptor (FXR)<sup>69</sup>. Conversely, PGC-1 $\alpha$  has been shown to target OGT to FoxO1 in fasting conditions in liver cells<sup>114,116</sup>. PGC-1 $\alpha$  also regulates SIRT1 and the enzyme AMP-activated protein kinase (AMPK) in various tissues, including skeletal muscles<sup>70</sup>. Additionally, PGC-1 $\alpha$ /OGT complex induces *O*-GlyNAcylation of the transcription factor FOXO1<sup>117</sup>, which upregulates genes coding for gluconeogenesis enzymes<sup>70</sup> and is upregulated in response to oxidative stress<sup>118,119</sup>. Besides PGC-1 $\alpha$ , *O*-GlcNAcylation can also activate CREB in liver cells which was shown to promote gluconeogenesis<sup>70</sup>. In addition, *in vitro* studies have demonstrated that OGT epigenetically regulates AMPK expression in skeletal muscle cells<sup>70</sup>. Given the role *O*-GlcNAcylation plays in signaling pathways involved in energy metabolism and mitochondrial function, epigenetic regulation of the *O*-GlyNAcylation function could be a potential therapeutic target for optimizing athletic performance.

Gut microbes also regulate *O*-GlcNAcylation. Notably, gut *A. muciniphila* and *Bacteroides thetaiotaomicron* produce OGT and OGA. They can hydrolyze *O*-GlcNAcyated proteins in epithelial cells and immune cells, which activates NF- $\kappa$ B signaling and regulates proinflammatory immune responses<sup>120</sup>, T-cell immune responses against viral bacterial, fungal, and parasitic infections<sup>121</sup>. Of interest is the fact that recent studies have reported a higher abundance of *Akkermansia* in athletes<sup>122–124</sup> making this bacteria a potential therapeutic target for exercise-induced intestinal inflammation and pathogen translocation.

Though research is in its infancy, there is a potential crosstalk between *O*-GlcNAcylation, SCFAs, and secondary bile acids, all of which are related to energy metabolism, mitochondrial function, and oxidative stress in and out of the intestines<sup>50,119,125</sup>. Oxidative stress, hypoxia, and ischemia, which occur in endurance athletes under extreme physical pressure, all affect *O*-GlcNAcylation levels, although the exact mechanisms are not yet fully understood<sup>119</sup>. Nevertheless, primary evidence shows that SCFAs can regulate *O*-GlcNAcylation glucose metabolism through various means, such as improving insulin sensitivity by directly activating AMPK, which induces PGC-1 $\alpha$  in skeletal muscles<sup>126</sup>, liver and adipose tissues<sup>127</sup>. SCFAs have been shown to regulate energy metabolism during exercise<sup>1,128</sup> through many of the same signaling pathways as *O*-GlcNAcylation, namely AMPK and PGC-1 $\alpha$ <sup>129</sup>. SCFAs also activate *SIRT1*, which plays a role in mitochondrial biogenesis via PGC-1 $\alpha$  deacetylation<sup>130</sup>. In skeletal muscle cells and brown adipose cells, butyrate has been shown to phosphorylate AMPK and p38, which then activates PGC-1 $\alpha$  resulting in FAO and ATP production<sup>127,131</sup>. The SCFA also regulates protein *O*-GlcNAcylation in intestinal L cells, a type of enteroendocrine cells that produce the hormones glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), therefore, regulating

glucose and lipid metabolism<sup>132</sup>. In the same line, intestinal epithelial levels of *O*-GlcNAc reduce in response to microbial depletion and can be partially rescued by SCFAs in mice<sup>133</sup>.

Secondary bile acids, produced by anaerobic bacteria that degrade bile acids<sup>134</sup>, are key signaling molecules for regulating energy metabolism by activating GLP-1 in L cells, regulating glucose and lipid metabolism, and acting as a ligand for the nuclear receptor FXR<sup>135</sup>. FXR is an essential metabolic regulator found in various tissues, including intestinal, liver, pancreatic, and muscles, and regulates the enteropathic circulation of bile acids, glycolysis, gluconeogenesis, lipogenesis, and lipoprotein biosynthesis and transport, and is sensitive to glucose levels held by *O*-GlcNAcylation<sup>136</sup>. Studies *in vitro* and *FRX*<sup>-/-</sup> C57BL/6J mice illustrated that *O*-GlcNAcylation increased FXR transcriptional activity<sup>136</sup>. FXR also targets the *SIRT1* gene<sup>135</sup>, which is directly regulated by bile acids, SCFAs, and *O*-GlcNAcylation<sup>115</sup>.

Given such complexity, we hypothesize that the mucin *O*-glycome and *O*-GlcNAcylation are essential during endurance exercise, especially when gut homeostasis is perturbed.

### **Nutritional strategies for gut mucosal health and remodeling in athletes**

Many endurance athletes are encouraged to consume high amounts of simple carbohydrates, protein, and low amounts of fat and fiber to provide quick energy and to avoid potential digestive issues such as gas and distension that high-fiber diets can sometimes cause<sup>1</sup>.

Interestingly, when dietary fiber is insufficiently available in the gut lumen, bacterial genomic regulation and intestinal microbial ecosystem equilibrium shifts toward mucus layer foraging<sup>137</sup>. A study by Desai et al. (2016)<sup>112</sup> illustrated how dietary fiber deficiencies could promote specific gut bacterial populations and their enzymes and cellular metabolism shift towards host mucin glycans as an energy source, leading to erosion of the colonic mucus layer. These findings have also been validated in a study of mice fed a fiber-free diet<sup>112</sup>. In line with these findings, excessive and aggressive mucus-degrading communities were observed in response to Western-style diets that are high in saturated fats and simple carbohydrates but low in dietary fiber, resulting in higher mucus layer permeability<sup>138</sup>. Lack of dietary fiber induced defects of the inner colonic mucus layer, which included a strongly reduced growth rate and higher mucus layer penetrability after changing from a chow diet to Western-style diets in mice. In support of this notion, Schroeder et al. (2018)<sup>66</sup> observed significantly decreased diversity and reduced abundance of Bacteroidetes and Actinobacteria in the absence of fiber for up to 40 days. The forced foraging on host glycans leads to thinning of the mucus layer<sup>66</sup> and increases the risk factor for infection by enteric pathogens<sup>138</sup>. In lack of dietary-derived carbohydrates, *Bacteroides thetaiotaomicron* revealed a capacity to turn to host mucus glycans when polysaccharides were absent from the diet<sup>139</sup>.

Since gut mucins are essential to ensure alternative energy sources during endurance exercise and enhance performance, mucosal glycosylation, composition, and thickness defects may worsen the athlete's performance. We thus propose that the new dietary frontiers in athletes should target modification of the gut glycome-microbiota axis throughout the administration of probiotics, prebiotics, or postbiotics (Figure 4; Table 1).

### **Probiotics that modify the gut glycome-microbiota axis**

Probiotic supplementation may be an effective strategy to improve or maintain intestinal barrier health and function in endurance athletes commonly affected by gastrointestinal complaints. The administration of *Lactobacillus* spp has been shown to stimulate *MUC3* expression in human intestinal epithelial cells and *MUC2* production and secretion<sup>140,141</sup>. In

line with this, *L. reuteri* increased mucus layer thickness after the dextran sulfate sodium-induced intestinal barrier damage<sup>142</sup>. Evidence also shows that *L. reuteri* can improve intestinal barrier integrity by increasing the expression of tight junction proteins in the intestinal epithelial cells<sup>143</sup>. Other examples include *Bifidobacterium longum* supplementation, which restored mucus growth in mice after four weeks of feeding with a Western-style diet<sup>144</sup>. The administration of the mucin-degrading *A. muciniphila*, which represents ~1-3% of the intestinal microbiota in humans, has shown promising probiotic effects, including metabolic modulation, immune system regulation, gut barrier function, and other physiological and homeostatic functions<sup>145-147</sup>. Mice treated with a high-fat diet and *A. muciniphila* presented restored mucus layer thickness. Although this bacteria is a mucus degrader, its oral supplementation increases the number and differentiation of Paneth cells and goblet cells in the small intestine and subsequently accelerates intestinal epithelial regeneration<sup>148</sup>. Most of these vital functions are determined via translational *O*-glycosylation events in the mucins<sup>100</sup>. Interestingly, co-culturing experiments of *A. muciniphila* with non-mucus-degrading butyrate-producing bacteria<sup>94</sup> can indirectly stimulate intestinal butyrate levels in the vicinity of the intestinal epithelial cells with potential health benefits to the host. These findings provide support for the use of different preparations of *A. muciniphila* as therapeutic options to target athletes and associated exercise-induced gastrointestinal disorders.

Beyond bacteria, the supplementation of *Gloiopeltis furcata*, an edible alga containing bioactive polysaccharides, improved the integrity of the colonic epithelial layer by remodeling the interactions between the gut microbiota and mucin *O*-glycans<sup>149</sup>. Specifically, this alga increased the proportions of complex long-chain mucin *O*-glycans in the epithelial layer with two terminal *N*-acetylneuraminic acid residues. It promoted the growth of probiotic bacteria, including *Roseburia* spp. and *Muribaculaceae*.

### **Prebiotics that modify the gut glycome-microbiota axis**

Prebiotics are non-digestible food sources that beneficial intestinal microbes feed on, promoting valuable species' growth and survival. Pruss and colleagues have recently shown that supplementing *O*-glycan-like human milk oligosaccharides (HMOs) or mucin-type *O*-glycans purified from commercially derived porcine gastric mucin modified the functionality of dysbiotic microbiota. After glycan supplementation, these dysbiotic communities were shifted back to a eubiotic (healthy-associated) state<sup>150</sup>. They also demonstrated that oral porcine mucin glycans suppressed *Clostridium difficile* abundance after antibiotic treatment, delayed the onset of diet-induced obesity, and increased the relative quantity of *A. muciniphila*<sup>36</sup>. Likewise, rats fed the *O*-glycan GlcNAc produced higher levels of the anti-inflammatory SCFA *n*-butyrate, ROR $\gamma$ t+Treg, and IgA-producing cells in colonic lamina propria than Wistar rats fed porcine stomach proteins<sup>151</sup>, demonstrating the fundamental role of the microbiota-glycome axis on the host immune responses<sup>152</sup>. The increased secretory IgA might prevent the toxic and pathogenic gut invasion<sup>153</sup>, a critical phenomenon in endurance athletes.

Studies show that inulin, a water-soluble dietary fiber obtained from chicory, and the long-chain arabinoxylans, an important non-starch polysaccharide in cereal grains, can increase colonic mucus levels as well as SCFAs<sup>144,154,155</sup>. Both inulin and long-chain arabinoxylans shifted the gut microbiota from acetate towards health-promoting propionate and butyrate-producers (*Roseburia intestinalis*, *Eubacterium rectale*, *Anaerostipes caccae*) and bifidobacteria (*B. longum*)<sup>155</sup>. These authors pointed to *A. muciniphila* as a potential marker of mucus layer status (the higher, the better). Additionally, (Bishara et al., 2013)<sup>138</sup> found

that mucus layer growth positively correlated with *Bifidobacterium* abundance. Furthermore, these long-chain arabinoxylans shifted mucin degradation to distal regions, where mucin-degraders may produce beneficial metabolites (e.g., propionate by *A. muciniphila*) so that prebiotics could improve gut health along the length of the intestine. This is of great interest since most digestive troubles in athletes originate in the distal colon. Interestingly, building a stable microbial community after administering prebiotics such as inulin could take several days to a week<sup>155</sup>.

As it has been shown that the gut microbiota is highly variable between individuals, new experiments are warranted to evaluate to which extent the described benefits can be extended to athletes. While increasing prebiotic intake may decrease the simple carbohydrate intake, the resulting prebiotic-SCFA production may improve muscle glycogen storage and metabolism during race<sup>29</sup>.

Overall, these studies suggest that using specifically-tailored glycans as a prebiotic contributes to the growth of beneficial gut bacteria and prevents mucus layer degradation and permeability, reducing the risk of gastrointestinal disorders.

### **Postbiotics that modify the gut glycome-microbiota axis**

Using non-living microbial materials (postbiotics), such as the outer-membrane pili proteins of *A. muciniphila*, might also increase intestinal barrier function<sup>156</sup>. Treating mice with only this protein (Amuc\_1100) and without the bacteria also recapitulates the effects of the bacterium, increasing the number of goblet cells and reinforcing the intestinal barrier<sup>93</sup>. Strikingly, this protein binds and activates TLR2, thereby possibly explaining the effects of the bacteria on both the regulation of immunity and the reinforcement of the gut barrier<sup>93</sup>.

Bioactive SCFA administration (primary butyrate) also promotes *MUC2* and *MUC5AC* gene expression and increases epithelial cell integrity after damage<sup>157</sup>. Analogously, the injection of gastric and intestinal SCFAs led to increased expression of intestinal tight junction proteins occludin and claudin-1 genes<sup>158</sup> and decreased the gene and protein abundances of IL-1 $\beta$  in the colon, coupled with reduced infiltration of neutrophils to the gut *lamina propria*. Whether administering other bacterial components (e.g., purified flagellin from Gram-negative bacteria, LPA from Gram-positive bacteria, and LPS) can regulate gut homeostasis needs to be yet tested. Using postbiotics instead of viable microbial cells (probiotics) has been shown to deliver many advantages in the upstream production and application possibilities of products that can be added to nutrition. Studying the diet-microbiota-mucus axis further might help design improved postbiotic options for treating conditions that involve mucus layer defects.

### **Conclusions**

An increasing number of clinical problems outside and inside the gut have emerged as one of the most critical factors affecting endurance athletes. Recent evidence shows a complex bidirectional interaction between the dietary and host-derived glycans and the gut microbiota, yet research has just scratched the surface in the context of exercise. While the microbiota's use of intestinal mucin glycans as a source of nutrients confers a competitive energy advantage for athletes, gut microbiota composition and function are important factors contributing to the protection of the intestinal mucus barrier function and gut homeostasis. This intertwined connection might be boosted by enhancing dietary glycan availability (e.g., dietary fiber, inulin, long-chain arabinoxylans), an uncommon practice in elite athletes, or via probiotics and postbiotics supplementation. This practice will likely change the gut

ecosystem, improving numerous functions required for commensal bacterial life, the gut environment, and the host's epithelial immune system and mucus layer thickness. The exact manner and how the gut microbiota composition and related compounds contribute to gut homeostasis in athletes is mainly unknown. Still, *Bifidobacterium* and *A. muciniphila* and its components appear as potential critical bacteria to maintain gut health and avoid intestinal disorders in athletes. Moreover, studies elucidating the respiratory and colon mucus systems and their interactions with the commensal microbiome are highly prioritized for endurance athletes, where gastrointestinal and respiratory comorbidities are commonly associated.

Many questions remain in every area of energy metabolism, the regulation of mucinome during exercise, optimal training interventions, and the potential for manipulating metabolic responses for ergogenic benefits. Exercise science combined with sports nutrition will thus continue to be a fruitful research area for many years as researchers seek a greater understanding of the mechanistic bases for athletic successes. New studies matching metagenomic, metabolomic, transcriptomics, and glycomic data are warranted to understand how diet and microbiota composition and function regulate the intestinal mucus barrier, energy production, immune responses, and dynamic and complex glycobiology during exercise.

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## TABLES

**Table 1: Nutritional strategies that regulate gut mucosal and immune health**

Nutrient	Experimental Model	Results	References
<b>Dietary fiber</b>			
	Gnotobiotic mouse model of mice colonized with 14 human gut microbiota species	Bacterial glycan metabolism switched from fiber degradation to mucus layer glycan foraging when fed a fiber-free diet. The gut microbiota used host-secreted mucus glycoproteins as a nutrient source, leading to the erosion of the colonic mucus barrier and increased susceptibility to <i>Citrobacter rodentium</i> infection.	Desai et al. (2016) <sup>112</sup>
	Mice fed a fiber-depleted, Western style diet for 10- 40 days.	Significantly decreased diversity and reduced abundance of Bacteroidetes and Actinobacteria, and reduced colonic mucus thickness.	Schroeder et al. (2018) <sup>66</sup>
	Applying the BacSpace pipeline to gnotobiotic and human-microbiota colonized mice	A diet free of microbiota accessible carbohydrates (MACs) resulted in thinner mucus in the distal colon, increased proximity of microbes to the epithelium, and increased expression of the inflammatory marker REG3 $\beta$ . Increased <i>Helicobacter pylori</i> invasion was also observed in mouse stomach gland cells.	Earle et al. (2015) <sup>159</sup>
<b>Probiotics</b>			
<i>Lactobacillus plantarum</i> 299v and <i>Lactobacillus rhamnosus</i> GG	<i>In vitro</i> model of human intestinal epithelial cell lines treated with <i>Lactobacillus plantarum</i> 299v and <i>Lactobacillus rhamnosus</i> GG	Both probiotics inhibited the attachment of pathogenic <i>Escherichia coli</i> to HT-29 intestinal epithelial cells possibly by inducing <i>MUC2</i> and <i>MUC3</i> gene expression.	Mack et al. (1999) <sup>160</sup>
<i>Lactobacillus reuteri</i> R2L	Mice were given rat-derived <i>Lactobacillus reuteri</i> R2LC or human-derived 4659 by gavage once daily for 14 days, and colitis was induced with 3% dextran sulphate sodium to drinking water for the last 7 days of this period.	The two <i>L. reuteri</i> strains protected against colitis in mice partly due to the increased mucus thickness and tightened intestinal epithelium junctions occludin and ZO-1 in the stem cell area of the colonic crypts.	Ahl et al. (2015) <sup>142</sup>
<i>Bifidobacterium longum</i>	<i>Bifidobacterium longum</i>	Mice were fed a Western style diet for 8 weeks. Supplementation with <i>Bifidobacterium longum</i> for 4 weeks restored intestinal mucus growth.	Schroeder et al. (2018) <sup>144</sup>
<i>Bifidobacterium longum</i> subsp. <i>Longum</i> OLP-01 (OLP-01)	<i>Bifidobacterium longum</i> subsp. <i>Longum</i> OLP-01 (OLP-01) was isolated from an elite Olympic athlete, was combined with a six-week	The exercise + OLP-01 group had higher endurance and improved fatigue-associated indexes (lactate, ammonia, creatine kinase, lactate dehydrogenase, and glycogen) and inflammation levels	Huang et al. (2020) <sup>161</sup>



	exercise training program. Institute of Cancer Research mice were assigned to sedentary, exercise, <i>OLP-01</i> , or exercise + <i>OLP-01</i> and administered probiotics or treadmill exercise for 6 weeks.	compared to the other groups. The exercise + <i>OLP-01</i> group also had significantly higher SCFA levels (acetate, butyrate, propionate)	
<i>Akkermansia muciniphila</i>	C57BL/6 mice were supplemented with <i>A. muciniphila</i> for 14 days and then injected with Concanavalin A to induce liver injury. Blood, liver, ileum, and colon samples were analyzed for liver injury, systemic inflammation, and intestinal barrier function.	Mice supplemented with <i>A. muciniphila</i> had lower levels of pro-inflammatory cytokines and chemokines (IL-2, IFN- $\gamma$ , IL-12p40, MCP-1, MIP-1a, MIP-1b) and increased expression of <i>Occludin</i> and <i>Tjp-1</i> which improved intestinal barrier function and reduced systemic LPS levels. restored mucus layer thickness and an increased number of goblet cells and the production of antimicrobial peptides. Fecal 16S rRNA sequence analysis showed that <i>A. muciniphila</i> increased microbial richness and diversity.	Wu et al., (2017) <sup>148</sup>
<i>Akkermansia muciniphila</i>	Human peripheral blood mononuclear cell lines HEK-Blue hTLR2, hTLR4, hTLR5, hTLR9 and hNOD2 were treated <i>in vitro</i> with the outer-membrane pili proteins of <i>A. muciniphila</i> .	<i>A. muciniphila</i> induced IL-8, IL-6, IL-1 $\beta$ , IL-10 and TNF- $\alpha$ . It also increased trans-epithelial resistance in a Caco-2 model system. The highly abundant pili-like protein Amuc_1100 of <i>A. muciniphila</i> modulated the host immunological homeostasis within the gut mucosa, which improved gut barrier function.	Ottman et al. (2017) <sup>156</sup>
<i>Akkermansia muciniphila</i>	Mice were fed a high fat diet and a purified form of <i>A. muciniphila</i> .	The outer membrane protein Amuc_1100 of <i>A. muciniphila</i> increasing the number of goblet cells and improved intestinal barrier function likely by binding to and activating TLR2.	Plovier et al. (2017) <sup>93</sup>
<i>Akkermansia muciniphila</i>	co-culturing experiments of <i>A. muciniphila</i> with non-mucus-degrading butyrate-producing bacteria <i>Anaerostipes caccae</i> , <i>Eubacterium hallii</i> , and <i>Faecalibacterium prausnitzii</i>	resulted in syntrophic growth and production of butyrate.	Belzer et al. (2017) <sup>94</sup>
<b>Prebiotics</b>			
Inulin and long-chain arabinosylans	Axenic rats were inoculated with a human faecal microbiota for 6 weeks were then fed inulin and/ or long-chain arabinosylans.	Inulin and long-chain arabinosylans stimulated the growth of butyrate-producing bacterial groups ( <i>Roseburia intestinalis</i> , <i>Eubacterium rectale</i> , <i>Anaerostipes caccae</i> ) and bifidobacteria ( <i>B. longum</i> ) respectively and shifted mucin degradation to distal regions where mucin-degraders may produce beneficial metabolites ( <i>e.g.</i> propionate by <i>Akkermansia muciniphila</i> ).	van den Abbeele et al. (2011) <sup>155</sup>
<i>O</i> -glycan-like human milk	6-week-old GF mice were bi-colonized with two HMO-	Supplementing with <i>O</i> -glycan-like HMOs or mucin-type <i>O</i> -glycans purified from	Pruss et al., (2021) <sup>150</sup>

oligosaccharides (HMOs)	utilizing taxa common to the infant gut: <i>Bacteroides</i> and <i>B. infantis</i> . Mice were fed a microbiota-accessible carbohydrates deficient diet supplemented with HMOs (1% in water for 1 week. Feces and cecal contents were collected and milk glycans were measured by high-performance liquid chromatography.	commercially derived porcine gastric mucin aided in recovering lost microbiota post antibiotic treatment and increased the relative abundance of <i>A. muciniphila</i> .	
O-glycan GlcNAc	6-week old Wistar rats were fed a control diet, control diet + porcine stomach mucin, control diet + GlcNAc, fucose, or N-acetylneuraminic acid, or control diet + porcine mucin or GlcNAc. Microbiota composition, SCFAs, cecal O-glycan content, and immune cells like IgA+, Tregs, and inflammatory cytokines were analyzed.	Rats fed the O-glycan GlcNAc produced higher levels of <i>n</i> -butyrate and IgA+ plasma cells and Tregs compared to the other groups.	Hino et al. (2020) <sup>151</sup>
<b>Postbiotics</b>			
Butyrate	C57BL/6 mice were fed a high fat diet (58% calories from fat supplemented with butyrate (5% wt/wt).	Butyrate supplementation increased energy expenditure and mitochondrial function and biogenesis in type I skeletal muscle fiber and brown fat by inducing PPAR mRNA expression.	Gao et al. (2009) <sup>162</sup>
SCFAs	<i>In vitro</i> model of human colon epithelium using HT29-MTX-E12 cells were differentiated for 42 days exposed to dextran sulphate sodium (DSS). The effects of SCFAs were tested to study proliferation, epithelial integrity and mucus production.	Cells treated with butyrate or a SCFA mixture significantly reduced DSS-induced intestinal epithelial damage. The SCFA mixture and butyrate alone induced <i>ZO-1</i> , <i>MUC2</i> and <i>MUC5AC</i> mRNA expression.	Giromini et al. (2022) <sup>157</sup>
SCFAs	Gastric infusion of short-chain fatty acids (SCFA) on gut barrier function in a pig model	Injection of gastric and intestinal SCFAs led to increased mRNA expression of intestinal tight junction proteins occludin and claudin-1.	Diao et al. (2019) <sup>158</sup>

## FIGURES

### Figure 1. Gut microbiota's impact on athlete's metabolism and physiology

The gut microbiota in athletes is critical for its positive contributions to host energy metabolism and physiology during exercise, such as hydration, heat production, cardiovascular fitness, gut-lung axis regulation, redox reactions, immune responses, mucus foraging and intestinal mucosa protection, and homeostasis. Additionally, the gut microbiota

and its metabolites regulate skeletal muscle function and neurotransmitter production related to fatigue, sensation, and motivation. This figure has been created with BioRender.com.

## **Figure 2. Gastrointestinal symptoms in endurance athletes: mucosal glycome damage matters**

Left panel: The mutualistic relationship between the gut glycome, gut microbiota, and derived metabolites under eubiosis. On the one hand, the gut microbiota and their metabolism induce the synthesis of large gel-forming mucins, including encapsulation, glycosylation, changes in fucosylation and sialidation patterns, and thickness. On the other hand, the mucin layer serves as an environmental niche and a food source for the microbiota. The high diversity of gut mucins impacts the gut microbiota composition, diversity, and stability, but also influences the immune and mitochondrial function.

Right panel: The mutualistic relationship between the gut glycome and the microbiota under a disrupted gut ecosystem and environment. Vigorous endurance training increases sympathetic nervous system activity and redistributes blood flow from the splanchnic organs to the working skeletal muscles and peripheral circulation. This results in gut hypoxia and hypoperfusion, reduced intestinal motility and absorption capacity, damage of the specialized antimicrobial protein-secreting cells (Paneth cells), the mucus-producing goblet cells, and increased loosening of tight junction protein structure and MUC2 destruction. The deterioration of the gastrointestinal mucosal barrier enables the translocation of bacteria and lipopolysaccharides (LPS) outside the gastrointestinal tract, triggering immune and inflammatory responses often resulting in increased intestinal permeability and, eventually, endotoxemia. Moreover, it induces oxidative stress via ROS production. Changes in the gut barrier integrity involve changes in the expression and glycosylation of mucins, and thus immune dysregulation and risk of disease onset. This figure has been created with BioRender.com.

## **Figure 3: The gut glycome-microbiota axis in the gut of athletes**

In healthy athletes, mucins (mainly MUC2) clear, contain, feed, prevent the proteolytic activity of bacteria and digestive enzymes, and continuously replenish the gut microbiome. The mucinome might modulate the gut microbiome composition and function (*e.g.*, SCFA and secondary bile acids production), and it may be the gateway for researchers aiming to influence gut health. Moreover, its *O*-glycosylation and the *O*-GalNAcylation, might control the nutrient and stress sensing during exercise and the mitochondrial function, illustrating how regulating the glycome-microbiota axis can be a decisive factor in improving exercise performance. Reciprocally, the athlete's gut microbiome possesses a functional capacity primed for tissue repair and a more remarkable ability to harness energy from mucins. This figure has been created with BioRender.com.

**Figure 4. New dietary frontiers in athletes: targeting the gut glycome-microbiota axis throughout the administration of probiotics, prebiotics, or postbiotics.** The administration of probiotics involving *Lactobacillus*, *Akkermansia*, and *Bifidobacterium* strains, as well as prebiotics such as inulin, long-chain arabinoxylans, and exogenous mucin-like glycans, appears as an effective strategy to improve or maintain the intestinal barrier health and function of athletes. These nutritional strategies increase the mucus thickness and the SCFA-producing bacteria. The SCFAs enter the bloodstream, modulating glucose and fat metabolism in the liver and skeletal muscles via transcription factor (PCG-1a, SIRT, FOXO1) and OGT regulation.

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## Authors' contributions

AC and NM conceptualized, designed, and drafted this review. The authors read and approved the final manuscript.

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