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

2  
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## 7 8 9 **Abstract**

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

25  
26 **Keywords:** gut microbiota, energy, endurance, dietary glycans, leaky gut, glycome, mucins,  
27 stress

## 28 29 **Introduction**

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

38  
39 It is estimated that 30-50% of endurance athletes suffer from gastrointestinal complaints,  
40 including diarrhea, vomiting, nausea, and ischemia <sup>7</sup>, in which individuals show temporary  
41 gut microbial imbalances, impaired mucus integrity, intestinal barrier damage, and  
42 inflammatory responses, the so-called, leaky gut. Moreover, the excessive strain on the  
43 respiratory system (relative to its capacity) leads to exercise-induced hypoxemia <sup>8</sup>. Therefore,  
44 excessive and prolonged high-intensity exercise may impart digestive and respiratory  
45 problems, reduced protection from many diseases <sup>9</sup>, and worse sports performance.

46  
47 New evidence has shown that the gut microbiome plays a role in athletic performance <sup>10-12</sup>.  
48 The human gut microbiome contains more than 100 trillion microorganisms and up to 10  
49 million non-redundant genes <sup>13</sup>. It spans over 2,000 bacterial species <sup>14,15</sup> and has a density of  
50 ~ 40 – 300 billion microorganisms in the colon <sup>16</sup>.

51  
52 The athlete's gut microbiome differs from non-athletes in composition and function<sup>10,11,17</sup>.  
53 Ultimately microbiota composition and function impact host athletic performance directly  
54<sup>11,12,18–20</sup>, and indirectly through myokines and other cytokines, modulating the activation of  
55 the hypothalamic-pituitary-adrenal (HPA) axis and metabolic pathways<sup>12,21,22</sup> (Figure 1). For  
56 example, changes in microbial composition and increased diversity correlate with improved  
57 performance and cardiorespiratory fitness (as predicted by  $VO_2$  max or the  $VO_2$  peak) in  
58 marathon runners regardless of sex, age, body mass index, and diet<sup>23</sup>. Increases in the  
59 Firmicutes-Bacteroidetes ratio<sup>24</sup> or depletion in *Eubacterium* spp enhance the cardiovascular  
60 capacity of athletes, as assessed by the maximum oxygen consumption<sup>25</sup>. Disruption of  
61 intestinal microbiota following antibiotic treatment can cause skeletal muscle endurance  
62 impairment<sup>26</sup>. Similar work demonstrated significant reductions in short-chain fatty acids  
63 (SCFAs), gut microbiota, and endurance capacity after antibiotic treatment<sup>27</sup>. Infusion of the  
64 SCFA acetate resulted in the restoration of endurance capacity. Deeper characterization of the  
65 links between athletic performance and gut microbiome revealed that just one bacterium  
66 (*Veillonella atypica*) was required to enhance the athletic outputs in treadmill mice  
67 experiments by producing propionate from exercise-induced lactate<sup>12,19</sup>. Enriching the genus  
68 *Bacteroides uniformis* and its preferred substrate,  $\alpha$ -cyclodextrin, enhanced endurance  
69 exercise performance in mice and humans, possibly by facilitating endogenous glucose  
70 production in the liver during exercise<sup>11</sup>. Lastly, exercise performance was enhanced in mice  
71 by augmenting dopamine signaling during physical activity, illustrating that gut-derived  
72 interoceptive circuits influence the rewarding properties of exercise<sup>28</sup>.

73  
74 Complementary to these results, compelling new evidence suggests that intestinal glycome -  
75 the ensemble of glycans - may be a critical factor in overall enteric health and energy  
76 production, serving as a potential missing factor in improving athletic performance. Using  
77 horses as an endurance exercise model and along with shotgun sequencing, our team showed  
78 better athletic performance in subjects who harbored highly diverse gut microbiomes with a  
79 greater capacity to cleave host glycosaminoglycans from the gut mucus layer<sup>29</sup>, mainly  
80 composed of mucin 2 (MUC2). This higher ability to use host gut mucins could offer  
81 complimentary or unique metabolic pathways that enhance fuel bioavailability for the  
82 mitochondria while improving aerobic work competencies and sparing glycogen usage. This  
83 was the case even after factors such as diet were accounted for. Mucins are critical for  
84 pathogen recognition, immune activation, selection of commensals to repress pathogens, and,  
85 even more importantly, building protective barriers<sup>30</sup>. Most of these mucin functions are  
86 governed via the *O*-glycome - the ensemble of glycans and their *O*-glycosylation<sup>31</sup>. Changes  
87 in the expression and glycosylation of mucins are associated with dysbiosis, immune  
88 dysregulation, and disease such as diabetes<sup>32,33</sup>. An ever-expanding field suggests that MUC-  
89 2 glycosylation and *O*-GlcNAcylation regulation of cell nucleocytoplasmic and  
90 mitochondrial proteins play a role in energy metabolism, intestinal barrier, and immune  
91 function<sup>33,34</sup>, representing a significant challenge endurance exercise biology today.

92  
93 There is a mutualistic relationship between the gut glycome, gut microbiota, and derived  
94 metabolites. The gut microbiota is required to synthesize large gel-forming mucins  
95 completely, including encapsulation, glycosylation, changes in fucosylation and sialidation  
96 patterns, and thickness<sup>35–37</sup>. Being both an environmental niche and a food source, this high  
97 diversity of gut mucins significantly impacts the gut microbiota composition, diversity, and  
98 stability, which can, in turn, stimulate mucosal expression and, together, form the basis of the  
99 host-microbial symbiosis<sup>37,38</sup>. Most bacteria use dietary fiber and starch as nutrient sources  
100<sup>34</sup>; however, many *Bacteroides* spp and *Akkermansia muciniphila* contain an extensive

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

107  
108 Given the increasing interest in exercise and the growing evidence of gastrointestinal  
109 complaints in endurance athletes, this review will provide insights into the crosstalk of the  
110 gut microbiome, the host glycome landscape, and its potential impact on exercise  
111 performance. Glycans, pre-, and post-biotics have a high potential to be applied in the dietary  
112 plans of athletes to prevent intestinal problems, while improving athletic performance in  
113 endurance events<sup>5</sup>. Therefore, we argue that the next phase in the gut microbiome and athlete  
114 research should be focused on the design of nutritional strategies that target the  
115 microorganisms able to regulate the *O*-glycome or the microbiota composition and function  
116 to maintain gastrointestinal mucosal health, reduce the prevalence of gastrointestinal  
117 symptoms and enhance athletic performance.

118  
119 **Gastrointestinal symptoms in endurance athletes: the mucosal glycome damage matters**  
120 Over the last decades, the increased participation of the general population in endurance  
121 events has raised concerns regarding the impact prolonged and intense exercise has on  
122 immune and gastrointestinal health, which can lead to worse performance<sup>41</sup> and recovery<sup>42</sup>.  
123 For example, 30-50% of endurance athletes<sup>5,43</sup> and 96% of ultramarathon runners experience  
124 gastrointestinal distress, in which nausea and vomiting are the main reasons for withdrawal  
125 from the competition<sup>44,45</sup>. Although most symptoms are mild and do not cause long-term  
126 health effects, epigastric pain, heartburn, nausea, vomiting, abdominal pain, bloody diarrhea  
127 episodes, and dehydration are typical responses in endurance athletes. Oesophagitis,  
128 hemorrhagic gastritis, gastric ulcer, gastrointestinal bleeding, and ischaemic bowel may  
129 involve severe medical complications in endurance athletes<sup>5</sup>.

130  
131 Vigorous endurance training ( $\geq 60$  min and  $\geq 70\%$  of maximum work capacity) increases  
132 sympathetic nervous system activity and redistributes blood flow from the splanchnic organs  
133 to the working skeletal muscles and peripheral circulation. This results in gut hypoxia and  
134 hypoperfusion, reduced intestinal motility and absorption capacity, damage of the specialized  
135 antimicrobial protein-secreting cells (Paneth cells), the mucus-producing cells (such as goblet  
136 cells), and increased loosening of tight junction protein structure<sup>46</sup>. The deterioration of the  
137 gastrointestinal mucosal barrier enables the translocation of bacteria and lipopolysaccharides  
138 (LPS) outside the gastrointestinal tract, triggering immune and inflammatory responses often  
139 resulting in increased intestinal permeability<sup>1</sup> and, eventually, endotoxemia<sup>47-49</sup>. Moreover,  
140 it induces oxidative stress via ROS production<sup>50</sup> and changes the oxygen concentration. A rat  
141 model showed that colonic ischemia destroyed intestinal MUC2, damaging the protective  
142 intestinal mucus layer<sup>51</sup> (Figure 2). Repairing the mucus layer is a time and energy-  
143 consuming process, and increased bacterial invasion of the lamina propria can trigger acute  
144 colitis<sup>52</sup>. Furthermore, emotional stress experienced by many endurance athletes can also  
145 decrease the intestinal mucus layer<sup>34</sup> and increase intestinal permeability<sup>1</sup>. Repeated bouts of  
146 stress decrease MUC2 synthesis and the number of goblet cells via notch signaling  
147 suppression in rats<sup>53</sup>. More recently, Silva and colleagues revealed that stress strongly affects  
148 the *O*-glycosylation of mucins, resulting in the flattening and loss of cohesive properties of  
149 the mucus layer<sup>34</sup>.

150

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

160

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

169

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

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

181

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

189

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

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

203

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

215

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

222

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

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

243

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

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

263

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

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

269

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

277

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

292

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

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

305  
306 Although the interaction between infection and glycan regulation isn't fully understood, it is  
307 known that pathogens not only reshape the host glycome on host cell surfaces and cause  
308 aberrant expression of host glycosyltransferases and glycosidases<sup>30</sup> but also create LPS  
309 sialylation. Interestingly, pathogens can add sialic acid units to their oligosaccharides and  
310 glycoproteins, allowing pathogens to evade host immune responses by disguising themselves  
311 as a host cell, otherwise called molecular mimicry. In other words, some bacteria can modify  
312 their capsule composition, promoting immune system evasion. This process also enables the  
313 pathogen to interact directly with sialic acid-specific siglecs found on the surface of host  
314 immune cells<sup>90</sup>. Complementary, other pathogens can degrade the mucus layer and penetrate  
315 or modify mucus pH, influencing its viscosity. This is possible thanks to mucin-degrading  
316 proteases, chemotaxis, and flagella, which allow pathogens to move inside the mucus, adhere  
317 to and produce infection<sup>68</sup>.

318

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

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

339

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

350



351 The glyco biome of Clostridia, *Bacteroides* spp., *Barnesiella intestinihominis*, *Eubacterium*  
352 spp., *Bifidobacterium* spp., *Vibrio cholerae*, *Ruminococcus* spp., and *Akkermansia*  
353 *muciniphila* represents one of the largest predicted enzyme arsenals for mucin metabolism  
354 <sup>31,52</sup>. For example, *A. muciniphila*, a symbiotic bacterium of the mucus layer, utilizes mucin  
355 as its sole carbon, nitrogen, and energy source via a combination of different enzymatic  
356 activities, including proteases, sulphatases, and GHs <sup>100</sup>. However, Ravcheev and Theile  
357 (2017) <sup>52</sup> reported that genes for the cleavage of mucin glycans were found in 86% of the 397  
358 genomes of gut microbes, which is significantly higher than a previous estimation. We expect  
359 those populations frequently found on the mucosal side (*e.g.*, *Lachnospiraceae*,  
360 *Enterobacteriaceae*, *Bacteroides*, *Eubacterium rectale*, *Faecalibacterium prausnitzii*,  
361 *Eubacterium cylindroides*, *Clostridium histolyticum*, *Clostridium lituseburense*, and *A.*  
362 *muciniphila*) harbor the enzymatic machinery to forage mucins. *In vivo* complete degradation  
363 of mucins in the gut likely relies on the cooperative action of several hosts glycan-consuming  
364 microbial species. It is also possible that many uncharacterized bacteria, archaea, or other  
365 microorganisms can grow on host glycan by cross-feeding activities dependent on other  
366 glycan utilizers.

367

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

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

393

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

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

405  
406 During exercise, it is estimated that most SCFA (60-70%) serve as an energy source for  
407 colonocytes<sup>98</sup>. The remaining SCFAs are sensed by G-coupled-protein-receptors, primarily  
408 GPCR41, GPCR42, and GPCR109A, located in enteroendocrine cells or can be transported  
409 to the bloodstream to hepatic, muscle cells and adipocytes where they can regulate host  
410 glucose and fat metabolism by stimulating the release of hormones such as glucagon-like  
411 peptide 1 (GPL-1)<sup>98</sup>, which are critical fuel sources for athletes<sup>109</sup>.

412  
413 Although SCFAs production from host glycan catabolism might be an effective and  
414 alternative way to fuel host during extraneous activity, the authors caution against the need to  
415 understand the consequences of excessive mucus layer foraging during exercise. The  
416 insufficient host replacement of the complex glycans following the microbiota consumption  
417 rate might impair mucosa functioning. A thinner mucus layer due to microbial degradation  
418 has been associated with severe gastrointestinal problems, including LPS and bacteria  
419 translocation<sup>110</sup>, dysglycemia<sup>111</sup>, and increased susceptibility to overt pathogens, including  
420 *Citrobacter rodentium*<sup>112</sup>. Nonetheless, Jakobsson et al. (2015)<sup>110</sup> showed that a thinner  
421 mucus layer is still functional and does not directly lead to a loss of tolerance defense toward  
422 the microbiota, as evidenced by a lack of overt idiopathic inflammation. This shows the  
423 power of MUC2 *O*-glycans in protecting against aggressive communities in these extreme  
424 conditions, allowing a co-existence with the gut microbiota, often mutualistic, despite the  
425 potential of the microbes to cause harm<sup>31</sup>.

426  
427 A symbiotic and coevolved relationship between the microbiome-encoded enzymatic  
428 machinery capable of digesting host mucins and the host's dynamic expression of mucin  
429 glycans seems to be established upon exercise. Considering the difficulty in obtaining these  
430 measurements *in vivo*, this assumption remains to be tested. These results raise the possibility  
431 that the makeup of the gut microbiome and its capacity to metabolize glycans may be a  
432 determiner of the effectiveness of exercise. In line with this, a recent study by Morita et al.<sup>11</sup>,  
433 shows that *Bacteroides uniformis*, a dominant component of the gut microbiota with an  
434 expanded glycolytic capability to utilize both dietary and endogenous glycans, enhances  
435 endurance exercise performance in mice and human males. Therefore, properly seeding the  
436 gut microbiota and mucosa early with those microorganisms might maximize athletic  
437 performance.

### 438 439 ***O*-GlcNAcylation, energy metabolism, and exercise: a potential key for optimizing** 440 **athletic performance**

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

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

455  
456 As previously mentioned, the *O*-GlcNAcylation of transcription factors and enzymes  
457 involved in oxidative stress signaling, glucose metabolism, gluconeogenesis, and  
458 mitochondrial function is needed to cope with stress and nutrient depletion during exercise,  
459 including transcriptional activator peroxisome proliferator-activated receptor one alpha  
460 (PGC-1 $\alpha$ ), AMPK, forkhead box 1 (FOXO1), and sirtin 1 (SIRT1)<sup>70,72,115</sup>. Precisely, OGT  
461 activates PGC-1 $\alpha$ , which promotes hepatic gluconeogenesis<sup>114</sup>, and regulates lipid and  
462 glucose metabolism via activating the transcription factors peroxisome proliferator-activated  
463 receptor (PPAR) $\alpha$ , PPAR $\delta$ , PPAR $\gamma$ , liver X receptor (LXR), and farnesoid X receptor (FXR)  
464<sup>69</sup>. Conversely, PGC-1 $\alpha$  has been shown to target OGT to FoxO1 in fasting conditions in liver  
465 cells<sup>114,116</sup>. PGC-1 $\alpha$  also regulates SIRT1 and the enzyme AMP-activated protein kinase  
466 (AMPK) in various tissues, including skeletal muscles<sup>70</sup>. Additionally, PGC-1 $\alpha$ / OGT  
467 complex induces *O*-GlyNAcylation of the transcription factor FOXO1<sup>117</sup>, which upregulates  
468 genes coding for gluconeogenesis enzymes<sup>70</sup> and is upregulated in response to oxidative  
469 stress<sup>118,119</sup>. Besides PGC-1 $\alpha$ , *O*-GlcNAcylation can also activate CREB in liver cells which  
470 was shown to promote gluconeogenesis<sup>70</sup>. In addition, *in vitro* studies have demonstrated  
471 that OGT epigenetically regulates AMPK expression in skeletal muscle cells<sup>70</sup>. Given the  
472 role *O*-GlcNAcylation plays in signaling pathways involved in energy metabolism and  
473 mitochondrial function, epigenetic regulation of the *O*-GlyNAcylation function could be a  
474 potential therapeutic target for optimizing athletic performance.

475  
476 Gut microbes also regulate *O*-GlcNAcylation. Notably, gut *A. muciniphila* and *Bacteroides*  
477 *thetaiotaomicron* produce OGT and OGA. They can hydrolyze *O*-GlcNAcyated proteins in  
478 epithelial cells and immune cells, which activates NF- $\kappa$ B signaling and regulates  
479 proinflammatory immune responses<sup>120</sup>, T-cell immune responses against viral bacterial,  
480 fungal, and parasitic infections<sup>121</sup>. Of interest is the fact that recent studies have reported a  
481 higher abundance of *Akkermansia* in athletes<sup>122-124</sup> making this bacteria a potential  
482 therapeutic target for exercise-induced intestinal inflammation and pathogen translocation.

483  
484 Though research is in its infancy, there is a potential crosstalk between *O*-GlcNAcylation,  
485 SCFAs, and secondary bile acids, all of which are related to energy metabolism,  
486 mitochondrial function, and oxidative stress in and out of the intestines<sup>50,119,125</sup>. Oxidative  
487 stress, hypoxia, and ischemia, which occur in endurance athletes under extreme physical  
488 pressure, all affect *O*-GlcNAcylation levels, although the exact mechanisms are not yet fully  
489 understood<sup>119</sup>. Nevertheless, primary evidence shows that SCFAs can regulate *O*-  
490 GlcNAcylation glucose metabolism through various means, such as improving insulin  
491 sensitivity by directly activating AMPK, which induces PGC-1 $\alpha$  in skeletal muscles<sup>126</sup>, liver  
492 and adipose tissues<sup>127</sup>. SCFAs have been shown to regulate energy metabolism during  
493 exercise<sup>1,128</sup> through many of the same signaling pathways as *O*-GlcNAcylation, namely  
494 AMPK and PGC-1 $\alpha$ <sup>129</sup>. SCFAs also activate *SIRT1*, which plays a role in mitochondrial  
495 biogenesis via PGC-1 $\alpha$  deacetylation<sup>130</sup>. In skeletal muscle cells and brown adipose cells,  
496 butyrate has been shown to phosphorylate AMPK and p38, which then activates PGC-1 $\alpha$   
497 resulting in FAO and ATP production<sup>127,131</sup>. The SCFA also regulates protein *O*-  
498 GlcNAcylation in intestinal L cells, a type of enteroendocrine cells that produce the  
499 hormones glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), therefore, regulating

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

502  
503 Secondary bile acids, produced by anaerobic bacteria that degrade bile acids<sup>134</sup>, are key  
504 signaling molecules for regulating energy metabolism by activating GLP-1 in L cells,  
505 regulating glucose and lipid metabolism, and acting as a ligand for the nuclear receptor FXR  
506<sup>135</sup>. FXR is an essential metabolic regulator found in various tissues, including intestinal,  
507 liver, pancreatic, and muscles, and regulates the enteropathic circulation of bile acids,  
508 glycolysis, gluconeogenesis, lipogenesis, and lipoprotein biosynthesis and transport, and is  
509 sensitive to glucose levels held by *O*-GlcNAcylation<sup>136</sup>. Studies *in vitro* and *FRX*<sup>-/-</sup>  
510 C57BL/6J mice illustrated that *O*-GlcNAcylation increased FXR transcriptional activity<sup>136</sup>.  
511 FXR also targets the *SIRT1* gene<sup>135</sup>, which is directly regulated by bile acids, SCFAs, and *O*-  
512 GlcNAcylation<sup>115</sup>.

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

515

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

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

520

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

538

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

544

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

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

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

569

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

576

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

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

592

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

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

607

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

613

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

617

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

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

625

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

638

### 639 **Conclusions**

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

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

658

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

**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>

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## FIGURES

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

674 The gut microbiota in athletes is critical for its positive contributions to host energy  
675 metabolism and physiology during exercise, such as hydration, heat production,  
676 cardiovascular fitness, gut-lung axis regulation, redox reactions, immune responses, mucus  
677 foraging and intestinal mucosa protection, and homeostasis. Additionally, the gut microbiota  
678

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

681

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

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

691

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

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

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

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719 **Figure 4. New dietary frontiers in athletes: targeting the gut glycome-microbiota axis**  
720 **throughout the administration of probiotics, prebiotics, or postbiotics.** The

721 administration of probiotics involving *Lactobacillus*, *Akkermansia*, and *Bifidobacterium*  
722 strains, as well as prebiotics such as inulin, long-chain arabinoxylans, and exogenous mucin-  
723 like glycans, appears as an effective strategy to improve or maintain the intestinal barrier  
724 health and function of athletes. These nutritional strategies increase the mucus thickness and  
725 the SCFA-producing bacteria. The SCFAs enter the bloodstream, modulating glucose and fat  
726 metabolism in the liver and skeletal muscles via transcription factor (PCG-1a, SIRT, FOXO1)  
727 and OGT regulation.

728

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732

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750 The authors declare no competing interests.

751

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