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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 ¹⁻³. Endurance exercise performance primarily depends on physiological adaptations
33 to sustain the metabolic and thermoregulatory demands of such activity ⁴, 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 ⁵. The development of fatigue limits sports performance, so the athletes with the
37 most outstanding fatigue resistance often succeed ⁶.

38
39 It is estimated that 30-50% of endurance athletes suffer from gastrointestinal complaints,
40 including diarrhea, vomiting, nausea, and ischemia ⁷, 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 ⁸. Therefore,
44 excessive and prolonged high-intensity exercise may impart digestive and respiratory
45 problems, reduced protection from many diseases ⁹, and worse sports performance.

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

51
52 The athlete's gut microbiome differs from non-athletes in composition and function ^{10,11,17}.
53 Ultimately microbiota composition and function impact host athletic performance directly
54 ^{11,12,18-20}, and indirectly through myokines and other cytokines, modulating the activation of
55 the hypothalamic-pituitary-adrenal (HPA) axis and metabolic pathways ^{12,21,22} (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 ²³. Increases in the
59 Firmicutes-Bacteroidetes ratio ²⁴ or depletion in *Eubacterium* spp enhance the cardiovascular
60 capacity of athletes, as assessed by the maximum oxygen consumption ²⁵. Disruption of
61 intestinal microbiota following antibiotic treatment can cause skeletal muscle endurance
62 impairment ²⁶. Similar work demonstrated significant reductions in short-chain fatty acids
63 (SCFAs), gut microbiota, and endurance capacity after antibiotic treatment ²⁷. 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 ^{12,19}. Enriching the genus
68 *Bacteroides uniformis* and its preferred substrate, α -cyclodextrin, enhanced endurance
69 exercise performance in mice and humans, possibly by facilitating endogenous glucose
70 production in the liver during exercise ¹¹. 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 ²⁸.

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 ²⁹, 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 ³⁰. Most of these mucin functions are
86 governed via the *O*-glycome - the ensemble of glycans and their *O*-glycosylation ³¹. Changes
87 in the expression and glycosylation of mucins are associated with dysbiosis, immune
88 dysregulation, and disease such as diabetes ^{32,33}. 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 ^{33,34}, 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 ³⁵⁻³⁷. 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 ^{37,38}. Most bacteria use dietary fiber and starch as nutrient sources
100 ³⁴; however, many *Bacteroides* spp and *Akkermansia muciniphila* contain an extensive

101 repertoire of enzymes that metabolize the host mucus layer^{32,39}. In turn, the fermentation of
102 these indigestible mucus layer glycans produces SCFAs³⁸, 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 function⁴⁰, 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 events⁵. 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 performance⁴¹ and recovery⁴².
123 For example, 30-50% of endurance athletes^{5,43} and 96% of ultramarathon runners experience
124 gastrointestinal distress, in which nausea and vomiting are the main reasons for withdrawal
125 from the competition^{44,45}. 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 athletes⁵.

130
131 Vigorous endurance training (≥ 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 structure⁴⁶. 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 permeability¹ and, eventually, endotoxemia⁴⁷⁻⁴⁹. Moreover,
140 it induces oxidative stress via ROS production⁵⁰ and changes the oxygen concentration. A rat
141 model showed that colonic ischemia destroyed intestinal MUC2, damaging the protective
142 intestinal mucus layer⁵¹ (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 colitis⁵². Furthermore, emotional stress experienced by many endurance athletes can also
145 decrease the intestinal mucus layer³⁴ and increase intestinal permeability¹. Repeated bouts of
146 stress decrease MUC2 synthesis and the number of goblet cells via notch signaling
147 suppression in rats⁵³. 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 layer³⁴.

150

151 On the other hand, moderate and regular physical activity improves gastrointestinal
152 symptoms in subjects with irritable bowel syndrome in humans⁵⁴. 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 (α -defensin, β -
156 defensin, Reg IIIb, and Reg IIIc)⁵⁵. 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)⁴⁶.

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 axis⁵⁶⁻⁵⁹. 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 stomach⁶⁰.
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^{61,62}.
175 The prominent gut mucin in the gastrointestinal tract is the gel-forming glycoprotein MUC2
176⁶³, 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)⁶⁴. Sialic acids protect underlying glycan chains from enzymatic
180 degradation⁶⁵.

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 body³⁶. Mucins clear, contain, feed, prevent the proteolytic activity of bacteria and
185 digestive enzymes, and continuously replenish the gut microbiome⁶⁴. Precisely, the MUC2
186 of the stratified inner mucus prevents microbes from reaching and translocating to the
187 intestinal epithelium⁶⁶. 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^{36,67}.

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 microbes³¹. *O*-glycosylation is the main
193 modification and type of glycosylation that affects mucins³⁰. *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 functions³¹.
197 *O*-glycosylation differs along the different gastrointestinal tract regions and individuals
198 (genetic factors partly determine them³⁷), depending on glycosyltransferase expression,
199 health or disease status, internal and external cues, microbiota composition, and function⁶⁸.
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 latter⁶⁰.

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 exercise⁶⁹. This
207 *O*-GalNAcylation is a dynamic process by the enzymes *O*-GlcNAc transferase (OGT) and the
208 *O*-GlcNAcase (OGA)⁷⁰. The *O*-GalNAc glycosylation is controlled by nutrient and stress-
209 sensing^{70,71}, 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^{70,72}. *O*-GlcNAcylation also regulates
212 protein synthesis/degradation balance via key signaling pathways such as Akt/GSK3 β ,
213 Akt/mTOR, Myogenin/Atrogin-1, Myogenin/Mef2D, Mrf4, and PGC-1 α in the skeletal
214 muscle, all of which might influence the response to exercise⁷³.

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 species⁶⁸. On the other hand, the gut
228 microbiota and its metabolites facilitate the creation of the mucus layer in the colon^{35,36} by
229 regulating *O*-glycosylation and *MUC2* expression in the colon goblet cells⁷⁴. Using germ-
230 free mice, Johansson and colleagues (2015)⁷⁵ 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 encapsulation⁷⁴. 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 enzymes³⁰. 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)- κ B⁷⁵. 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 expression⁷⁶.

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 cells⁷⁷, which help maintain intestinal barrier function. In
248 line with these findings, an elegant study by Bergstrom and Xia³¹ showed that the SCFAs
249 resulting from *O*-glycans fermentation drove Foxp3-derived regulatory T cell (Treg)
250 production and regulated intestinal O₂ levels and barrier function. Another study in

251 inflammatory bowel disease patients in Japan ⁷⁸ 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 ⁷⁹. 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 ⁷⁸. *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 ⁸⁰. 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 ⁸¹. 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 ^{31,74}.

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 ⁸², 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 ⁸³.

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 ⁷⁹. SIgA is heavily glycosylated in which
281 carbohydrates comprise 25% of the secretory component ⁸⁴. 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 ⁸⁵. Some commensals express highly strain-
284 specific superantigens that induce SIgA responses, whereas IgA glycosylation enables the
285 recognition of Gram-positive bacterial glycans ⁸⁶. Besides SIgA, intestinal IgA interacts
286 directly with intestinal microbial glycans in which microbes can bind to *N* and *O*-linked
287 glycans ⁸⁶, 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 ⁸⁷. 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 ³¹. Pathogens utilize mucin *O*-
295 glycans as receptors to evade host immune responses and act as a food source ^{30,62,82,88}. 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 ³¹. For example, *Neisseria meningitidis*, *Haemophilus*
299 *influenzae*, *Campylobacter jejuni*, *Pseudomonas aeruginosa*, *Group B Streptococcus*, and
300 *Escherichia coli* K1 ^{89,90} 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 recognition⁸⁹. *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 sources⁶⁵.

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 glycosidases³⁰ 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 cells⁹⁰. 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 infection⁶⁸.

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 site⁷⁴. Mucins also
322 serve as metabolic substrates, providing a nutritional advantage to microorganisms adapted to
323 the mucosal environment and favoring their replication and colonization⁹¹. 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 SCFA⁹². These
327 bacteria encode thousands of carbohydrate-active-enzymes (CAZymes) that are strain or
328 subspecies-dependent⁹³⁻⁹⁵ and associated transport systems, enabling complex
329 polysaccharide chain uptake, depolymerization, and fermentation into CO₂ and host-
330 absorbable SCFA, namely acetate, propionate, and butyrate⁹⁶. 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 body⁹⁷. 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 body⁹⁸. 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 mucins⁹⁷.

339

340 The primary enzymatic activity needed for MUC2 breakdown is glycosyl hydrolases (GHs),
341 such as endo- β -*N*-acetylgalactosaminidase, fucosidases, *N*-acetyl- β -hexosaminidases, β -
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 humans⁹⁹. Sialic acid, therefore, represents a much-coveted source of nutrients for the gut
347 bacteria that inhabit the mucus niche in the large intestine⁶⁷. 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 microbes³⁷.

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 ^{31,52}. 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 ¹⁰⁰. However, Ravcheev and Theile
357 (2017) ⁵² 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 ^{12,17,101,102}. 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 ²¹. 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 ¹⁰³. Barton and colleagues (2018) ¹⁰¹ 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 ^{22,102}. 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 ²⁹. For instance, GH29, which encodes fucosidases, and GH20 families,
386 which include hexosaminidase and Lacto-*N*-biosidase activities ¹⁰⁴, 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 ^{104,105}.

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 ¹⁰⁶. Recent data also showed that athletes harbor higher abundances of
397 *Bifidobacterium* ¹⁰⁷. 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 ¹⁰⁸. Barton

401 et al.¹⁰¹ 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 sports²¹.

405
406 During exercise, it is estimated that most SCFA (60-70%) serve as an energy source for
407 colonocytes⁹⁸. 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)⁹⁸, which are critical fuel sources for athletes¹⁰⁹.

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 translocation¹¹⁰, dysglycemia¹¹¹, and increased susceptibility to overt pathogens, including
420 *Citrobacter rodentium*¹¹². Nonetheless, Jakobsson et al. (2015)¹¹⁰ 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 harm³¹.

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.¹¹,
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^{72,73,113,114}. In an elegant study, Shi and colleagues (2018)⁷² 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 α in the
453 skeletal muscles⁷³. Thus, exercise is a crucial regulator of the *O*-GlcNAc gene expression,
454 signaling, and protein modification, all of which modulate energy metabolism⁶⁹.

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 α), AMPK, forkhead box 1 (FOXO1), and sirtin 1 (SIRT1)^{70,72,115}. Precisely, OGT
461 activates PGC-1 α , which promotes hepatic gluconeogenesis¹¹⁴, and regulates lipid and
462 glucose metabolism via activating the transcription factors peroxisome proliferator-activated
463 receptor (PPAR) α , PPAR δ , PPAR γ , liver X receptor (LXR), and farnesoid X receptor (FXR)
464⁶⁹. Conversely, PGC-1 α has been shown to target OGT to FoxO1 in fasting conditions in liver
465 cells^{114,116}. PGC-1 α also regulates SIRT1 and the enzyme AMP-activated protein kinase
466 (AMPK) in various tissues, including skeletal muscles⁷⁰. Additionally, PGC-1 α / OGT
467 complex induces *O*-GlyNAcylation of the transcription factor FOXO1¹¹⁷, which upregulates
468 genes coding for gluconeogenesis enzymes⁷⁰ and is upregulated in response to oxidative
469 stress^{118,119}. Besides PGC-1 α , *O*-GlcNAcylation can also activate CREB in liver cells which
470 was shown to promote gluconeogenesis⁷⁰. In addition, *in vitro* studies have demonstrated
471 that OGT epigenetically regulates AMPK expression in skeletal muscle cells⁷⁰. 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- κ B signaling and regulates
479 proinflammatory immune responses¹²⁰, T-cell immune responses against viral bacterial,
480 fungal, and parasitic infections¹²¹. Of interest is the fact that recent studies have reported a
481 higher abundance of *Akkermansia* in athletes¹²²⁻¹²⁴ 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^{50,119,125}. 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 understood¹¹⁹. 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 α in skeletal muscles¹²⁶, liver
492 and adipose tissues¹²⁷. SCFAs have been shown to regulate energy metabolism during
493 exercise^{1,128} through many of the same signaling pathways as *O*-GlcNAcylation, namely
494 AMPK and PGC-1 α ¹²⁹. SCFAs also activate *SIRT1*, which plays a role in mitochondrial
495 biogenesis via PGC-1 α deacetylation¹³⁰. In skeletal muscle cells and brown adipose cells,
496 butyrate has been shown to phosphorylate AMPK and p38, which then activates PGC-1 α
497 resulting in FAO and ATP production^{127,131}. 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 metabolism¹³². 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 mice¹³³.

502
503 Secondary bile acids, produced by anaerobic bacteria that degrade bile acids¹³⁴, 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
506¹³⁵. 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*-GlcNAcylation¹³⁶. Studies *in vitro* and *FRX*^{-/-}
510 C57BL/6J mice illustrated that *O*-GlcNAcylation increased FXR transcriptional activity¹³⁶.
511 FXR also targets the *SIRT1* gene¹³⁵, which is directly regulated by bile acids, SCFAs, and *O*-
512 GlcNAcylation¹¹⁵.

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 cause¹.

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 foraging¹³⁷. A study by Desai et al. (2016)¹¹² 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 diet¹¹². 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 permeability¹³⁸. 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)⁶⁶ 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 layer⁶⁶ and increases the risk factor for infection by enteric pathogens
536¹³⁸. In lack of dietary-derived carbohydrates, *Bacteroides thetaiotaomicron* revealed a
537 capacity to turn to host mucus glycans when polysaccharides were absent from the diet¹³⁹.

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^{140,141}. In

550 line with this, *L. reuteri* increased mucus layer thickness after the dextran sulfate sodium-
551 induced intestinal barrier damage¹⁴². 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 cells¹⁴³. Other examples include *Bifidobacterium longum*
554 supplementation, which restored mucus growth in mice after four weeks of feeding with a
555 Western-style diet¹⁴⁴. 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 functions¹⁴⁵⁻¹⁴⁷. 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 regeneration¹⁴⁸. Most of these vital functions are determined via translational *O*-
563 glycosylation events in the mucins¹⁰⁰. Interestingly, co-culturing experiments of *A.*
564 *muciniphila* with non-mucus-degrading butyrate-producing bacteria⁹⁴ 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*-glycans¹⁴⁹.
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) state¹⁵⁰. 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*³⁶. Likewise, rats fed the *O*-glycan GlcNAc produced higher levels of the anti-
587 inflammatory SCFA *n*-butyrate, ROR γ t+Treg, and IgA-producing cells in colonic lamina
588 propria than Wistar rats fed porcine stomach proteins¹⁵¹, demonstrating the fundamental role
589 of the microbiota-glycome axis on the host immune responses¹⁵². The increased secretory
590 IgA might prevent the toxic and pathogenic gut invasion¹⁵³, 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^{144,154,155}. 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*)¹⁵⁵. These authors pointed to *A. muciniphila* as a potential marker
599 of mucus layer status (the higher, the better). Additionally, (Bishara et al., 2013)¹³⁸ 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 week¹⁵⁵.

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 race²⁹.

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 function¹⁵⁶. 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 barrier⁹³.
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 barrier⁹³.

625

626 Bioactive SCFA administration (primary butyrate) also promotes *MUC2* and *MUC5AC* gene
627 expression and increases epithelial cell integrity after damage¹⁵⁷. Analogously, the injection
628 of gastric and intestinal SCFAs led to increased expression of intestinal tight junction
629 proteins occludin and claudin-1 genes¹⁵⁸ and decreased the gene and protein abundances of
630 IL-1 β 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
Dietary fiber			
	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) ¹¹²
	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) ⁶⁶
	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 REG3β. Increased <i>Helicobacter pylori</i> invasion was also observed in mouse stomach gland cells.	Earle et al. (2015) ¹⁵⁹
Probiotics			
<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) ¹⁶⁰
<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) ¹⁴²
<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) ¹⁴⁴
<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) ¹⁶¹

	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- γ , 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) ¹⁴⁸
<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 β , IL-10 and TNF- α . 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) ¹⁵⁶
<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) ⁹³
<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) ⁹⁴
Prebiotics			
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) ¹⁵⁵
<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) ¹⁵⁰

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) ¹⁵¹
Postbiotics			
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) ¹⁶²
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) ¹⁵⁷
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) ¹⁵⁸

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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

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.

706

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.

718

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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751

752 **Reference**

- 753 1. Clark A, Mach N. Exercise-induced stress behavior, gut-microbiota-brain axis and
754 diet: a systematic review for athletes. *J Int Soc Sports Nutr* 2016;**13**:43.
755 <https://doi.org/10.1186/s12970-016-0155-6>.
- 756 2. Joyner MJ, Coyle EF. Endurance exercise performance: The physiology of champions.
757 *J Physiol* 2008;**586**:35–44. <https://doi.org/10.1113/jphysiol.2007.143834>.
- 758 3. Mach N, Fuster-Botella D. Endurance exercise and gut microbiota: A review. *J Sport*
759 *Heal Sci* 2017;**6**:179–97. <https://doi.org/10.1016/j.jshs.2016.05.001>.
- 760 4. Shave R, Howatson G, Dickson D, Young L. Exercise-induced cardiac remodeling:
761 Lessons from humans, horses, and dogs. *Vet Sci* 2017;**4**:9.
762 <https://doi.org/10.3390/vetsci4010009>.
- 763 5. De Oliveira EP, Burini RC, Jeukendrup A. Gastrointestinal complaints during
764 exercise: Prevalence, etiology, and nutritional recommendations. *Sport Med*
765 2014;**44**:79–85. <https://doi.org/10.1007/s40279-014-0153-2>.
- 766 6. Hargreaves M, Spriet LL. Skeletal muscle energy metabolism during exercise. *Nat*
767 *Metab* 2020;**2**:817–28. <https://doi.org/10.1038/s42255-020-0251-4>.
- 768 7. de Oliveira EP, Burini RC. Carbohydrate-dependent, exercise-induced gastrointestinal
769 distress. *Nutrients* 2014;**6**:4191–9. <https://doi.org/10.3390/nu6104191>.
- 770 8. Durand F, Raberin A. Exercise-Induced Hypoxemia in Endurance Athletes:
771 Consequences for Altitude Exposure. *Front Sport Act Living* 2021;**3**.
772 <https://doi.org/10.3389/fspor.2021.663674>.
- 773 9. Neuffer PD, Bamman MM, Muoio DM, Bouchard C, Cooper DM, Goodpaster BH, et
774 al. Understanding the Cellular and Molecular Mechanisms of Physical Activity-
775 Induced Health Benefits. *Cell Metab* 2015;**22**:4–11.
776 <https://doi.org/10.1016/j.cmet.2015.05.011>.
- 777 10. Mohr AE, Jäger R, Carpenter KC, Kerksick CM, Purpura M, Townsend JR, et al. The
778 athletic gut microbiota. Vol. 17, Journal of the International Society of Sports

- 779 Nutrition. *Journal of the International Society of Sports Nutrition*, 2020. 1–33 p.
780 <https://doi.org/10.1186/s12970-020-00353-w>.
- 781 11. Morita H, Kano C, Ishii C, Kagata N, Ishikawa T, Hirayama A, et al. *Bacteroides*
782 *uniformis* and its preferred substrate, α -cyclodextrin, enhance endurance exercise
783 performance in mice and human males. *Sci Adv* 2023;**9**.
784 <https://doi.org/10.1126/sciadv.add2120>.
- 785 12. Scheiman J, Lubber JM, Chavkin TA, MacDonald T, Tung A, Pham LD, et al. Meta-
786 omics analysis of elite athletes identifies a performance-enhancing microbe that
787 functions via lactate metabolism. *Nat Med* 2019;**25**:1104–9.
788 <https://doi.org/10.1038/s41591-019-0485-4>.
- 789 13. Forster SC, Browne HP, Kumar N, Hunt M, Denise H, Mitchell A, et al. HPMCD: The
790 database of human microbial communities from metagenomic datasets and microbial
791 reference genomes. *Nucleic Acids Res* 2016;**44**:D604–9.
792 <https://doi.org/10.1093/nar/gkv1216>.
- 793 14. Jousset A, Bienhold C, Chatzinotas A, Gallien L, Gobet A, Kurm V, et al. Where less
794 may be more: How the rare biosphere pulls ecosystems strings. *ISME J* 2017;**11**:853–
795 62. <https://doi.org/10.1038/ismej.2016.174>.
- 796 15. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial
797 microbiota. *Nat Rev Microbiol* 2015;**14**:20–32. <https://doi.org/10.1038/nrmicro3552>.
- 798 16. Vandeputte D, Falony G, Vieira-Silva S, Tito RY, Joossens M, Raes J. Stool
799 consistency is strongly associated with gut microbiota richness and composition,
800 enterotypes and bacterial growth rates. *Gut* 2016;**65**:57–62.
801 <https://doi.org/10.1136/gutjnl-2015-309618>.
- 802 17. Barton W, Cronin O, Garcia- Perez I, Whiston R, Holmes E, Woods T, et al. The
803 effects of sustained fitness improvement on the gut microbiome: A longitudinal,
804 repeated measures case- study approach. *Transl Sport Med* 2021;**4**:174–92.
805 <https://doi.org/10.1002/tsm2.215>.
- 806 18. Frampton J, Murphy KG, Frost G, Chambers ES. Short-chain fatty acids as potential
807 regulators of skeletal muscle metabolism and function. *Nat Metab* 2020;**2**:840–8.
808 <https://doi.org/10.1038/s42255-020-0188-7>.
- 809 19. Lundberg JO, Moretti C, Benjamin N, Weitzberg E. Symbiotic bacteria enhance
810 exercise performance. *Br J Sports Med* 2021;**55**:243. <https://doi.org/10.1136/bjsports-2020-102094>.
- 811 20. Ticinesi A, Lauretani F, Tana C, Nouvenne A, Ridolo E, Meschi T. Exercise and
812 immune system as modulators of intestinal microbiome: Implications for the gut-
813 muscle axis hypothesis. *Exerc Immunol Rev* 2019;**25**:84–95.
- 814 21. Barton W, Penney NC, Cronin O, Garcia-Perez I, Molloy MG, Holmes E, et al. The
815 microbiome of professional athletes differs from that of more sedentary subjects in
816 composition and particularly at the functional metabolic level. *Gut* 2017:gutjnl-2016-
817 313627. <https://doi.org/10.1136/gutjnl-2016-313627>.
- 818 22. Petersen LM, Bautista EJ, Nguyen H, Hanson BM, Chen L, Lek SH, et al. Community
819 characteristics of the gut microbiomes of competitive cyclists. *Microbiome* 2017;**5**:98.
820 <https://doi.org/10.1186/s40168-017-0320-4>.
- 821 23. Estaki M, Pither J, Baumeister P, Little JP, Gill SK, Ghosh S, et al. Cardiorespiratory
822 fitness as a predictor of intestinal microbial diversity and distinct metagenomic
823 functions. *Microbiome* 2016;**4**:42. <https://doi.org/10.1186/s40168-016-0189-7>.
- 824 24. Durk R, Castillo E, Marquez-Magaña L, Grosicki G, Bolter N, Lee M, et al. Gut
825 Microbiota Composition is Related to Cardiorespiratory Fitness. *Int J Sport Nutr Exerc*
826 *Metab* 2019;**29**:249–253. <https://doi.org/10.1123/ijsnem.2018-0024>.
- 827 25. Yang Y, Shi Y, Wiklund P, Tan X, Wu N, Zhang X, et al. The association between
828

- 829 cardiorespiratory fitness and gut microbiota composition in premenopausal women.
830 *Nutrients* 2017;**9**:1–11. <https://doi.org/10.3390/nu9080792>.
- 831 26. Nay K, Jollet M, Goustard B, Baati N, Vernus B, Pontones M, et al. Gut bacteria are
832 critical for optimal muscle function: A potential link with glucose homeostasis. *Am J*
833 *Physiol - Endocrinol Metab* 2019;**317**:E158–71.
834 <https://doi.org/10.1152/ajpendo.00521.2018>.
- 835 27. Okamoto T, Morino K, Ugi S, Nakagawa F, Lemecha M, Ida S, et al. Microbiome
836 potentiates endurance exercise through intestinal acetate production. *Am J Physiol -*
837 *Endocrinol Metab* 2019;**316**:E956–66. <https://doi.org/10.1152/ajpendo.00510.2018>.
- 838 28. Dohnalová L, Lundgren P, Carty JRE, Goldstein N, Wenski SL, Nanudorn P, et al. A
839 microbiome-dependent gut–brain pathway regulates motivation for exercise. *Nature*
840 2022;**612**:739–47. <https://doi.org/10.1038/s41586-022-05525-z>.
- 841 29. Mach N, Midoux C, Leclercq S, Pennarun S, Moyec L Le, Rué O, et al. Mining the
842 equine gut metagenome: poorly-characterized taxa associated with cardiovascular
843 fitness in endurance athletes. *Commun Biol* 2022;**5**:1032.
844 <https://doi.org/10.1038/s42003-022-03977-7>.
- 845 30. Qin R, Mahal LK. The host glycomic response to pathogens. *Curr Opin Struct Biol*
846 2021;**68**:149–56. <https://doi.org/10.1016/j.sbi.2020.12.011>.
- 847 31. Bergstrom K, Xia L. The barrier and beyond: Roles of intestinal mucus and mucin-
848 type O-glycosylation in resistance and tolerance defense strategies guiding host-
849 microbe symbiosis. *Gut Microbes* 2022;**14**.
850 <https://doi.org/10.1080/19490976.2022.2052699>.
- 851 32. Taleb V, Liao Q, Narimatsu Y, García-García A, Compañón I, Borges RJ, et al.
852 Structural and mechanistic insights into the cleavage of clustered O-glycan patches-
853 containing glycoproteins by mucinases of the human gut. *Nat Commun* 2022;**13**:1–15.
854 <https://doi.org/10.1038/s41467-022-32021-9>.
- 855 33. van Putten JPM, Strijbis K. Transmembrane Mucins: Signaling Receptors at the
856 Intersection of Inflammation and Cancer. *J Innate Immun* 2017;**9**:281–99.
857 <https://doi.org/10.1159/000453594>.
- 858 34. Silva S Da, Robbe-Masselot C, Ait-Belgnaoui A, Mancuso A, Mercade-Loubière M,
859 Salvador-Cartier C, et al. Stress disrupts intestinal mucus barrier in rats via mucin O -
860 glycosylation shift: prevention by a probiotic treatment. *Am J Physiol Liver Physiol*
861 2014;**307**:G420–9. <https://doi.org/10.1152/ajpgi.00290.2013>.
- 862 35. Arike L, Holmén-Larsson J, Hansson GC. Intestinal Muc2 mucin O-glycosylation is
863 affected by microbiota and regulated by differential expression of glycosyltransferases.
864 *Glycobiology* 2017;**27**:318–28. <https://doi.org/10.1093/glycob/cww134>.
- 865 36. Bergstrom K, Shan X, Casero D, Batushansky A, Lagishetty V, Jacobs JP, et al.
866 Proximal colon-derived O-glycosylated mucus encapsulates and modulates the
867 microbiota. *Science (80-)* 2020;**370**:467–72. <https://doi.org/10.1126/science.aay7367>.
- 868 37. Belzer C. Nutritional strategies for mucosal health: the interplay between microbes and
869 mucin glycans. *Trends Microbiol* 2022;**30**:13–21.
870 <https://doi.org/10.1016/j.tim.2021.06.003>.
- 871 38. Koropatkin NM, Cameron E a., Martens EC. How glycan metabolism shapes the
872 human gut microbiota. *Nat Rev Microbiol* 2012;**10**:323–35.
873 <https://doi.org/10.1038/nrmicro2746>.
- 874 39. Crouch LI, Liberato M V., Urbanowicz PA, Baslé A, Lamb CA, Stewart CJ, et al.
875 Prominent members of the human gut microbiota express endo-acting O-glycanases to
876 initiate mucin breakdown. *Nat Commun* 2020;**11**. <https://doi.org/10.1038/s41467-020-17847-5>.
- 877
878 40. Mach N, Moroldo M, Rau A, Lecardonnell J, Le Moyec L, Robert C, et al.

- 879 Understanding the Holobiont: Crosstalk Between Gut Microbiota and Mitochondria
880 During Long Exercise in Horse. *Front Mol Biosci* 2021;**8**:656204.
- 881 41. Svendsen IS, Taylor IM, Tønnessen E, Bahr R, Gleeson M. Training-related and
882 competition-related risk factors for respiratory tract and gastrointestinal infections in
883 elite cross-country skiers. *Br J Sports Med* 2016;**50**:809–15.
884 <https://doi.org/10.1136/bjsports-2015-095398>.
- 885 42. Gordon L, Schweltnus M, Swanevelder S, Jordaan E, Derman W. Recent acute pre-race
886 systemic illness in runners increases the risk of not finishing the race: SAFER study v.
887 *Br J Sports Med* 2017;**51**:1295–300. <https://doi.org/10.1136/bjsports-2016-096964>.
- 888 43. Jeukendrup AE, Vet-joop K, Sturk A, Stegen JHJC, Senden J, Saris WHM, et al.
889 Relationship between gastro-intestinal complaints and endotoxaemia, cytokine release
890 and the acute-phase reaction during and after a long-distance triathlon in highly trained
891 men. *Clin Sci* 2000;**98**:47–55. <https://doi.org/10.1042/cs0980047>.
- 892 44. Hoffman MD, Fogard K. Factors related to successful completion of a 161-km
893 ultramarathon. *Int J Sports Physiol Perform* 2011;**6**:25–37.
894 <https://doi.org/10.1123/ijspp.6.1.25>.
- 895 45. Urwin CS, Main LC, Mikocka-Walus A, Skvarc DR, Roberts SSH, Condo D, et al.
896 The Relationship Between Psychological Stress and Anxiety with Gastrointestinal
897 Symptoms Before and During a 56 km Ultramarathon Running Race. *Sport Med -*
898 *Open* 2021;**7**. <https://doi.org/10.1186/s40798-021-00389-5>.
- 899 46. Ribeiro FM, Petriz B, Marques G, Kamilla LH, Franco OL. Is There an Exercise-
900 Intensity Threshold Capable of Avoiding the Leaky Gut? *Front Nutr* 2021;**8**.
901 <https://doi.org/10.3389/fnut.2021.627289>.
- 902 47. Stuempfle KJ, Hoffman MD. Gastrointestinal distress is common during a 161-km
903 ultramarathon. *J Sports Sci* 2015;**33**:1814–21.
904 <https://doi.org/10.1080/02640414.2015.1012104>.
- 905 48. Koehler K, Peake J, Franco OL, Ribeiro FM, Petriz B, Marques G, et al. Is There an
906 Exercise-Intensity Threshold Capable of Avoiding the Leaky Gut? *Front Nutr /*
907 *www.frontiersin.org* 2021;**1**:627289. <https://doi.org/10.3389/fnut.2021.627289>.
- 908 49. Ruiz-Iglesias P, Estruel-Amades S, Camps-Bossacoma M, Massot-Cladera M, Castell
909 M, Pérez-Cano FJ. Alterations in the mucosal immune system by a chronic exhausting
910 exercise in Wistar rats. *Sci Rep* 2020;**10**:1–14. <https://doi.org/10.1038/s41598-020-74837-9>.
- 912 50. Pagesy P, Bouaboud A, Feng Z, Hulin P, Issad T. Short O-GlcNAcase Is Targeted to
913 the Mitochondria and Regulates Mitochondrial Reactive Oxygen Species Level. *Cells*
914 2022;**11**:1827. <https://doi.org/10.3390/cells11111827>.
- 915 51. Grootjans J, Hundscheid IHR, Lenaerts K, Boonen B, Renes IB, Verheyen FK, et al.
916 Ischaemia-induced mucus barrier loss and bacterial penetration are rapidly
917 counteracted by increased goblet cell secretory activity in human and rat colon. *Gut*
918 2013;**62**:250–8. <https://doi.org/10.1136/gutjnl-2011-301956>.
- 919 52. Ravcheev DA, Thiele I. Comparative genomic analysis of the human gut microbiome
920 reveals a broad distribution of metabolic pathways for the degradation of host-
921 synthesized mucin glycans and utilization of mucin-derived monosaccharides. *Front*
922 *Genet* 2017;**8**:1–22. <https://doi.org/10.3389/fgene.2017.00111>.
- 923 53. Pfeiffer CJ, Qiu B, Lam SK. Reduction of colonic mucus by repeated short-term stress
924 enhances experimental colitis in rats. *J Physiol* 2001;**95**:81–7.
925 [https://doi.org/10.1016/S0928-4257\(01\)00012-2](https://doi.org/10.1016/S0928-4257(01)00012-2).
- 926 54. Kim B, Chae J, Kim EH, Yang HI, Cheon JH, Il Kim T, et al. Physical activity and
927 quality of life of patients with inflammatory bowel disease. *Med (United States)*
928 2021;**100**. <https://doi.org/10.1097/MD.00000000000026290>.

- 929 55. Luo B, Xiang D, Nieman DC, Chen P. The effects of moderate exercise on chronic
930 stress-induced intestinal barrier dysfunction and antimicrobial defense. *Brain Behav*
931 *Immun* 2014;**39**:99–106. <https://doi.org/10.1016/j.bbi.2013.11.013>.
- 932 56. Dang AT, Marsland BJ. Microbes, metabolites, and the gut–lung axis. *Mucosal*
933 *Immunol* 2019;**12**:843–50. <https://doi.org/10.1038/s41385-019-0160-6>.
- 934 57. Mach N, Baranowski E, Nouvel LX, Citti C. The Airway Pathobiome in Complex
935 Respiratory Diseases: A Perspective in Domestic Animals. *Front Cell Infect Microbiol*
936 2021;**11**:1–22. <https://doi.org/10.3389/fcimb.2021.583600>.
- 937 58. Budden KF, Gellatly SL, Wood DLA, Cooper MA, Morrison M, Hugenholtz P, et al.
938 Emerging pathogenic links between microbiota and the gut-lung axis. *Nat Rev*
939 *Microbiol* 2017;**15**:55. <https://doi.org/10.1038/nrmicro.2016.142>.
- 940 59. Wypych TP, Wickramasinghe LC, Marsland BJ. The influence of the microbiome on
941 respiratory health. *Nat Immunol* 2019;**20**:1279–90. [https://doi.org/10.1038/s41590-](https://doi.org/10.1038/s41590-019-0451-9)
942 [019-0451-9](https://doi.org/10.1038/s41590-019-0451-9).
- 943 60. Cone RA. Barrier properties of mucus. *Adv Drug Deliv Rev* 2009;**61**:75–85.
944 <https://doi.org/10.1016/j.addr.2008.09.008>.
- 945 61. Bansil R, Turner BS. The biology of mucus: Composition, synthesis and organization.
946 *Adv Drug Deliv Rev* 2018;**124**:3–15. <https://doi.org/10.1016/j.addr.2017.09.023>.
- 947 62. Nason R, Büll C, Konstantinidi A, Sun L, Ye Z, Halim A, et al. Display of the human
948 mucinome with defined O-glycans by gene engineered cells. *Nat Commun* 2021;**12**:1–
949 16. <https://doi.org/10.1038/s41467-021-24366-4>.
- 950 63. Carvalho FA, Aitken JD, Vijay-Kumar M, Gewirtz AT. Toll-like receptor-gut
951 microbiota interactions: Perturb at your own risk! *Annu Rev Physiol* 2012;**74**:177–98.
952 <https://doi.org/10.1146/annurev-physiol-020911-153330>.
- 953 64. Bergstrom KSB, Xia L. Mucin-type O-glycans and their roles in intestinal
954 homeostasis. *Glycobiology* 2013;**23**:1026–37. <https://doi.org/10.1093/glycob/cwt045>.
- 955 65. Robinson LS, Lewis WG, Lewis AL. The sialate O-acetyltransferase EstA from gut
956 Bacteroidetes species enables sialidase-mediated cross-species foraging of 9-O-
957 acetylated sialoglycans. *J Biol Chem* 2017;**292**:11861–72.
958 <https://doi.org/10.1074/jbc.M116.769232>.
- 959 66. Schroeder BO, Birchenough GMH, Ståhlman M, Arike L, Johansson MEV, Hansson
960 GC, et al. Bifidobacteria or Fiber Protects against Diet-Induced Microbiota-Mediated
961 Colonic Mucus Deterioration. *Cell Host Microbe* 2018;**23**:27-40.e7.
962 <https://doi.org/10.1016/j.chom.2017.11.004>.
- 963 67. Bell A, Brunt J, Crost E, Vaux L, Nepravishta R, Owen CD, et al. Elucidation of a
964 sialic acid metabolism pathway in mucus-foraging Ruminococcus gnavus unravels
965 mechanisms of bacterial adaptation to the gut. *Nat Microbiol* 2019;**4**:2393–404.
966 <https://doi.org/10.1038/s41564-019-0590-7>.
- 967 68. Paone P, Cani PD. Mucus barrier, mucins and gut microbiota: the expected slimy
968 partners? *Gut* 2020;**69**:2232–43. <https://doi.org/10.1136/gutjnl-2020-322260>.
- 969 69. Myslicki JP, Belke DD, Shearer J. Role of O-GlcNAcylation in nutritional sensing,
970 insulin resistance and in mediating the benefits of exercise. *Appl Physiol Nutr Metab*
971 2014;**39**:1205–13. <https://doi.org/10.1139/apnm-2014-0122>.
- 972 70. Bullen JW, Balsbaugh JL, Chanda D, Shabanowitz J, Hunt DF, Neumann D, et al.
973 Cross-talk between two essential nutrient-sensitive enzymes O-GlcNAc Transferase
974 (OGT) and Amp-Activated Protein Kinase (AMPK). *J Biol Chem* 2014;**289**:10592–
975 606. <https://doi.org/10.1074/jbc.M113.523068>.
- 976 71. Issad T, Al-Mukh H, Bouaboud A, Pagesy P. Protein O-GlcNAcylation and the
977 regulation of energy homeostasis: lessons from knock-out mouse models. *J Biomed Sci*
978 2022;**29**:1–15. <https://doi.org/10.1186/s12929-022-00851-w>.

- 979 72. Shi H, Munk A, Nielsen TS, Daughtry MR, Larsson L, Li S, et al. Skeletal muscle O-
980 GlcNAc transferase is important for muscle energy homeostasis and whole-body
981 insulin sensitivity. *Mol Metab* 2018;**11**:160–77.
982 <https://doi.org/10.1016/j.molmet.2018.02.010>.
- 983 73. Lambert M, Bastide B, Cieniewski-Bernard C. Involvement of O-GlcNAcylation in
984 the skeletal muscle physiology and physiopathology: Focus on muscle metabolism.
985 *Front Endocrinol (Lausanne)* 2018;**9**:1–12. <https://doi.org/10.3389/fendo.2018.00578>.
- 986 74. Goto Y, Uematsu S, Kiyono H. Epithelial glycosylation in gut homeostasis and
987 inflammation. *Nat Immunol* 2016;**17**:1244–51. <https://doi.org/10.1038/ni.3587>.
- 988 75. Johansson M, Jakobsson H, Holmén-Larsson J, Schutte A, Ermund A, Rodríguez-
989 Piñeiro A, et al. Normalization of host intestinal mucus layers requires long-term
990 microbial colonization. *Cell Host Microbe* 2015;**18**:582–92.
991 <https://doi.org/10.1016/j.chom.2015.10.007>.Normalization.
- 992 76. Smirnova MG, Guo L, Birchall JP, Pearson JP. LPS up-regulates mucin and cytokine
993 mRNA expression and stimulates mucin and cytokine secretion in goblet cells. *Cell*
994 *Immunol* 2003;**221**:42–9. [https://doi.org/10.1016/S0008-8749\(03\)00059-5](https://doi.org/10.1016/S0008-8749(03)00059-5).
- 995 77. Burger-van Paassen N, Vincent A, Puiman PJ, van der Sluis M, Bouma J, Boehm G, et
996 al. The regulation of intestinal mucin MUC2 expression by short-chain fatty acids:
997 Implications for epithelial protection. *Biochem J* 2009;**420**:211–9.
998 <https://doi.org/10.1042/BJ20082222>.
- 999 78. Larsson JMH, Karlsson H, Crespo JG, Johansson MEV, Eklund L, Sjövall H, et al.
1000 Altered O-glycosylation profile of MUC2 mucin occurs in active ulcerative colitis and
1001 is associated with increased inflammation. *Inflamm Bowel Dis* 2011;**17**:2299–307.
1002 <https://doi.org/10.1002/ibd.21625>.
- 1003 79. Pabst O, Slack E. IgA and the intestinal microbiota: the importance of being specific.
1004 *Mucosal Immunol* 2020;**13**:12–21. <https://doi.org/10.1038/s41385-019-0227-4>.
- 1005 80. Xia B, Zhong R, Wu W, Luo C, Meng Q, Gao Q, et al. Mucin O-glycan-microbiota
1006 axis orchestrates gut homeostasis in a diarrheal pig model. *Microbiome* 2022;**10**:1–21.
1007 <https://doi.org/10.1186/s40168-022-01326-8>.
- 1008 81. Hiippala K, Jouhten H, Ronkainen A, Hartikainen A, Kainulainen V, Jalanka J, et al.
1009 The potential of gut commensals in reinforcing intestinal barrier function and
1010 alleviating inflammation. *Nutrients* 2018;**18**:e988.
1011 <https://doi.org/10.3390/nu10080988>.
- 1012 82. Reily C, Stewart TJ, Renfrow MB, Novak J. Glycosylation in health and disease. *Nat*
1013 *Rev Nephrol* 2019;**15**:346–66. <https://doi.org/10.1038/s41581-019-0129-4>.
- 1014 83. Rollenske T, Szijarto V, Lukasiewicz J, Guachalla LM, Stojkovic K, Hartl K, et al.
1015 Cross-specificity of protective human antibodies against *Klebsiella pneumoniae* LPS
1016 O-antigen. *Nat Immunol* 2018;**19**:617–24. <https://doi.org/10.1038/s41590-018-0106-2>.
- 1017 84. Mathias A, Corthésy B. N-glycans on secretory component: Mediators of the
1018 interaction between secretory IgA and gram-positive commensals sustaining intestinal
1019 homeostasis. *Gut Microbes* 2011;**2**:287–93. <https://doi.org/10.4161/gmic.2.5.18269>.
- 1020 85. Michaud E, Waackel L, Gayet R, Goguyer- Deschaumes R, Chanut B, Jospin F, et al.
1021 Alteration of microbiota antibody- mediated immune selection contributes to dysbiosis
1022 in inflammatory bowel diseases. *EMBO Mol Med* 2022;**14**:1–21.
1023 <https://doi.org/10.15252/emmm.202115386>.
- 1024 86. Huus KE, Petersen C, Finlay BB. Diversity and dynamism of IgA–microbiota
1025 interactions. *Nat Rev Immunol* 2021;**21**:514–25. <https://doi.org/10.1038/s41577-021-00506-1>.
- 1026
1027 87. McKune A, Starzak D, Semple S. Repeated bouts of eccentrically biased endurance
1028 exercise stimulate salivary IgA secretion rate. *Biol Sport* 2014;**32**:21–5.

- 1029 <https://doi.org/10.5604/20831862.1126324>.
- 1030 88. Anderluh M, Berti F, Bzducha-Wróbel A, Chiodo F, Colombo C, Compostella F, et al.
 1031 Emerging glyco-based strategies to steer immune responses. *FEBS J* 2021;**288**:4746–
 1032 72. <https://doi.org/10.1111/febs.15830>.
- 1033 89. Chang Y-C, Olson J, Beasley FC, Tung C, Zhang J, Crocker PR, et al. Group B
 1034 Streptococcus Engages an Inhibitory Siglec through Sialic Acid Mimicry to Blunt
 1035 Innate Immune and Inflammatory Responses In Vivo. Wessels MR, editor. *PLoS*
 1036 *Pathog* 2014;**10**:e1003846. <https://doi.org/10.1371/journal.ppat.1003846>.
- 1037 90. Severi E, Hood DW, Thomas GH. Sialic acid utilization by bacterial pathogens.
 1038 *Microbiology* 2007;**153**:2817–22. <https://doi.org/10.1099/mic.0.2007/009480-0>.
- 1039 91. Ndeh D, Gilbert HJ. Biochemistry of complex glycan depolymerisation by the human
 1040 gut microbiota. *FEMS Microbiol Rev* 2018;**42**:146–64.
 1041 <https://doi.org/10.1093/femsre/fuy002>.
- 1042 92. Tailford LE, Crost EH, Kavanaugh D, Juge N. Mucin glycan foraging in the human
 1043 gut microbiome. *Front Genet* 2015;**5**. <https://doi.org/10.3389/fgene.2015.00081>.
- 1044 93. Plovier H, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, et al. A purified
 1045 membrane protein from Akkermansia muciniphila or the pasteurized bacterium
 1046 improves metabolism in obese and diabetic mice. *Nat Med* 2017;**23**:107–13.
 1047 <https://doi.org/10.1038/nm.4236>.
- 1048 94. Belzer C, Chia LW, Aalvink S, Chamlagain B, Piironen V, Knol J, et al. Microbial
 1049 metabolic networks at the mucus layer lead to diet-independent butyrate and vitamin
 1050 B12 production by intestinal symbionts. *MBio* 2017;**8**:1–14.
 1051 <https://doi.org/10.1128/mBio.00770-17>.
- 1052 95. Sicard J-F, Le Bihan G, Vogelee P, Jacques M, Harel J. Interactions of Intestinal
 1053 Bacteria with Components of the Intestinal Mucus. *Front Cell Infect Microbiol* 2017;**7**.
 1054 <https://doi.org/10.3389/fcimb.2017.00387>.
- 1055 96. Kaoutari A El, Armougom F, Gordon JI, Raoult D, Henrissat B. The abundance and
 1056 variety of carbohydrate-active enzymes in the human gut microbiota. *Nat Rev*
 1057 *Microbiol* 2013;**11**:497–504. <https://doi.org/10.1038/nrmicro3050>.
- 1058 97. Hansson GC. Mucins and the Microbiome. *Annu Rev Biochem* 2020;**89**:769–93.
 1059 <https://doi.org/10.1146/annurev-biochem-011520-105053>.
- 1060 98. van der Hee B, Wells JM. Microbial Regulation of Host Physiology by Short-chain
 1061 Fatty Acids. *Trends Microbiol* 2021;**29**:700–12.
 1062 <https://doi.org/10.1016/j.tim.2021.02.001>.
- 1063 99. Gabius HJ, Roth J. An introduction to the sugar code. *Histochem Cell Biol*
 1064 2017;**147**:111–7. <https://doi.org/10.1007/s00418-016-1521-9>.
- 1065 100. Trastoy B, Naegeli A, Anso I, Sjögren J, Guerin ME. Structural basis of mammalian
 1066 mucin processing by the human gut O-glycopeptidase OgpA from Akkermansia
 1067 muciniphila. *Nat Commun* 2020;**11**:4844. <https://doi.org/10.1038/s41467-020-18696->
 1068 [y](https://doi.org/10.1038/s41467-020-18696-y).
- 1069 101. Barton W, Penney NC, Cronin O, Garcia-Perez I, Molloy MG, Holmes E, et al. The
 1070 microbiome of professional athletes differs from that of more sedentary subjects in
 1071 composition and particularly at the functional metabolic level. *Gut* 2018;**67**:625–33.
 1072 <https://doi.org/10.1136/gutjnl-2016-313627>.
- 1073 102. Clarke SF, Murphy EF, O’Sullivan O, Lucey AJ, Humphreys M, Hogan A, et al.
 1074 Exercise and associated dietary extremes impact on gut microbial diversity. *Gut*
 1075 2014;**63**:1913–20. <https://doi.org/10.1136/gutjnl-2013-306541>.
- 1076 103. Hooper L V, Gordon JI. Commensal host-bacterial relationships in the gut. *Science*
 1077 2001;**292**:1115–8. <https://doi.org/10.1126/science.1058709>.
- 1078 104. Turrone F, Milani C, Duranti S, Mahony J, van Sinderen D, Ventura M. Glycan

- 1079 Utilization and Cross-Feeding Activities by Bifidobacteria. *Trends Microbiol*
1080 2018;**26**:339–50. <https://doi.org/10.1016/j.tim.2017.10.001>.
- 1081 105. D’Souza G, Shitut S, Preussger D, Yousif G, Waschina S, Kost C. Ecology and
1082 evolution of metabolic cross-feeding interactions in bacteria. *Nat Prod Rep*
1083 2018;**35**:455–88. <https://doi.org/10.1039/c8np00009c>.
- 1084 106. Milani C, Lugli GA, Duranti S, Turrone F, Mancabelli L, Ferrario C, et al.
1085 Bifidobacteria exhibit social behavior through carbohydrate resource sharing in the
1086 gut. *Nat Publ Gr* 2015. <https://doi.org/10.1038/srep15782>.
- 1087 107. O’Donovan CM, Madigan SM, Garcia-Perez I, Rankin A, O’ Sullivan O, Cotter PD.
1088 Distinct microbiome composition and metabolome exists across subgroups of elite
1089 Irish athletes. *J Sci Med Sport* 2020;**23**:63–8.
1090 <https://doi.org/10.1016/j.jsams.2019.08.290>.
- 1091 108. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-talk
1092 between Akkermansia muciniphila and intestinal epithelium controls diet-induced
1093 obesity. *Proc Natl Acad Sci U S A* 2013;**110**:9066–71.
1094 <https://doi.org/10.1073/pnas.1219451110>.
- 1095 109. Hu Y, Li C, Hou Y. Possible regulation of liver glycogen structure through the gut-
1096 liver axis by resistant starch: a review. *Food Funct* 2021;**12**:11154–64.
1097 <https://doi.org/10.1039/D1FO02416G>.
- 1098 110. Jakobsson HE, Rodríguez- Piñeiro AM, Schütte A, Ermund A, Boysen P, Bemark M,
1099 et al. The composition of the gut microbiota shapes the colon mucus barrier. *EMBO*
1100 *Rep* 2015;**16**:164–77. <https://doi.org/10.15252/embr.201439263>.
- 1101 111. Chassaing B, Raja SM, Lewis JD, Srinivasan S, Gewirtz AT. Colonic Microbiota
1102 Encroachment Correlates With Dysglycemia in Humans. *Cmgh* 2017;**4**:205–21.
1103 <https://doi.org/10.1016/j.jcmgh.2017.04.001>.
- 1104 112. Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, et al. A
1105 Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and
1106 Enhances Pathogen Susceptibility. *Cell* 2016;**167**:1339-1353.e21.
1107 <https://doi.org/10.1016/j.cell.2016.10.043>.
- 1108 113. Hortemo KH, Lunde PK, Anonsen JH, Kvaløy H, Munkvik M, Rehn TA, et al.
1109 Exercise training increases protein O-GlcNAcylation in rat skeletal muscle. *Physiol*
1110 *Rep* 2016;**4**:1–20. <https://doi.org/10.14814/phy2.12896>.
- 1111 114. Wang X, Feng Z, Wang X, Yang L, Han S, Cao K, et al. O-GlcNAcase deficiency
1112 suppresses skeletal myogenesis and insulin sensitivity in mice through the modulation
1113 of mitochondrial homeostasis. *Diabetologia* 2016;**59**:1287–96.
1114 <https://doi.org/10.1007/s00125-016-3919-2>.
- 1115 115. Chattopadhyay T, Maniyadath B, Bagul HP, Chakraborty A, Shukla N, Budnar S, et al.
1116 Spatiotemporal gating of SIRT1 functions by O-GlcNAcylation is essential for liver
1117 metabolic switching and prevents hyperglycemia. *Proc Natl Acad Sci U S A*
1118 2020;**117**:6890–900. <https://doi.org/10.1073/pnas.1909943117>.
- 1119 116. Housley MP, Udeshi ND, Rodgers JT, Shabanowitz J, Puigserver P, Hunt DF, et al. A
1120 PGC-1 α -O-GlcNAc Transferase Complex Regulates FoxO Transcription Factor
1121 Activity in Response to Glucose. *J Biol Chem* 2009;**284**:5148–57.
1122 <https://doi.org/10.1074/jbc.M808890200>.
- 1123 117. Housley MP, Rodgers JT, Udeshi ND, Kelly TJ, Shabanowitz J, Hunt DF, et al. O-
1124 GlcNAc regulates FoxO activation in response to glucose. *J Biol Chem*
1125 2008;**283**:16283–92. <https://doi.org/10.1074/jbc.M802240200>.
- 1126 118. Butkinaree C, Park K, Hart GW. Transcription in Response to Nutrients and Stress.
1127 *Biochim Biophys Acta* 2011;**1800**:1–23.
1128 <https://doi.org/10.1016/j.bbagen.2009.07.018.O-Linked>.

- 1129 119. Chen PH, Chi JT, Boyce M. Functional crosstalk among oxidative stress and O-
1130 GlcNAc signaling pathways. *Glycobiology* 2018;**28**:556–64.
1131 <https://doi.org/10.1093/glycob/cwy027>.
- 1132 120. Vidal-Veuthey B, González D, Cárdenas JP. Role of microbial secreted proteins in gut
1133 microbiota-host interactions. *Front Cell Infect Microbiol* 2022;**12**:1–8.
1134 <https://doi.org/10.3389/fcimb.2022.964710>.
- 1135 121. Quik M, Hokke CH, Everts B. The role of O-GlcNAcylation in immunity against
1136 infections. *Immunology* 2020;**161**:175–85. <https://doi.org/10.1111/imm.13245>.
- 1137 122. Hintikka JE, Munukka E, Valtonen M, Luoto R, Ihalainen JK, Kallonen T, et al. Gut
1138 Microbiota and Serum Metabolome in Elite Cross-Country Skiers: A Controlled
1139 Study. *Metabolites* 2022;**12**. <https://doi.org/10.3390/metabo12040335>.
- 1140 123. Clarke SF, Murphy EF, O’Sullivan O, Lucey AJ, Humphreys M, Hogan A, et al.
1141 Exercise and associated dietary extremes impact on gut microbial diversity. *Gut*
1142 2014;**63**:1913–20. <https://doi.org/10.1136/gutjnl-2013-306541>.
- 1143 124. Munukka E, Ahtiainen JP, Puigbó P, Jalkanen S, Pahkala K, Kesitalo A, et al. Six-
1144 week endurance exercise alters gut metagenome that is not reflected in systemic
1145 metabolism in over-weight women. *Front Microbiol* 2018;**9**:2323.
1146 <https://doi.org/10.3389/fmicb.2018.02323>.
- 1147 125. Radak Z, Zhao Z, Koltai E, Ohno H, Atalay M. Oxygen consumption and usage during
1148 physical exercise: The balance between oxidative stress and ROS-dependent adaptive
1149 signaling. *Antioxidants Redox Signal* 2013;**18**:1208–46.
1150 <https://doi.org/10.1089/ars.2011.4498>.
- 1151 126. Nay K, Jollet M, Goustard B, Baati N, Vernus B, Pontones M, et al. Gut bacteria are
1152 critical for optimal muscle function: A potential link with glucose homeostasis. *Am J*
1153 *Physiol - Endocrinol Metab* 2019;**317**:E158-171.
1154 <https://doi.org/10.1152/ajpendo.00521.2018>.
- 1155 127. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud D-J, Bakker BM. The
1156 role of short-chain fatty acids in the interplay between diet, gut microbiota, and host
1157 energy metabolism. *J Lipid Res* 2013;**54**:2325–40.
1158 <https://doi.org/10.1194/jlr.R036012>.
- 1159 128. Mishra SP, Karunakar P, Taraphder S, Yadav H. Free fatty acid receptors 2 and 3 as
1160 microbial metabolite sensors to shape host health: Pharmacophysiological view.
1161 *Biomedicines* 2020;**8**. <https://doi.org/10.3390/BIOMEDICINES8060154>.
- 1162 129. Li G, Jin B, Fan Z. Mechanisms Involved in Gut Microbiota Regulation of Skeletal
1163 Muscle. *Oxid Med Cell Longev* 2022;**2022**:15–9.
1164 <https://doi.org/10.1155/2022/2151191>.
- 1165 130. Radak Z, Chung HY, Koltai E, Taylor AW, Goto S. Exercise, oxidative stress and
1166 hormesis. *Ageing Res Rev* 2008;**7**:34–42. <https://doi.org/10.1016/j.arr.2007.04.004>.
- 1167 131. Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, et al. Butyrate Improves
1168 Insulin Sensitivity and Increases Energy Expenditure in Mice. *Diabetes* 2009;**58**:1509–
1169 17. <https://doi.org/10.2337/db08-1637>.
- 1170 132. Zhao M, Ren K, Xiong X, Cheng M, Zhang Z, Huang Z, et al. Protein O-GlcNAc
1171 Modification Links Dietary and Gut Microbial Cues to the Differentiation of
1172 Enteroendocrine L Cells. *Cell Rep* 2020;**32**:108013.
1173 <https://doi.org/10.1016/j.celrep.2020.108013>.
- 1174 133. Ruan H-B, Zhao M. Microbiota-sensing O-GlcNAc signaling in intestinal and
1175 metabolic homeostasis. *Biochem Mol Biol* 2017;**31**:784.4-784.4.
1176 https://doi.org/10.1096/fasebj.31.1_supplement.784.4.
- 1177 134. Gérard P. Metabolism of cholesterol and bile acids by the gut microbiota. *Pathogens*
1178 2013;**3**:14–24. <https://doi.org/10.3390/pathogens3010014>.

- 1179 135. Kuipers F, Bloks VW, Groen AK. Beyond intestinal soap—bile acids in metabolic
1180 control. *Nat Rev Endocrinol* 2014;**10**:488–98. <https://doi.org/10.1038/nrendo.2014.60>.
- 1181 136. Berrabah W, Aumercier P, Gheeraert C, Dehondt H, Bouchaert E, Alexandre J, et al.
1182 Glucose sensing O-GlcNAcylation pathway regulates the nuclear bile acid receptor
1183 farnesoid X receptor (FXR). *Hepatology* 2014;**59**:2022–33.
1184 <https://doi.org/10.1002/hep.26710>.
- 1185 137. Egan M, Motherway MO, Kilcoyne M, Kane M, Joshi L, Ventura M, et al. Cross-
1186 feeding by *Bifidobacterium breve* UCC2003 during co-cultivation with
1187 *Bifidobacterium bifidum* PRL2010 in a mucin-based medium (ene bifido profiteert van
1188 glucosidase activiteit van de andere). *BMC Microbiol* 2014;**14**:1–14.
- 1189 138. Bishara J, Farah R, Mograbi J, Khalaila W, Abu-Elheja O, Mahamid M, et al. Obesity
1190 as a risk factor for *Clostridium difficile* infection. *Clin Infect Dis* 2013;**57**:489–93.
1191 <https://doi.org/10.1093/cid/cit280>.
- 1192 139. Sonnenburg JL, Xu J, Leip DD, Chen C-H, Westover BP, Weatherford J, et al. Glycan
1193 Foraging in Vivo by an Intestine-Adapted Bacterial Symbiont. *Science (80-)*
1194 2005;**307**:1955–9. <https://doi.org/10.1126/science.1109051>.
- 1195 140. Bron PA, Kleerebezem M, Brummer R-J, Cani PD, Mercenier A, MacDonald TT, et
1196 al. Can probiotics modulate human disease by impacting intestinal barrier function? *Br*
1197 *J Nutr* 2017;**117**:93–107. <https://doi.org/10.1017/S0007114516004037>.
- 1198 141. Sicard JF, Bihan G Le, Vogeleeer P, Jacques M, Harel J. Interactions of intestinal
1199 bacteria with components of the intestinal mucus. *Front Cell Infect Microbiol* 2017;**7**.
1200 <https://doi.org/10.3389/fcimb.2017.00387>.
- 1201 142. Ahl D, Liu H, Schreiber O, Roos S, Phillipson M, Holm L. *Lactobacillus reuteri*
1202 increases mucus thickness and ameliorates dextran sulphate sodium-induced colitis in
1203 mice. *Acta Physiol* 2016;**217**:300–10. <https://doi.org/10.1111/apha.12695>.
- 1204 143. Gou H-Z, Zhang Y-L, Ren L-F, Li Z-J, Zhang L. How do intestinal probiotics restore
1205 the intestinal barrier? *Front Microbiol* 2022;**13**.
1206 <https://doi.org/10.3389/fmicb.2022.929346>.
- 1207 144. Schroeder BO, Birchenough GMH, Ståhlman M, Arike L, Johansson ME V, Hansson
1208 GC, et al. Bifidobacteria or fiber protect against diet-induced microbiota-mediated
1209 colonic mucus deterioration HHS Public Access The defects can be prevented by
1210 application of a probiotic bifidobacteria or the prebiotic fiber inulin. *Cell Host Microbe*
1211 2018;**23**:27–40. <https://doi.org/10.1016/j.chom.2017.11.004>. Bifidobacteria.
- 1212 145. Zhai Q, Feng S, Arjan N, Chen W. A next generation probiotic, *Akkermansia*
1213 *muciniphila*. *Crit Rev Food Sci Nutr* 2019;**59**:3227–36.
1214 <https://doi.org/10.1080/10408398.2018.1517725>.
- 1215 146. Shin N-R, Lee J-C, Lee H-Y, Kim M-S, Whon TW, Lee M-S, et al. An increase in the
1216 *Akkermansia* spp. population induced by metformin treatment improves glucose
1217 homeostasis in diet-induced obese mice. *Gut* 2014;**63**:727–35.
1218 <https://doi.org/10.1136/gutjnl-2012-303839>.
- 1219 147. Wu W, Lv L, Shi D, Ye J, Fang D, Guo F, et al. Protective Effect of *Akkermansia*
1220 *muciniphila* against Immune-Mediated Liver Injury in a Mouse Model. *Front*
1221 *Microbiol* 2017;**8**. <https://doi.org/10.3389/fmicb.2017.01804>.
- 1222 148. Kim S, Shin Y-C, Kim T-Y, Kim Y, Lee Y-S, Lee S-H, et al. Mucin degrader
1223 *Akkermansia muciniphila* accelerates intestinal stem cell-mediated epithelial
1224 development. *Gut Microbes* 2021;**13**. <https://doi.org/10.1080/19490976.2021.1892441>.
- 1225 149. Pan L, Fu T, Cheng H, Mi J, Shang Q, Yu G. Polysaccharide from edible alga
1226 *Gloiopeltis furcata* attenuates intestinal mucosal damage by therapeutically remodeling
1227 the interactions between gut microbiota and mucin O-glycans. *Carbohydr Polym*
1228 2022;**278**:118921. <https://doi.org/10.1016/j.carbpol.2021.118921>.

- 1229 150. Pruss KM, Marcobal A, Southwick AM, Dahan D, Smits SA, Ferreyra JA, et al.
1230 Mucin-derived O-glycans supplemented to diet mitigate diverse microbiota
1231 perturbations. *ISME J* 2021;**15**:577–91. <https://doi.org/10.1038/s41396-020-00798-6>.
1232 151. Hino S, Mizushima T, Kaneko K, Kawai E, Kondo T, Genda T, et al. Mucin-Derived
1233 O-Glycans act as endogenous fiber and sustain mucosal immune homeostasis via
1234 short-Chain fatty acid production in rat cecum. *J Nutr* 2020;**150**:2656–65.
1235 <https://doi.org/10.1093/jn/nxaa097>.
1236 152. Yamada T, Hino S, Iijima H, Genda T, Aoki R, Nagata R, et al. Mucin O-glycans
1237 facilitate symbiosynthesis to maintain gut immune homeostasis. *EBioMedicine*
1238 2019;**48**:513–25. <https://doi.org/10.1016/j.ebiom.2019.09.008>.
1239 153. Raskova Kafkova L, Brokesova D, Krupka M, Stehlikova Z, Dvorak J, Coufal S, et al.
1240 Secretory IgA N-glycans contribute to the protection against E. coli O55 infection of
1241 germ-free piglets. *Mucosal Immunol* 2021;**14**:511–22. [https://doi.org/10.1038/s41385-](https://doi.org/10.1038/s41385-020-00345-8)
1242 [020-00345-8](https://doi.org/10.1038/s41385-020-00345-8).
1243 154. Everard A, Lazarevic V, Derrien M, Girard M, Muccioli GM, Neyrinck AM, et al.
1244 Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic
1245 obese and diet-induced leptin-resistant mice. *Diabetes* 2011;**60**:2775–86.
1246 <https://doi.org/10.2337/db11-0227>.
1247 155. van den Abbeele P, Gérard P, Rabot S, Bruneau A, El Aidy S, Derrien M, et al.
1248 Arabinoxylans and inulin differentially modulate the mucosal and luminal gut
1249 microbiota and mucin-degradation in humanized rats. *Environ Microbiol*
1250 2011;**13**:2667–80. <https://doi.org/10.1111/j.1462-2920.2011.02533.x>.
1251 156. Ottman N, Reunanen J, Meijerink M, Pietila TE, Kainulainen V, Klievink J, et al. Pili-
1252 like proteins of Akkermansia muciniphila modulate host immune responses and gut
1253 barrier function. *PLoS One* 2017;**12**:1–18.
1254 <https://doi.org/10.1371/journal.pone.0173004>.
1255 157. Giromini C, Baldi A, Rebucci R, Lanzoni D, Policardi M, Sundaram TS, et al. Role of
1256 Short Chain Fatty Acids to Counteract Inflammatory Stress and Mucus Production in
1257 Human Intestinal HT29-MTX-E12 Cells. *Foods* 2022;**11**.
1258 <https://doi.org/10.3390/foods11131983>.
1259 158. Diao H, Jiao AR, Yu B, Mao XB, Chen DW. Gastric infusion of short-chain fatty acids
1260 can improve intestinal barrier function in weaned piglets. *Genes Nutr* 2019;**14**:4.
1261 <https://doi.org/10.1186/s12263-019-0626-x>.
1262 159. Earle KA, Billings G, Sigal M, Lichtman JS, Hansson GC, Elias JE, et al. Quantitative
1263 Imaging of Gut Microbiota Spatial Organization. *Cell Host Microbe* 2015;**18**:478–88.
1264 <https://doi.org/10.1016/j.chom.2015.09.002>.
1265 160. Mack D, Michail S, McDougall L, Hollingsworth M. Probiotics inhibit
1266 enteropathogenic E. coli adherence in vitro by inducing intestinal mucin gene
1267 expression. *Am J Physiol - Endocrinol Metab Physiol* 1999;**276**:G941-50.
1268 161. Huang W-C, Hsu Y-J, Huang C-C, Liu H-C, Lee M-C. Exercise Training Combined
1269 with Bifidobacterium longum OLP-01 Supplementation Improves Exercise
1270 Physiological Adaption and Performance. *Nutrients* 2020;**12**:1145.
1271 <https://doi.org/10.3390/nu12041145>.
1272 162. Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, et al. Butyrate improves
1273 insulin sensitivity and increases energy expenditure in mice. *Diabetes* 2009;**58**:1509–
1274 1517. <https://doi.org/10.2337/db08-1637>.
1275