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## Somatic complications and nutritional management of anorexia nervosa

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1 **Intestinal microbiota and Anorexia Nervosa**

2

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4

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16

17 **KEYWORDS**

18 microbiota; anorexia nervosa; eating disorder; functional gastrointestinal disorders; anxiety;

19 depression

20 **ABSTRACT**

21

22         Anorexia nervosa is an eating disorder characterized by a severe malnutrition, an  
23 intense fear of gaining weight and a disturbed self-body image. Anorexia is conventionally  
24 considered as a multifactorial disease depending on biological, psychological and socio-  
25 cultural factors. Among the different biological factors involved in this eating disorder, the  
26 gut microbiota has recently gained a lot of attention. Indeed, the identification of an intestinal  
27 microbiota dysbiosis in individuals with anorexia has opened new and promising lines of  
28 research. These researches focus in particular on the role of intestinal micro-organisms in the  
29 functional gastrointestinal disorders associated with Anorexia, in anxiety and depression-  
30 related disorders as well as in the regulation of eating behavior. This review aims to present  
31 the current knowledge about the putative role of the gut microbiota in the pathogenesis,  
32 course and treatment of Anorexia nervosa.

33 *Introduction*

34

35 Anorexia Nervosa (AN) is a highly morbid disease characterized by a significant  
36 malnutrition (Body Mass Index (BMI) below  $18.5 \text{ kg.m}^{-2}$ ), an intense fear of gaining weight,  
37 and a disturbed self-body image (DSM-V diagnostic criteria) (1). This disease mainly occurs  
38 in adolescent girls or young women. Prevalence of AN has been estimated to 1.4% for women  
39 and 0.2% for men, and steadily increases in most countries (2). Anorexia Nervosa has the  
40 highest mortality rate of any psychiatric illness, with a standardized mortality ratio  $>5$  (ratio  
41 of observed deaths in anorectic patients to expected deaths in the general population) (3). Two  
42 subtypes of AN can be distinguished: restricting type (where patients limit their food intake to  
43 decrease body weight) and purging type (where patients use self-induced vomiting or take  
44 laxatives to counter act food intake) (1). Anorectic patients often present with comorbid  
45 anxiety and depression (4,5). Treatment of Anorexia Nervosa is currently based on  
46 psychological approaches and refeeding approaches. Refeeding is unfortunately reported to be  
47 uncomformable and distressing to patients, and often fail to restore a healthy body fat  
48 composition (6). Weight restoration is however of critical importance to the healing process.  
49 Indeed, higher BMI correlates with better outcome after treatment and prevent depression and  
50 severe somatic sequalae, including osteoporosis and infertility (7-9). Anorexia Nervosa is  
51 conventionally considered as a multifactorial disease depending on biological, psychological  
52 and socio-cultural factors. Unfortunately, the etiology and the pathophysiology of this disease  
53 remain incompletely understood and treatments targeting the causal factors of AN are still  
54 lacking (10). As a consequence, current treatment efficacy remains limited since 40% of  
55 anorectic patients after 10 years of medical care still show prolonged symptoms and  
56 disabilities (11). This highlights the importance to develop new alternative therapies against  
57 AN.

58           Among the different biological factors potentially involved in this eating disorder, the  
59 gut microbiota has recently gained a lot of attention. Indeed, the intestinal microbiota, which  
60 corresponds to the community of micro-organisms living in the intestine, has recently been  
61 involved in weight regulation, fat storage and energy harvest from diet, as well as in eating  
62 behavior, anxiety and depression (12-15). As these parameters constitute key facets of AN,  
63 this has logically raised the question of the potential role of the gut microbiota in Anorexia  
64 Nervosa onset and maintenance, which constitutes a new and promising area of research (16-  
65 25). This review aims to present the current knowledge about the putative role of the gut  
66 microbiota in the pathogenesis, course and treatment of AN.

67

68 *Why gut microbiota may be a critical player during AN ?*

69

70           The intestinal microbiota is an ecosystem composed by bacteria, archaea,  
71 microeukaryotes (including fungi and protozoans) and viruses, living in symbiotic  
72 relationships within the human host. Bacteria outnumber archaea and microeukaryotes in the  
73 human microbiome (26). Recent estimations determined that the number of bacteria in the gut  
74 is of the same order as the number of human cells in the whole body (i.e.  $\sim 4.0 \times 10^{13}$  bacteria  
75 per individual (27)). The dominant bacterial phyla in the gut are Firmicutes ( $\sim 60-65\%$ ),  
76 Bacteroidetes ( $\sim 20-25\%$ ), Proteobacteria ( $\sim 5-10\%$ ) and Actinobacteria ( $\sim 3\%$ )(13). Despite the  
77 consistency of these phyla, the hundreds of bacterial species present in the gut greatly vary  
78 between individuals. In contrast to bacterial species variability, the functional gene profiles of  
79 bacteria are quite similar between individuals, suggesting the existence of shared core  
80 functions (28). The functions performed by intestinal micro-organisms include digestion and  
81 fermentation of nutrients, in particular carbohydrates and amino acids, and production of  
82 essential metabolites such as short chain fatty acids (SCFAs) and vitamin B and K. In

83 addition, the microbiota plays a key role in the development and the stimulation of the  
84 immune system, and constitutes an efficient barrier against exogenous pathogens (29).

85         The abundance and the composition of the gut microbiota is influenced by many  
86 factors, including diet (30-32). Indeed, long-term as well as short-term dietary changes can  
87 induce measurable microbial shifts (30,32). Particular cases such as severe malnutrition were  
88 reported to trigger gut microbiota imbalance (also called dysbiosis). Kwashiorkor, for  
89 example, is a severe acute form of malnutrition often observed in developing countries  
90 characterized by generalized edema, hepatic steatosis, ulcerative rashes and anorexia. This  
91 malnutrition was correlated to an altered composition of the gut microbiota, which, in turn,  
92 can influence the consequences of this malnutrition (33). Indeed, transplantation of bacterial  
93 species from children with Kwashiorkor into germ-free mice fed with a low caloric density  
94 and nutrient-deficient diet resulted in a significantly greater weight loss in recipient mice than  
95 those harboring the microbiota of healthy infants (33). Given that diet strongly influences gut  
96 microbiota composition, it is not surprising that many studies have now established that  
97 anorectic patients' intestinal microbiota differ in composition from healthy individuals (see  
98 below). Indeed, the diet of anorectic patients is strongly altered, both quantitatively and  
99 qualitatively (low in fat and calorie intakes, and high in fiber). An obvious interpretation  
100 would be that this altered diet reshapes anorectic patients gut microbiota, which may  
101 exacerbate or perpetuate AN by modulating weight loss, eating behavior, mood and intestinal  
102 physiology. Another interesting hypothesis would be that a gut dysbiosis may precede the  
103 onset of AN and triggers the start of a vicious cycle where the aberrant diet of these patients  
104 further alter the composition of the intestinal ecosystem, which may then exacerbate the  
105 clinical symptoms of this disease.

106

107

109

110           The first results suggesting that the intestinal microbiota from anorectic patients may  
111 differ from healthy individuals came from the quantification of specific bacterial and archaeal  
112 species, using quantitative PCR, in stools from anorectic patients compared to healthy  
113 individuals (34). These studies observed an increased level in the methanogen  
114 *Methanobrevibacter smithii* in the intestinal microbiota from 9 AN patients compared to 20  
115 normal-weight healthy controls (34). Interestingly, *M. smithii*, as well as other methanogens,  
116 improves microbial fermentation and the transformation of nutrients into calories by  
117 metabolizing H<sub>2</sub> excess from the gut into methane. This increase in *M. smithii* could thus  
118 reflect an adaptive response towards energy extraction optimization from the very low calory  
119 diet absorbed by anorectic patients (34). This increase in *M. smithii* could also be related to  
120 constipation, a frequent functional intestinal disorder observed in anorectic patients (see  
121 below). Indeed, increase in methane producing-bacteria has been reported in patients  
122 suffering from constipation, and more particularly in patients with a constipation-predominant  
123 Irritable Bowel Syndrome (C-IBS) (35,36). In these patients, the load of *M. smithii* is  
124 negatively correlated with the stool frequency and there is now some evidence suggesting  
125 that methane slows gastrointestinal motility down and thus may contribute to constipation  
126 (35-37).

127           Following this first evidence of gut dysbiosis occurring in anorectic patients, a study  
128 confirmed the increased levels of *M. smithii* in fecal samples from individuals with BMI<25  
129 kg.m<sup>-2</sup> compared to individuals with BMI>25 kg.m<sup>-2</sup> (38). Another analysis, using culture-  
130 based experiments, identified 11 previously undescribed bacterial species in a stool sample  
131 from a single AN patient (39). Whether these species are uniquely associated with AN was  
132 not addressed. These three first studies were rapidly completed by several large-scale analysis

133 of the microbiota composition in AN patients, that provided new insights in the dysbiosis  
134 associated with anorexia (40-46) (Fig. 2). Of note, the dysbiosis occurring in AN patients  
135 does not affect only Eubacteria and Archaea but most probably all the intestinal ecosystem of  
136 these individuals, which includes viruses and eukaryotes. Consistently, a study focusing on  
137 the gut microeukaryotes of a patient suffering from AN identified a decrease in fungi diversity  
138 and detected four species previously unreported in the human gut (47). Again, these results  
139 need to be generalized to other AN patients and completed by large-scale analysis of the  
140 diversity of viruses and eukaryotes in these patients.

141         Measurement of bacterial abundance and alpha diversity (*i.e.* bacterial species richness  
142 in one individual) of anorectic patients' microbiota are diverging between studies: some  
143 studies observed decreases while other studies showed no alteration of these parameters  
144 compared to healthy individuals (40-43,45,46). It is thus difficult to draw definitive  
145 conclusions on the modification of these parameters during AN. Regarding the  
146 Firmicutes/Bacteroidetes ratio, or the identity of bacterial taxa showing altered levels in  
147 anorectic patients, diverging results are also observed between studies (Fig. 2). No clear  
148 pattern was actually consistently observed in anorectic patients in currently published studies.  
149 There are several reasons that may explain these discrepancies such as the sample collection  
150 procedures, the quantification method of bacterial species in feces samples, the selection  
151 method of the control group, the choice of timepoint for investigation, or the level of calorie  
152 intake by patients (21). It is also important to note that the number of patients included in  
153 these cohorts is quite low (from 10 to 60 patients) compared to other studies focusing on  
154 disease-related alteration of gut microbiota (such as obesity or type 1 diabetes, where several  
155 hundreds of patients are included).

156         Thus, there is now evidence that the gut microbiota composition is altered in anorectic  
157 patients. However, the exact features of this dysbiosis remain to be unraveled. This will



158 require large and controlled studies where anorectic patients will be precisely phenotyped, in  
159 particular regarding their intestinal transit time (stool consistency) or the occurrence of  
160 functional gastrointestinal disorder or comorbidities, such as anxiety and depression (see  
161 below). This better characterization of the gut dysbiosis in anorectic patients would be  
162 instrumental to understand the exact role of the intestinal microbiota in the different features  
163 of AN.

164         Of note, most of the studies discussed here focused on bacteria recovered from faeces  
165 samples. It should be kept in mind that faeces samples only constitute proxies to characterize  
166 the gut microbiome and that the actual ecosystems present in the colon or in the small  
167 intestine may significantly diverge. It is also important to consider the importance of  
168 longitudinal studies in the case of anorexia. In particular, refeeding protocols during  
169 hospitalization of these patients may strongly affect their gut microbiota composition. Indeed,  
170 the diet of AN patients after hospitalization is quickly changed to a high-calorie diet rich in fat  
171 and carbohydrates. Although some studies have started to dissect microbiota changes before  
172 and after refeeding during anorexia, more systematic longitudinal studies would be  
173 informative to identify specific micro-organisms associated with either health recovery or  
174 relapse (22,41,42,44).

175         Finally, in addition to these studies of patients' gut microbiota, analysis of gut  
176 dysbiosis can be performed in animal models of AN. Interestingly, several changes in  
177 bacterial species levels have been observed in the Activity-based Anorexia (ABA) rat model  
178 (48) (Fig. 2). This suggests that animal models may constitute a reliable tool to study  
179 microbiota-dependent mechanisms involved in AN.

180

181 *Potential involvement of gut microbiota in anorexia nervosa pathophysiology*

182

183 • Functional gastrointestinal disorders

184

185 AN is frequently associated with functional gastrointestinal disorders, which  
186 exacerbate patient distress, decrease their quality of life and complicate disease treatment  
187 (49,50). In particular, anorectic patients frequently suffer from delayed gastric emptying,  
188 constipation or visceral hypersensitivity. These disorders challenge refeeding strategies  
189 resulting in low treatment acceptance and high dropout, increased anxiety linked to food  
190 consumption, and finally lead to prolonged nutritional and digestive impairments (49,50).

191 A link between gut microbiota and gastrointestinal disorders has been proposed in  
192 other pathological processes such as Irritable Bowel Syndrome or chronic constipation  
193 (51,52,53). Indeed, it has been shown that transplantation of fecal microbiota from patients  
194 with chronic constipation into recipient mice leads to a reduction of intestinal peristalsis, a  
195 decrease in defecation frequency and an increase in gastrointestinal transit time (51).  
196 Similarly, transplantation of fecal microbiota from patients suffering from IBS with diarrhea  
197 (IBS-D) into recipient mice is correlated with a faster gastrointestinal transit, dysfunctions of  
198 the intestinal barrier as well as anxiety-like behavior (53). Finally, hypersensitivity to colonic  
199 distention of IBS patients can be transferred to rats through their fecal microbiota (54).  
200 Disruption of the intestinal barrier and increased intestinal permeability has been involved in  
201 the pathophysiology of functional gastrointestinal disorders (55). Interestingly, the gut  
202 microbiota plays a pivotal role in the regulation of the gut barrier permeability, in particular  
203 via the regulation of tight junctions proteins expression (55). As a consequence, dysbiosis  
204 may lead to gene expression dysregulation followed by an increase in intestinal barrier  
205 permeability and a low-grade inflammation. Increased intestinal permeability and low-grade  
206 inflammation in the colon have been observed in the Activity-Based Anorexia rodent model  
207 (56,57). In AN patients, a decrease in intestinal permeability has been reported in the small

208 intestine, based on urinary recovery of lactulose (58). Whether intestinal permeability is  
209 increased in the colon of anorectic patients remains currently unknown.

210         According to these established links between intestinal micro-organisms and  
211 gastrointestinal dysfunctions, we can hypothesize that the observed gut dysbiosis in anorectic  
212 patients may contribute to the onset or maintenance of functional gastrointestinal disorders  
213 associated with AN. As already discussed above, the observed increase in *M. smithii* in the  
214 intestine of anorectic patients may for instance contribute to constipation through the  
215 production of methane by this Archaea, which slows down intestinal motility (36). The  
216 putative role of the microbiota in functional gastrointestinal disorders has recently been  
217 highlighted by a study on severely malnourished anorectic patients, which identified  
218 correlations between changes in specific bacterial genera with severity of functional  
219 gastrointestinal disorders (46). Characterization of the involvement of gut microbes in these  
220 functional intestinal disorders is essential as it would help to identify new putative therapeutic  
221 targets in order to decrease these disorders and improve quality of life and treatment  
222 efficiency of AN.

223

- 224         • Anxiety- and depression-related disorders

225

226         Psychiatric disorders such as anxiety, depression, affective disorders, substance use  
227 disorder or personality disorders are often observed in anorectic patients. For example, up to  
228 80% of individuals with AN will experience major depression at some point in their lifetime,  
229 and up to 75% will suffer from anxiety disorders (4,5). These disorders constitute risk factors  
230 for poorer long-term outcomes (4,59,60). In particular, anorectic patients with a psychiatric  
231 comorbidity have higher mortality rates than those without comorbidities (60).

232 As in the case of functional gastrointestinal disorders, several evidence are now  
233 linking gut microbes with psychiatric disorders such as anxiety or depression (14,61). Fecal  
234 content transfer from an innately stress-sensitive mouse strain to non-anxious mice, for  
235 example, is sufficient to induce anxiety-like phenotypes in recipient mice (62). Along the  
236 same line, fecal microbiota transfer from depressed patients to rats induce behavioral and  
237 psychological features characteristic of depression in recipient rodents (63). In addition,  
238 comparisons of fecal samples from patients with depression with healthy controls highlighted  
239 alterations in the levels of several bacterial genera, although no clear consensus has yet  
240 emerged from existing human studies (64).

241 Identifying whether specific intestinal micro-organisms correlate with anxiety and  
242 depression in anorectic patients is of interest as it will help understanding the role of the  
243 intestinal microbes in these comorbidities of AN. Interestingly, preliminary studies correlating  
244 anorectic patients dysbiosis and their level of anxiety and depression identified negative  
245 association between the number of observed bacterial species and the level of depression in  
246 patients, thereby strengthening the hypothesis of a role of gut microbiota in psychiatric  
247 disorders during AN (41).

248

- 249 • Regulation of eating behavior

250

251 Appetite in humans is regulated by the balance between energy intake and  
252 expenditure. A shift towards low food intake and high energy expenditure normally increases  
253 appetite. In the case of AN, patients are unable to adapt their feeding behavior to the body's  
254 energy expenditure. This raised the hypothesis that anorectic patients exhibit dysfunctions in  
255 appetite regulation and more particularly in the hypothalamic orexigenic/anorexigenic  
256 neuropeptides pathways (10,65). Of course, this dysregulation in neuropeptides controlling

257 appetite may not be the only determinant in this complex diseases and other mechanisms such  
258 as abnormally high reward process for starvation and maintenance of thinness have also been  
259 proposed for AN (10,66).

260         Interestingly, recent evidence suggests that appetite may not only be regulated by the  
261 body's energy expenditure but also by the energy needs from gut bacteria, which may be  
262 distinct from those of the host (15). Indeed, there might be conflicts between host and  
263 microbes in the gut which are fully dependent on their hosts for providing the nutrients  
264 necessary for their survival. Thus, intestinal micro-organisms may manipulate host eating  
265 behavior to promote their own fitness (67). Several mechanisms have now been characterized  
266 showing how gut microbes manipulate eating behavior of their host (15,67). These  
267 mechanisms rely on the production of a variety of molecules that may either regulate the  
268 production of neurohormones involved in mood and eating behavior or act directly as  
269 neurohormone-like molecules. For instance, the production of SCFAs, as a result of  
270 polysaccharides fermentation by intestinal bacteria, can directly act on enteroendocrine cells  
271 of the intestinal epithelium and activate the release of hormones contributing to satiation such  
272 as PYY (Peptide tyrosine tyrosine) or GLP1 (Glucagon-Like Peptide 1) (68). Bacteria may  
273 also affect eating behavior by producing compounds reaching the bloodstream and acting at  
274 distal sites. The targets of these bacteria-derived molecules are thus not only the local enteric  
275 nervous system but many other distant organs including the brain (69).

276         In contrast to other endocrine organs of the human body, the gut microbiota thus  
277 constitutes a “metabolic organ” that has the potential to produce hundreds of different  
278 “signaling” molecules. Some of these molecules can be identical to human neurotransmitters  
279 or hormones. This is the case for gamma-aminobutyric acid (GABA), produced by strains of  
280 *Lactobacillus* and *Bifidobacterium* (70), or for monoamines such as noradrenaline, dopamine  
281 or serotonin, produced by diverse strains of intestinal bacteria (69). The level of these

282 molecules in the body can thus, at least in part, be regulated by gut microbes. Other molecules  
283 produced by bacteria can mimic host hormones. This is the case for the bacterial ClpB protein  
284 (for Caseinolytic peptidase B protein homologue), that shares some homology with the alpha-  
285 melanocyte-stimulating (alpha-MSH) hormone, involved in appetite control (15,71). Whereas  
286 intragastric gavage of *E. coli* in mice reduces food intake and body weight, gavage of ClpB-  
287 deficient *E. coli* failed to modulate food consumption, suggesting an important role of this  
288 protein in host eating behavior (71). ClpB, which is produced by intestinal bacteria, can be  
289 detected in the plasma of both rodents and humans and can directly activate neurons  
290 producing pro-opiomelanocortin (POMC), a precursor of the anorexigenic alpha-MSH  
291 neuropeptide. ClpB thus constitutes an example of a bacteria-derived molecule linking the gut  
292 microbiota with hypothalamic circuits controlling host appetite (72,73).

293         Taken together, these data on the role of the gut microbiota in the regulation of eating  
294 behavior suggest that the observed dysbiosis in anorectic patients may be involved in the  
295 disbalance of food intake control and may favor the maintenance or onset of this eating  
296 disorder. This hypothesis is strengthened by the recent observation of elevated ClpB levels in  
297 a variety of eating disorders (73). Comparing the levels of bacterial metabolites between  
298 anorectic patients and healthy individuals will help to identify new putative bacteria-derived  
299 molecules involved in the aberrant eating behavior of individuals with AN.

300

### 301 *Conclusion*

302

303         Whereas several studies established that anorectic patients have a dysbiotic intestinal  
304 microbiota, the putative consequences of this dysbiosis remain hypothetical. A major  
305 challenge in the microbiota field is how to move from observational to mechanistic studies. In  
306 other words, how to move from correlations to the demonstration of the role of the microbiota

307 in a given pathology? Much efforts is now required to identify how remodeling of the gut  
308 microbiota may impact the onset or the course of anorexia. Animal models of AN may  
309 constitute interesting tools to tackle these questions (74). Transplantation of microbiota from  
310 anorectic patients to mice and measurement of intestinal physiology alterations, metabolic and  
311 behavioral changes specific to the composition of the grafted microbiota would also greatly  
312 help to identify enteric micro-organisms that have a detrimental or beneficial impact in AN.  
313 This would in particular help to identify bacterial species whose promotion or elimination  
314 would improve the efficiency of weight restoration, as well as patients' quality of life. These  
315 bacteria may in particular constitute targets to decrease functional gastrointestinal disorders  
316 and anxiety or depression disorders associated with AN. The publication of preliminary data  
317 on the clinical efficacy of fecal microbiota transfer in anorectic patients has recently  
318 confirmed the therapeutic potential of gut microbiota targeting in Anorexia Nervosa (75).  
319 Taken together, these experiments will pave the way for the development of innovative  
320 treatments, including pharmaconutrition, microbiota modulation or the use of microbiota-  
321 derived drugs, as a complement to refeeding and psychological strategies to fight this frequent  
322 and highly morbid disease.

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328

329 **AUTHOR CONTRIBUTIONS**

330

331 **Jonathan Breton:** Writing-Original draft, Review & editing. **Pierre Déchelotte :** Writing-  
332 Original draft, Review & editing. **David Ribet:** Writing-Original draft, Review & editing.

333

334 **CONFLICT OF INTEREST**

335 The authors declare that they have no conflicts of interest with the contents of this article.



336 **REFERENCES**

- 337 1. Call C, Walsh BT, Attia E. From DSM-IV to DSM-5: changes to eating disorder  
338 diagnoses. *Curr Opin Psychiatry*. 2013;26(6):532-6.
- 339 2. Galmiche M, Déchelotte P, Lambert G, Tavalacci M. Prevalence of eating disorders  
340 over the 2000-2018 period: a systematic literature review. *Am J Clin Nutr*. (in press)
- 341 3. Arcelus J, Mitchell AJ, Wales J, Nielsen S. Mortality rates in patients with anorexia  
342 nervosa and other eating disorders. A meta-analysis of 36 studies. *Arch Gen Psychiatry*.  
343 2011;68(7):724-31.
- 344 4. Kaye WH, Bulik CM, Thornton L, Barbarich N, Masters K. Comorbidity of anxiety  
345 disorders with anorexia and bulimia nervosa. *Am J Psychiatry*. 2004;161(12):2215-21.
- 346 5. Fernandez-Aranda F, Pinheiro AP, Tozzi F, Thornton LM, Fichter MM, Halmi KA, et  
347 al. Symptom profile of major depressive disorder in women with eating disorders. *Aust N Z J*  
348 *Psychiatry*. 2007;41(1):24-31.
- 349 6. Mayer L, Walsh BT, Pierson RN, Jr., Heymsfield SB, Gallagher D, Wang J, et al.  
350 Body fat redistribution after weight gain in women with anorexia nervosa. *Am J Clin Nutr*.  
351 2005;81(6):1286-91.
- 352 7. Kaplan AS, Walsh BT, Olmsted M, Attia E, Carter JC, Devlin MJ, et al. The slippery  
353 slope: prediction of successful weight maintenance in anorexia nervosa. *Psychol Med*.  
354 2009;39(6):1037-45.
- 355 8. Misra M, Golden NH, Katzman DK. State of the art systematic review of bone disease  
356 in anorexia nervosa. *Int J Eat Disord*. 2016;49(3):276-92.
- 357 9. Meehan KG, Loeb KL, Roberto CA, Attia E. Mood change during weight restoration  
358 in patients with anorexia nervosa. *Int J Eat Disord*. 2006;39(7):587-9.
- 359 10. Gorwood P, Blanchet-Collet C, Chartrel N, Duclos J, Dechelotte P, Hanachi M, et al.  
360 New Insights in Anorexia Nervosa. *Front Neurosci*. 2016;10:256.
- 361 11. Rigaud D, Pennacchio H, Bizeul C, Reveillard V, Verges B. Outcome in AN adult  
362 patients: a 13-year follow-up in 484 patients. *Diabetes Metab*. 2011;37(4):305-11.
- 363 12. Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF. The microbiota-gut-brain axis  
364 in obesity. *Lancet Gastroenterol Hepatol*. 2017;2(10):747-56.
- 365 13. Rosenbaum M, Knight R, Leibel RL. The gut microbiota in human energy  
366 homeostasis and obesity. *Trends Endocrinol Metab*. 2015;26(9):493-501.
- 367 14. Foster JA, McVey Neufeld KA. Gut-brain axis: how the microbiome influences  
368 anxiety and depression. *Trends Neurosci*. 2013;36(5):305-12.

- 369 15. Fetissov SO. Role of the gut microbiota in host appetite control: bacterial growth to  
370 animal feeding behaviour. *Nat Rev Endocrinol.* 2017;13(1):11-25.
- 371 16. Kleiman SC, Carroll IM, Tarantino LM, Bulik CM. Gut feelings: A role for the  
372 intestinal microbiota in anorexia nervosa? *Int J Eat Disord.* 2015;48(5):449-51.
- 373 17. Carr J, Kleiman SC, Bulik CM, Bulik-Sullivan EC, Carroll IM. Can attention to the  
374 intestinal microbiota improve understanding and treatment of anorexia nervosa? *Expert Rev*  
375 *Gastroenterol Hepatol.* 2016;10(5):565-9.
- 376 18. Karakula-Juchnowicz H, Pankowicz H, Juchnowicz D, Valverde Piedra JL, Malecka-  
377 Massalska T. Intestinal microbiota - a key to understanding the pathophysiology of anorexia  
378 nervosa? *Psychiatr Pol.* 2017;51(5):859-70.
- 379 19. Herpertz-Dahlmann B, Seitz J, Baines J. Food matters: how the microbiome and gut-  
380 brain interaction might impact the development and course of anorexia nervosa. *Eur Child*  
381 *Adolesc Psychiatry.* 2017;26(9):1031-41.
- 382 20. Aurigemma NC, Koltun KJ, VanEvery H, Rogers CJ, De Souza MJ. Linking the Gut  
383 Microbiota to Bone Health in Anorexia Nervosa. *Curr Osteoporos Rep.* 2018;16(1):65-75.
- 384 21. Schwensen HF, Kan C, Treasure J, Hoiby N, Sjogren M. A systematic review of  
385 studies on the faecal microbiota in anorexia nervosa: future research may need to include  
386 microbiota from the small intestine. *Eat Weight Disord.* 2018;23(4):399-418.
- 387 22. Ruusunen A, Rocks T, Jacka F, Loughman A. The gut microbiome in anorexia  
388 nervosa: relevance for nutritional rehabilitation. *Psychopharmacology (Berl).* 2019.
- 389 23. Seitz J, Trinh S, Herpertz-Dahlmann B. The Microbiome and Eating Disorders.  
390 *Psychiatr Clin North Am.* 2019;42(1):93-103.
- 391 24. Mendez-Figueroa V, Biscaia JM, Mohedano RB, Blanco-Fernandez A, Bailen M,  
392 Bressa C, et al. Can Gut Microbiota and Lifestyle Help Us in the Handling of Anorexia  
393 Nervosa Patients? *Microorganisms.* 2019;7(2).
- 394 25. Roubalova R, Prochazkova P, Papezova H, Smitka K, Bilej M, Tlaskalova-Hogenova  
395 H. Anorexia nervosa: Gut microbiota-immune-brain interactions. *Clin Nutr.* 2019.
- 396 26. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut  
397 microbial gene catalogue established by metagenomic sequencing. *Nature.*  
398 2010;464(7285):59-65.
- 399 27. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria  
400 Cells in the Body. *PLoS Biol.* 2016;14(8):e1002533.
- 401 28. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability  
402 and resilience of the human gut microbiota. *Nature.* 2012;489(7415):220-30.

- 403 29. Libertucci J, Young VB. The role of the microbiota in infectious diseases. Nat  
404 Microbiol. 2019;4(1):35-45.
- 405 30. Dore J, Blottiere H. The influence of diet on the gut microbiota and its consequences  
406 for health. Curr Opin Biotechnol. 2015;32:195-9.
- 407 31. Graf D, Di Cagno R, Fak F, Flint HJ, Nyman M, Saarela M, et al. Contribution of diet  
408 to the composition of the human gut microbiota. Microb Ecol Health Dis. 2015;26:26164.
- 409 32. Zmora N, Suez J, Elinav E. You are what you eat: diet, health and the gut microbiota.  
410 Nat Rev Gastroenterol Hepatol. 2018.
- 411 33. Smith MI, Yatsunenkov T, Manary MJ, Trehan I, Mkakosya R, Cheng J, et al. Gut  
412 microbiomes of Malawian twin pairs discordant for kwashiorkor. Science.  
413 2013;339(6119):548-54.
- 414 34. Armougom F, Henry M, Vialettes B, Raccach D, Raoult D. Monitoring bacterial  
415 community of human gut microbiota reveals an increase in *Lactobacillus* in obese patients  
416 and Methanogens in anorexic patients. PLoS One. 2009;4(9):e7125.
- 417 35. Fiedorek SC, Pumphrey CL, Casteel HB. Breath methane production in children with  
418 constipation and encopresis. J Pediatr Gastroenterol Nutr. 1990;10(4):473-7.
- 419 36. Ghoshal U, Shukla R, Srivastava D, Ghoshal UC. Irritable Bowel Syndrome,  
420 Particularly the Constipation-Predominant Form, Involves an Increase in *Methanobrevibacter*  
421 *smithii*, Which Is Associated with Higher Methane Production. Gut Liver. 2016;10(6):932-8.
- 422 37. Gottlieb K, Wachter V, Sliman J, Pimentel M. Review article: inhibition of  
423 methanogenic archaea by statins as a targeted management strategy for constipation and  
424 related disorders. Aliment Pharmacol Ther. 2016;43(2):197-212.
- 425 38. Million M, Angelakis E, Maraninchi M, Henry M, Giorgi R, Valero R, et al.  
426 Correlation between body mass index and gut concentrations of *Lactobacillus reuteri*,  
427 *Bifidobacterium animalis*, *Methanobrevibacter smithii* and *Escherichia coli*. Int J Obes  
428 (Lond). 2013;37(11):1460-6.
- 429 39. Pfliederer A, Lagier JC, Armougom F, Robert C, Vialettes B, Raoult D. Culturomics  
430 identified 11 new bacterial species from a single anorexia nervosa stool sample. Eur J Clin  
431 Microbiol Infect Dis. 2013;32(11):1471-81.
- 432 40. Morita C, Tsuji H, Hata T, Gondo M, Takakura S, Kawai K, et al. Gut Dysbiosis in  
433 Patients with Anorexia Nervosa. PLoS One. 2015;10(12):e0145274.
- 434 41. Kleiman SC, Watson HJ, Bulik-Sullivan EC, Huh EY, Tarantino LM, Bulik CM, et al.  
435 The Intestinal Microbiota in Acute Anorexia Nervosa and During Renourishment:

436 Relationship to Depression, Anxiety, and Eating Disorder Psychopathology. *Psychosom Med.*  
437 2015;77(9):969-81.

438 42. Mack I, Cuntz U, Gramer C, Niedermaier S, Pohl C, Schwiertz A, et al. Weight gain  
439 in anorexia nervosa does not ameliorate the faecal microbiota, branched chain fatty acid  
440 profiles, and gastrointestinal complaints. *Sci Rep.* 2016;6:26752.

441 43. Borgo F, Riva A, Benetti A, Casiraghi MC, Bertelli S, Garbossa S, et al. Microbiota in  
442 anorexia nervosa: The triangle between bacterial species, metabolites and psychological tests.  
443 *PLoS One.* 2017;12(6):e0179739.

444 44. Kleiman SC, Glenny EM, Bulik-Sullivan EC, Huh EY, Tsilimigras MCB, Fodor AA,  
445 et al. Daily Changes in Composition and Diversity of the Intestinal Microbiota in Patients  
446 with Anorexia Nervosa: A Series of Three Cases. *Eur Eat Disord Rev.* 2017;25(5):423-7.

447 45. Morkl S, Lackner S, Muller W, Gorkiewicz G, Kashofer K, Oberascher A, et al. Gut  
448 microbiota and body composition in anorexia nervosa inpatients in comparison to athletes,  
449 overweight, obese, and normal weight controls. *Int J Eat Disord.* 2017;50(12):1421-31.

450 46. Hanachi M, Manichanh C, Schoenenberger A, Pascal V, Levenez F, Cournede N, et al.  
451 Altered host-gut microbes symbiosis in severely malnourished anorexia nervosa (AN) patients  
452 undergoing enteral nutrition: An explicative factor of functional intestinal disorders? *Clin*  
453 *Nutr.* 2018.

454 47. Gouba N, Raoult D, Drancourt M. Gut microeukaryotes during anorexia nervosa: a  
455 case report. *BMC Res Notes.* 2014;7:33.

456 48. Queipo-Ortuno MI, Seoane LM, Murri M, Pardo M, Gomez-Zumaquero JM, Cardona  
457 F, et al. Gut microbiota composition in male rat models under different nutritional status and  
458 physical activity and its association with serum leptin and ghrelin levels. *PLoS One.*  
459 2013;8(5):e65465.

460 49. Sato Y, Fukudo S. Gastrointestinal symptoms and disorders in patients with eating  
461 disorders. *Clin J Gastroenterol.* 2015;8(5):255-63.

462 50. Hetterich L, Mack I, Giel KE, Zipfel S, Stengel A. An update on gastrointestinal  
463 disturbances in eating disorders. *Mol Cell Endocrinol.* 2018.

464 51. Cao H, Liu X, An Y, Zhou G, Liu Y, Xu M, et al. Dysbiosis contributes to chronic  
465 constipation development via regulation of serotonin transporter in the intestine. *Sci Rep.*  
466 2017;7(1):10322.

467 52. Tap J, Derrien M, Tornblom H, Brazeilles R, Cools-Portier S, Dore J, et al.  
468 Identification of an Intestinal Microbiota Signature Associated With Severity of Irritable  
469 Bowel Syndrome. *Gastroenterology.* 2017;152(1):111-23 e8.

- 470 53. De Palma G, Lynch MD, Lu J, Dang VT, Deng Y, Jury J, et al. Transplantation of  
471 fecal microbiota from patients with irritable bowel syndrome alters gut function and behavior  
472 in recipient mice. *Sci Transl Med.* 2017;9(379).
- 473 54. Crouzet L, Gaultier E, Del'Homme C, Cartier C, Delmas E, Dapoigny M, et al. The  
474 hypersensitivity to colonic distension of IBS patients can be transferred to rats through their  
475 fecal microbiota. *Neurogastroenterol Motil.* 2013;25(4):e272-82.
- 476 55. Shin A, Preidis GA, Shulman R, Kashyap P. The Gut Microbiome in Adult and  
477 Pediatric Functional Gastrointestinal Disorders. *Clin Gastroenterol Hepatol.* 2019;17(2):256-  
478 274.
- 479 56. Jesus P, Ouelaa W, Francois M, Riachy L, Guerin C, Aziz M, et al. Alteration of  
480 intestinal barrier function during activity-based anorexia in mice. *Clin Nutr.* 2014;33(6):1046-  
481 53.
- 482 57. Belmonte L, Achamrah N, Nobis S, Guerin C, Riou G, Bole-Feysot C, et al. A role for  
483 intestinal TLR4-driven inflammatory response during activity-based anorexia. *Sci Rep.*  
484 2016;6:35813.
- 485 58. Monteleone P, Carratu R, Carteni M, Generoso M, Lamberti M, Magistris LD, et al.  
486 Intestinal permeability is decreased in anorexia nervosa. *Mol Psychiatry.* 2004;9(1):76-80.
- 487 59. Calugi S, El Ghoch M, Conti M, Dalle Grave R. Depression and treatment outcome in  
488 anorexia nervosa. *Psychiatry Res.* 2014;218(1-2):195-200.
- 489 60. Kask J, Ekselius L, Brandt L, Kollia N, Ekblom A, Papadopoulos FC. Mortality in  
490 Women With Anorexia Nervosa: The Role of Comorbid Psychiatric Disorders. *Psychosom*  
491 *Med.* 2016;78(8):910-9.
- 492 61. Sarkar A, Harty S, Lehto SM, Moeller AH, Dinan TG, Dunbar RIM, et al. The  
493 Microbiome in Psychology and Cognitive Neuroscience. *Trends Cogn Sci.* 2018;22(7):611-  
494 36.
- 495 62. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The intestinal microbiota  
496 affect central levels of brain-derived neurotropic factor and behavior in mice.  
497 *Gastroenterology.* 2011;141(2):599-609, e1-3.
- 498 63. Kelly JR, Borre Y, C OB, Patterson E, El Aidy S, Deane J, et al. Transferring the  
499 blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. *J*  
500 *Psychiatr Res.* 2016;82:109-18.
- 501 64. Cheung SG, Goldenthal AR, Uhlemann AC, Mann JJ, Miller JM, Sublette ME.  
502 Systematic Review of Gut Microbiota and Major Depression. *Front Psychiatry.* 2019;10:34.

- 503 65. Kaye WH, Fudge JL, Paulus M. New insights into symptoms and neurocircuit  
504 function of anorexia nervosa. *Nat Rev Neurosci.* 2009;10(8):573-84.
- 505 66. Kaye WH, Wierenga CE, Bailer UF, Simmons AN, Bischoff-Grethe A. Nothing tastes  
506 as good as skinny feels: the neurobiology of anorexia nervosa. *Trends Neurosci.*  
507 2013;36(2):110-20.
- 508 67. Alcock J, Maley CC, Aktipis CA. Is eating behavior manipulated by the  
509 gastrointestinal microbiota? Evolutionary pressures and potential mechanisms. *Bioessays.*  
510 2014;36(10):940-9.
- 511 68. Chambers ES, Morrison DJ, Frost G. Control of appetite and energy intake by SCFA:  
512 what are the potential underlying mechanisms? *Proc Nutr Soc.* 2015;74(3):328-36.
- 513 69. Clarke G, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG. Minireview: Gut  
514 microbiota: the neglected endocrine organ. *Mol Endocrinol.* 2014;28(8):1221-38.
- 515 70. Barrett E, Ross RP, O'Toole PW, Fitzgerald GF, Stanton C. gamma-Aminobutyric  
516 acid production by culturable bacteria from the human intestine. *J Appl Microbiol.*  
517 2012;113(2):411-7.
- 518 71. Tennoune N, Chan P, Breton J, Legrand R, Chabane YN, Akkermann K, et al.  
519 Bacterial ClpB heat-shock protein, an antigen-mimetic of the anorexigenic peptide alpha-  
520 MSH, at the origin of eating disorders. *Transl Psychiatry.* 2014;4:e458.
- 521 72. Breton J, Tennoune N, Lucas N, Francois M, Legrand R, Jacquemot J, et al. Gut  
522 Commensal *E. coli* Proteins Activate Host Satiety Pathways following Nutrient-Induced  
523 Bacterial Growth. *Cell Metab.* 2016;23(2):324-34.
- 524 73. Breton J, Legrand R, Akkermann K, Jarv A, Harro J, Dechelotte P, et al. Elevated  
525 plasma concentrations of bacterial ClpB protein in patients with eating disorders. *Int J Eat*  
526 *Disord.* 2016;49(8):805-8.
- 527 74. Mequinion M, Chauveau C, Viltart O. The use of animal models to decipher  
528 physiological and neurobiological alterations of anorexia nervosa patients. *Front Endocrinol*  
529 *(Lausanne).* 2015;6:68.
- 530 75. de Clercq NC, Frissen MN, Davids M, Groen AK, Nieuwdorp M. Weight Gain after  
531 Fecal Microbiota Transplantation in a Patient with Recurrent Underweight following Clinical  
532 Recovery from Anorexia Nervosa. *Psychother Psychosom.* 2019;88(1):58-60.
- 533 76. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal Database  
534 Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res.*  
535 2014;42(Database issue):D633-42.

536 **FIGURE LEGENDS**

537

538 **Figure 1 : Putative role of the gut microbiota in the onset and maintenance of Anorexia**  
539 **nervosa**

540 Dramatic changes in diet may profoundly impact the composition of the gut microbiota. The  
541 resulting dysbiosis may participate to the onset and/or maintenance of disorders frequently  
542 associated with Anorexia nervosa such as functional gastrointestinal disorders, anxiety- and  
543 depression-related disorders, as well as appetite dysregulation.

544

545 **Figure 2 : Alterations in bacteria levels observed in individuals with Anorexia nervosa**

546 List of bacteria with significant increased (blue) or decreased (yellow) levels in patients with  
547 AN compared to normal weight individuals (NW) (summary from eight independent studies).  
548 Alterations in bacteria levels in a rat model of anorexia (Activity-Based Anorexia model,  
549 ABA) are shown in parallel. The phylogeny of bacteria was obtained from the Ribosomal  
550 Database Project (76).