Impact of dietary fiber and fat on gut microbiota re-modeling and metabolic health

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Abstract

Background. Scientific evidence suggests that diet plays a role in obesity and its comorbidities, partly via its interactions with the individual's gut microbiota. Likewise, the individual's microbiota influences the efficacy of dietary interventions to reduce body weight. However, we require a better understanding of the key components of the gut microbiota that are responsive to specific diets and of their effects on energy balance in order to use this information in practice.

Scope and Approach. This review provides an up-to-date description of the influence of dietary fibers and fat on gut microbiota and the mechanisms presumably mediating their effects on metabolic health. We also discuss the main knowledge gaps and the need to gain greater understanding of the role of diet-microbe interactions in obesity and the associated comorbidities.

Key Findings and Conclusions. Dietary fibers are major drivers of gut microbiota composition and function, stimulating the dominance of bacteria able to utilize these substrates as energy source, although effects vary depending on both the type of fiber and the individual's microbiota. However, the key bacteria and the primary and secondary metabolic pathways mediating specific fiber-induced effects on the metabolic phenotype remain unclear, and this information is necessary to personalize fiber-based interventions. The literature also shows that gut microbiota contributes to the adverse consequences of high-fat diets on the metabolic phenotype; however, little is known about the effects of dietary fat type. Further progress is expected from translational approaches integrating controlled dietary intervention human trials, combining functional omics technologies and physiological/clinical endpoints, and mechanistic studies in experimental models. This will

ultimately help us to progress towards establishing informed microbiome-based dietary recommendations and interventions, which can contribute to tackling the obesity epidemic and its comorbidities.

Key words: Gut microbiota, microbiome, fiber, fat, diet-related diseases, obesity.

Introduction

Obesity has reached pandemic dimensions affecting a vast number of people worldwide. In 2014, approximately 39% of adults (1.9 billion) were overweight and 13% of these (600 million) were obese. Moreover, 42 million children under the age of 5 were reported as overweight or obese in 2013(World Health Organization, 2015). It is well known that obesity is not only associated with populations in high-income countries, but the prevalence is continuously growing in low- and mid-income countries, particularly in urban settings (World Health Organization, 2015). Obesity is a result of an unbalance between energy intake and expenditure, to which over-nutrition and a sedentary lifestyle are major contributors (Coppinger, Jeanes, Dabinett, Vogele, & Reeves, 2010). Obesity is associated with a state of chronic low-grade inflammation, which partly explains the insulin resistance phenotype observed in many obese individuals. In turn, insulin resistance is a component of the metabolic syndrome that often precedes the development of type 2 diabetes (T2D) and cardiovascular disease (CVD) (Jia, DeMarco, & Sowers, 2016). This metabolic inflammation is characterized by infiltration of macrophages and lymphocytes in peripheral tissues. This is accompanied by an increased production of pro-inflammatory cytokines, adipokines, acute-phase proteins and other immune mediators as a consequence of the activation of several signalling pathways, including the nuclear factor kappa B (NFκB)/Inhibitor of theκ kinase (IKK), c-jun N-terminal kinase (JNK), protein kinase R (PKR) and theToll-Like receptors (TLRs) (Gregor & Hotamisligil, 2011). Adipose tissue from obese individuals is considered to be the main contributor to obesity-related metabolic inflammation, with the highest accumulation of infiltrating macrophages and tissue

concentrations of cytokines, with similar events occurring in the liver and central nervous system, contributing to systemic insulin resistance (Johnson & Olefsky, 2013).

In the last decade, an increasing number of studies have reported that obesity is associated with alterations in gut microbiota structure, suggesting that specific microbial taxa could be contributing factors to the obesity epidemic, although results are not fully consistent across human observational studies (Sanz, Rastmanesh, & Agostoni, 2013). Animal studies have provided information about the mechanisms by which gut microbiota could play a role in obesity, including contribution to nutrient digestion and absorption and to regulation of immune and neuro-endocrine functions (Moya-Perez, Neef, & Sanz, 2015). Experimental models have also demonstrated that gut microbiota can transmit the obesity-associated metabolic phenotype of its original human host when transferred to a germ-free recipient, providing a first evidence of causality (Turnbaugh, et al., 2006). Furthermore, a unique fecal transplantation study in humans has also demonstrated that the transference of feces from a lean donor into subjects with metabolic syndrome beneficially influence glucose metabolism, confirming the causal role of gut microbiota (Vrieze, et al., 2010). Nonetheless, the role of gut microbiota in obesity seems largely dependent on diet-microbe interactions due to the fact that diet is a major modifiable factor influencing gut microbiota composition and function (De Filippis, et al., 2015; Flint, Duncan, Scott, & Louis, 2015). Indeed, experimental models revealed that such interactions contribute to obesity, for example, by increasing lipid absorption or aggravating adipose tissue inflammation independently of adiposity in the context of diets rich in saturated lipids (Caesar, Tremaroli, Kovatcheva-Datchary, Cani, & Backhed, 2015; Semova, et al., 2012). Furthermore, dietary reprograming of microbiota ameliorates development of metabolic dysfunction despite susceptible genotypes (Ussar, et al., 2015). Nevertheless, our understanding of how diet-

microbe interactions influence energy balance, eating behavior and obesity in humans is still insufficient to transform this information into practical solutions to tackle obesity-associated disorders.

This review discusses the most recent data regarding the potential role of dietary fiber and fat in remodeling gut microbiota composition and function and, thereby, in programming metabolic health. It also addresses the main limitations that must be overcome to progress our understanding of the microbiome's role in the chain of events causing obesity. Only on gaining a better understanding of the above, will we be able to speed up the translation of this information into informed microbiome-based dietary interventions and recommendations.

1. Impact of dietary fiber on human physiology

1.1. Dietary fiber: role in metabolic health and as main fuel for gut microbiota.

Dietary fiber is generally defined as non-digestible carbohydrates plus lignin, which include structurally different components including non-starch polysaccharides, resistant oligosaccharides (e.g. fructo-oligosaccharides [FOS], galacto-oligosaccharides [GOS]) and resistant starch (EFSA NDA Panel, 2010). Prebiotics are defined as dietary fibers that modify the composition and/or metabolic activity of gut microbiota, thereby conferring a benefit to the host (G. R. Gibson, 2004; G. R. Gibson, Probert, Loo, Rastall, & Roberfroid, 2004). According to this definition, a wide variety of food ingredients can be classified as prebiotics such as GOS, FOS and longer inulin-derived fructans, xylo-oligosaccharides (XOS) and arabinoxylan oligosaccharides (AXOS); however this is based mainly on their impact on gut microbiota rather than on robust evidence of their effects on health-related

endpoints (Hutkins, et al., 2016). Dietary fiber is not digested by human enzymes and thus it reaches proximal colonic regions, where it constitutes the main energy source for obligate anaerobic bacteria, whose fermentative activity leads to the generation of organic acids (lactic, succinic acid) and short-chain fatty acids (SCFA) (acetate, propionate and butyrate). Consequently, the quantity and quality of fiber is considered to be one of the main dietary determinants of gut microbiota composition and function (Scott, Gratz, Sheridan, Flint, & Duncan, 2013). The current recommendations on dietary fiber intake (25 g per day for adults) are based on their well-known role in regulating bowel habits (frequency of defecation), including native chicory inulin considered to be prebiotic (Hutkins, et al., 2016). In addition, there is evidence for a role of dietary fiber and some prebiotics (inulin and oligofructose) in the reduction of dietary glycemic responses and glycemic load, with favorable effects on metabolic risk factors. Furthermore, consumption of fiber-rich diets with fiber intake above recommendations is associated with a reduced risk of coronary heart disease and type 2 diabetes as well as improved weight maintenance (Bes-Rastrollo, Martinez-Gonzalez, Sanchez-Villegas, de la Fuente Arrillaga, & Martinez, 2006; EFSA NDA Panel, 2010; S. Liu, et al., 2000; Ludwig, et al., 1999; Ye, Chacko, Chou, Kugizaki, & Liu, 2012). Dietary fiber is thought to positively influence metabolic health through multiple mechanisms, although effects cannot be generalized as they vary depending on the type of fiber. The mechanisms of action include direct effects related to its physicochemical and structural properties (e.g. indigestibility, viscosity, etc.) and indirect effects mediated by the individual's gut microbiota. For example, compared to digestible carbohydrates, insoluble and soluble fibers reach distal portion of colon with no major degradation by human enzymes leading to a significant reduction in postprandial glycemic responses due to their slower digestion (EFSA, 2014). Consequently, consumption of fiber improves the

142 glucose metabolism as a whole, which have direct impact on satiety and tip the balance
143 towards oxidation instead storage metabolism (reviewed in (Koh-Banerjee & Rimm,
144 2003)). Moreover, dietary fiber is considered to be very useful for weight loss/maintenance
145 aims given its low energetics estimated to be ~1.91 kcal/g (8 kJ/g) in comparison with other
146 macronutrients as digestible carbohydrates, (~4.06 kcal/g), proteins (~4.06 kcal/g), and fat
147 (~8.84 kcal/g) (Menezes, et al., 2016). Soluble viscous fibers may also exert beneficial
148 metabolic effects by their ability to form gels that delay gastric emptying, inhibit nutrient
149 absorption and bile acid (BA) binding; altogether this may contribute to a decreased
150 postprandial glycemic response and a reduction in body cholesterol stores due to increased
151 synthesis of new BAs from cholesterol in the liver (Dikeman & Fahey, 2006). In addition,
152 dietary fiber is thought to mediate other effects (e.g. satiety and anti-inflammatory effects)
153 through activation of the fermentative activity of gut bacteria, and the generation of
154 potentially beneficial metabolites (e.g. SCFAs), as explained in greater detail in section 3.
155
156 *1.2. Evidence of the influence of dietary fiber on gut microbiota from observational studies.*
157 The role of non-digestible carbohydrates in the gut microbiota is well exemplified by the
158 differences in the infant's gut microbiota between breast-fed and formula-fed infants and
159 between infant formula supplemented or not with oligosaccharides, which mainly stimulate
160 the growth of bifidobacteria (Closa-Monasterolo, et al., 2013; Hascoet, et al., 2011). These
161 effects have also been well-established by comparing the gut microbiota of individuals
162 from different geographical regions that consume rural diets (Africa and South America)
163 rich in dietary fiber or Western diets (Europe and North America) rich in animal protein
164 and fat(De Filippo, et al., 2010; Yatsunenko, et al., 2012). Acomparison of the microbiota
165 between European and African children, consuming a fiber-rich diet, showed that the latter

have reduced abundance of Firmicutes and increased abundance of Bacteroidetes, particularly the *Prevotella* and *Xylanibacter* genera, known to have genes specialized in cellulose and xylan utilization, with parallel increased fecal concentrations of SCFAs. In contrast, Enterobacteriaceae species (Proteobacteria) were reduced in African compared with European children (De Filippo, et al., 2010). Another large study including healthy children and adults also revealed important differences in bacterial communities and functional gene repertoires between US subjects from metropolitan areas and those from countries with a rural lifestyle (Amazonas of Venezuela and Malawi), finding the genus *Prevotella* to be abundant in humans with a diet rich in corn and cassava and in US children not following a full western diet (Yatsunenko, et al., 2012). A more recent study comparing African Americans and rural South Africans, found that animal protein and fat intake was 2-3 times higher in Americans whereas carbohydrate and fiber (mainly resistant starch) intake was higher in Africans. The same authors also reported diet-associated microbiota and metabolite changes that were related to colon cancer risk. While the American microbiota was dominated by *Bacteroides*, the African microbiota was dominated by *Prevotella* and higher levels of starch degraders, carbohydrate fermenters, and butyrate producers. Moreover, the American microbiota had higher levels of potentially pathogenic Proteobacteria (*Escherichia* and *Acinetobacter*) and BA deconjugators (Ou, et al., 2013). A recent Dutch population-based metagenomic study involving 1,135 subjects has associated higher diversity, functional microbiome richness and abundance of Bacteroidetes with higher intake of fruits and vegetables (source of dietary fiber), higher concentrations of high-density lipoprotein (HDL) and lower concentrations of fecal chromogranin A (Zhernakova, et al., 2016). The total amount of carbohydrates in the diet was also positively associated with *Bifidobacterium* but negatively associated with *Lactobacillus* and

190 microbiome diversity (Zhernakova, et al., 2016). All in all, these observational studies
191 reveal that long-term consumption of fiber-rich diets promotes the dominance of fiber-
192 degraders of the phylum Bacteroidetes and Actinobacteria (*Bifidobacterium*spp.) and, more
193 consistently, of *Prevotella* spp. and reductions in Proteobacteria; nevertheless, *Bacteroides*
194 spp. seem to be adapted to both fiber-rich diets and diets rich in animal protein and fat,
195 probably due to their versatile metabolic capabilities. Notwithstanding, these observational
196 data only provide associations but not causal relationships between specific dietary habits
197 and the predominance of specific bacterial taxa, which limits their value in practice.
198 Furthermore, other relevant environmental factors such as hygiene, geography, and
199 ethnicity that could be involved in the respective gut microbiota profile observed are not
200 well assessed.
201 A recent experimental study in animal models also suggests that the lack of dietary fiber
202 leads to a substantial loss in gut microbiota diversity, which influences the ability of gut
203 bacteria to be transferred from parents to offspring. It also revealed that simply restoring
204 fiber consumption was not enough to reverse these effects since some bacterial groups
205 failed to return to their previous levels (Sonnenburg, et al., 2016). These results have led to
206 hypothesize that long-term dietary changes in industrialized countries could have altered
207 the host-microbiota partnership and microbiome functionality, with an adverse long-term
208 impact on health that could be transmitted from generation to generation (Sonnenburg, et
209 al., 2016). Notwithstanding, evidence from systematic studies in humans is required to
210 confirm this hypothesis.
211
212 *1.3. Evidence for the influence of dietary fiber on gut microbiota from intervention studies.*

A summary is given in Table 1 of recent representative human dietary interventions investigating how most common types of dietary fibers contribute to remodeling the gut microbiota. The responsiveness and effects of dietary fibers may differ depending on the individual's gut microbiota profile (Korpela, et al., 2014), suggesting the need to work towards defining more specific and personalized dietary interventions and recommendations.

1.3.1. Effects of wholegrain (WG)-rich foods. Wholegrain cereals are composed of starch-rich endosperm, germ, and bran with high plant-fiber content. During harvesting and food processing, these components must preserve their relative proportions as in the intact kernel (HEALTHGRAIN Consortium - http://www.healthgrain.org). Rice, wheat, maize, oats, and barley are the main whole grains consumed worldwide and some of them have been proven to reduce the risk of certain diet-related diseases such as obesity and CVD. A controlled cross-over study showeda bifidogenic effectupon consumption of 48 g/day maize-based WG breakfast cereals during 21 days (Carvalho-Wells, et al., 2010). This effect was observed exclusively for the intervention period and not sustained after completion of the WG diet, strongly indicating that WG fiber is predominantly used by *Bifidobacterium* spp. (Carvalho-Wells, et al., 2010). Similar results were obtained by Costabile and coworkers who reported increased bifidobacteria and lactobacilli in feces after daily consumption of WG wheat breakfast cereals (48 g/day) in comparison with non-WG cereal (Costabile, et al., 2008). More recent results have shown that a four-week dietary intervention with 60 g/day WG barley flakes in healthy adults induced a significant increase in the genus *Blautia* and a less pronounced increase in the abundance of the genera *Roseburia, Bifidobacterium* and *Dialister* (Martinez, et al., 2013). Additionally, this study showed that WG barley,

237 brown rice and specially the combination of WG barley and brown rice reduced plasma
238 interleukin-6 (IL-6) and postprandial glucose. Interestingly, *Eubacterium rectale* was
239 significantly more abundant in volunteers showing improvements in postprandial blood
240 glucose and insulin response, whereas abundance of *Dialister* species was associated with
241 the highest improvements in IL-6 levels (Martinez, et al., 2013).
242
243 *1.3.2. Resistant starch (RS).* Starch is the major component of the plant-derived foods and
244 comprises an important part of the human diet. The starch is referred as resistant when it
245 cannot be hydrolyzed by digestive enzymes of the human GIT. The RS can be classified
246 into several types (RS1 to RS5) according to the physical or chemical reasons to be
247 indigestible. The RS1 is contained inside whole grains and is physically inaccessible for
248 digestion; the RS2 is also native starch but remains indigestible by its compact structure;
249 the RS3, also known as retrograde starch, is obtained by slow re-crystallization prior to heat
250 disruption on water; the RS4 is the chemically modified starch by cross-linking or
251 esterification; and the RS5 is a mixture of starch with lipids with high stability (Ma &
252 Boye, 2016). Early studies about the RS impact on gut microbiota indicated that
253 administration of controlled diet including 22 g/day RS induces changes in gut microbiota
254 mainly in the clostridia cluster including members of the *Ruminococcus* genus (Abell,
255 Cooke, Bennett, Conlon, & McOrist, 2008). Interventions with 50-60 g/day RS3 increased
256 the abundance of several *Ruminococcus* spp. and especially *Ruminococcus bromii* and
257 *Eubacterium rectale* (Walker, et al., 2011). Similar results were obtained when 33 g/day
258 RS2 or RS4 were administrated in baked crakers to volunteers during 3 weeks. In this case,
259 increased proportions of *Bifidobacterium adolescentis* and *Parabacteroides distasonis* were
260 found to be induced particularly by RS4 intake, whereas increased proportions of

Ruminococcus bromii and *Eubacterium rectale* were induced by RS2 consumption (Martinez, Kim, Duffy, Schlegel, & Walter, 2010). In addition, RS intake of has been found to improve lipid metabolism in individuals with metabolic syndrome and help to control waist circumference and fat mass in non metabolic syndrome individuals (Nichenametla, et al., 2014). These beneficial effects of RS on metabolic aspects are thought to be at least partially mediated by the microbiota induced changes but direct evidence still has to be provided.

1.3.3. Inulin and FOS. Inulin and FOS, also called oligofructose or oligofructans, are types of fructo-polysaccharides that consist of several β-linked D-fructosyl residues with a D-glucose group at end of the extended saccharide chain. These differ in the polymerization degree, which may range from 2 to 60 fructose units. FOS are usually produced by degradation of inulin obtained primarily from artichoke and chicory plants. These are used in the food industry as sweeteners, texture modifiers and fibers. A number of intervention studies have shown that the effects of inulin and FOS on gut microbiota composition can be associated with modifications on health related outcomes or subrogated biomarkers (Table 1). In adults and infants, it is generally reported that inulin and FOS intake increases the number of bifidobacteria, sometimes associated with changes in metabolic products (e.g. lactate) (Closa-Monasterolo, et al., 2013; Garcia-Peris, et al., 2012; Petry, Egli, Chassard, Lacroix, & Hurrell, 2012). In some studies, inulin or FOS-induced microbiota changes have also been correlated with indicators of metabolic health. For example, a three-month double-blind placebo-controlled intervention with a mixture ofinulin/oligofructose or maltodextrin (8 g twice daily in powder to be dissolved in warm drinks) in obese women, showed increased abundances of *Bifidobacterium* spp. and *Faecalibacterium prausnitzii,*

284 which correlated to reduced serum LPS (lipopolysacchraide) levels. Additionally, the
285 researchers observed reductions of *Bacteroides intestinalis*, *Bacteroides vulgatus* and
286 *Propionibacterium* spp., which correlated to modest changes in fat mass. Additionally, they
287 found reductions in plasma LPS, fecal acetate and propionate concentrations, and fasting
288 insulinemia (Dewulf, et al., 2013; Salazar, et al., 2015). A recent study has evaluated the
289 role of agave inulin showing a dose-dependent bifidogenic effect. The consumption of 5 or
290 7.5 g/day agave inulin in chocolate chews, primarily promoted the presence of *B.*
291 *adolescentis, B. breve, B. longum*, and *B. pseudolongum* (Holscher, et al., 2015). Positive
292 correlations were also detected between fecal butyrate concentrations and the dose of fiber,
293 and between fecal butyrate concentration and *Faecalibacterium* abundance. These effects
294 could be explained by cross-feeding interactions disclosed between bifidobacteria and
295 *Faecalibacterium* (Moens, Weckx, & De Vuyst, 2016). Interestingly, a depletion of
296 *Desulfovibrio* species was also identified as a consequence of agave inulin consumption
297 (Holscher, et al., 2015), which could be of clinical relevance because increased
298 *Desulfovibrio* species have been related to obesity and the associated endotoxemia (Xiao, et
299 al., 2014; Zhang-Sun, Augusto, Zhao, & Caroff, 2015; Zhang, et al., 2009).
300
301 *1.3.4. GOS*
302 GOS are mainly produced through transgalactosylation reactions mediated by β-
303 galactosidases using lactose or derivatives as substrate. GOS are often used to supplement
304 infant formula due to their chemical and structural resemblance to human milk
305 oligosaccharides. In infant formula, GOS have been shown to exert a bifidogenic effect
306 (Giovannini, et al., 2014). In adults, the six-week administration of 5.5 g/day GOS powder

307 mixture dissolved in water to subjects with metabolic syndrome has been shown to reduce
308 levels of *Clostridium histolyticum, Desulfovibrio* spp. and *Bacteroides* spp.(Vulevic, Juric,
309 Tzortzis, & Gibson, 2013). These changes were accompanied by increases in
310 *Bifidobacterium* spp. and reductions in inflammatory markers, including fecal calprotectin
311 and plasma C-reactive protein (CRP) and in some metabolic parameters (e.g. plasma
312 insulin, total cholesterol and triglycerides in males).
313
314 *1.3.5. Xylans and arabinoxylans.* Arabinoxylans (AX) from cereals are cell wall
315 components that constitute a major part of the dietary fiber fraction of cereal grains and
316 thus, an important fiber source in the diet (McCleary, 2003). Enzymatic hydrolysis of AX
317 either in the production of processed foods or by bacteria in the colon yields
318 arabinoxylanoligosaccharides (AXOS) and xylooligosaccharides (XOS), both of which are
319 proposed to be prebiotic fibers (Broekaert, et al., 2011). Additionally to the well known
320 bifidogenic effect of AX, a fact in which is based its prebiotic potential (reviewed in
321 (Riviere, Selak, Lantin, Leroy, & De Vuyst, 2016)), other AX-degrading bacteria in the
322 human colon belong to the genera *Roseburia* and *Bacteroides* and include the butyrate
323 producing *Roseburia intestinalis* (Chassard, Goumy, Leclerc, Del'homme, & Bernalier-
324 Donadille, 2007). These data are of interest since a higher relative abundance of butyrate-
325 producing bacteria and *Bacteroides* spp. has been reported in healthy individuals compared
326 to patients with T2D or pre-diabetic subjects in some studies (reviewed in (Sanz, Olivares,
327 Moya-Perez, & Agostoni, 2015)). Human intervention trials have also shown increased
328 fecal abundance of *Bifidobacterium* spp. following intake of 4 g/day XOS during three
329 weeks (Chung, Hsu, Ko, & Chan, 2007) and from 2.14 to 10 g/day AXOS (Cloetens, et al.,
330 2010; Francois, et al., 2012; Maki, et al., 2012). Furthermore, a higher abundance of this

genus has been reported in normal weight subjects compared to obese and T2D subjects in some observational studies (Schwiertz, et al., 2010; Wu, et al., 2010).

2. Microbiome components involved in the utilization of dietary fiber

Dietary intake of fibers may lead to enrichment and altered expression of microbial genes which encode proteins/enzymes of metabolic pathways involved in the utilization of dietary fiber and the production of potentially beneficial metabolites (e.g. SCFAs). It is necessary to identify and characterize these pathways in order to understand the components of the microbiota and the microbiome that may underlie health effects associated with dietary fiber intake. Members of the phyla Bacteroidetes and Firmicutes are specialized in the utilization of complex carbohydrates and are the main producers of SCFAs. Butyrate and propionate are the two most thoroughly investigated SCFAs in terms of their potential role in metabolic health. The production of these SCFAs may require the participation of different bacterial genera and species via cross-feeding mechanisms. For example, *Bacteroides thetaiotaomicron* can directly produce propionate and acetate, which then can be used by *Eubacterium halli* to produce butyrate (Mahowald, et al., 2009). Similar cross-feeding mechanisms have been described between some *Bifidobacterium* spp. and *Faecalibacterium prausnitzii* leading to increased butyrate production (Rios-Covian, Gueimonde, Duncan, Flint, & de los Reyes-Gavilan, 2015). Figure 1 shows the pathways identified for bacterial production of butyrate by genomic and metagenomic analysis of the human gut microbiota (Mahowald, et al., 2009; Reichardt, et al., 2014; Vital, Howe, & Tiedje, 2014). A conventional genetic signature to explore both the enrichment and variability of butyrate producers is via analyzing the butyryl-CoA:acetate CoA-transferase gene (*BCoAT* gene) encoding the respective enzyme responsible for the last step in butyrate

355 production. Quantitative approaches indicate *BCoAT* gene enrichment in gut microbiota
356 from individuals with a high intake of plant fiber, which is indicative of increased colonic
357 butyrate production (Hippe, et al., 2011; Louis, Young, Holtrop, & Flint, 2010; Remely, et
358 al., 2014; Vital, Gao, Rizzo, Harrison, & Tiedje, 2015).
359 Additionally to genes encoding enzymes of pathways responsible for SCFA production, the
360 detection of other genes involved in the uptake and degradation of complex polysaccharides
361 could be useful to define the active bacteria and their mode of action in response to fiber
362 intake. Pioneer studies regarding characterization of proteins involved in the utilization of
363 complex carbohydrates by anaerobe gut bacteria have revealed the essential role of
364 polypeptides encoded by Sus genes, extensively studied in *B. thetaiotaomicron* (Reeves,
365 Wang, & Salyers, 1997). The Sus products were originally described as outer membrane
366 proteins able to bind complex starch. Notwithstanding, the genetic context of their encoding
367 genes has enabled the inclusion of glycoside hydrolases (GH) enzymes in the Sus repertoire
368 of proteins, which collectively work to produce small oligosaccharides that are more easily
369 imported by bacteria. Consequently, Sus genes have become useful to detect different
370 polysaccharide utilization loci (PULs) in other Bacteroides species by comparative
371 genomics approaches, allowing them to be studied in response to a wide variety of complex
372 polysaccharides (reviewed in (White, Lamed, Bayer, & Flint, 2014)). Nowadays, research
373 on carbohydrate utilization by gut bacteria is conceived as a cornerstone to understand their
374 physiology and potential interactions and bidirectional communication with the host in
375 health and disease. In this regard, the Carbohydrate Active Enzymes (CAZy) database
376 (http://www.cazy.org/) is one of the most complete repositories describing the families of
377 structurally-related catalytic and carbohydrate-binding functional domains of enzymes that
378 bind, degrade, modify or create glycosidic bonds (Lombard, Golaconda Ramulu, Drula,

379 Coutinho, & Henrissat, 2013). Hierarchical classification of CAZy comprises 4 main
380 families such as the Glycoside Hydrolase (GH, with 135 subfamilies reported at Nov 2016),
381 the Glycosyltransferase (GT, with 101 subfamilies), the Polysaccharide Lyase (PL, with 24
382 subfamilies), and the Carbohydrate Esterase (CE, with 16 subfamilies) family. All GH
383 reported are classified according to the functional modules they contain, with the aim to
384 determine sites of action (exo or endo-acting enzymes) or type of cleavage (α- or β-
385 glycosilases). Members of the phyla Bacteroidetes and Firmicutes are characterized by
386 encoding the largest set of GH in their genomes, thus exhibiting a remarkable versatility for
387 the utilization of different polysaccharides as carbon source (White et al 2014). These
388 features convert species of such bacterial phyla into key players for degradation of complex
389 polysaccharides in the human colon. Proof of this can be found in the studies performed in
390 Flint's lab with *Ruminoccocus bromii* in which this bacteria was observed to present a
391 specialized extracellular polypeptide complex, known as amylosome (Ze, et al., 2015). It
392 was also found to be an indispensable member of the human gut microbiota, having a direct
393 effect on energy recovery from a central component of diet, i.e., RS (Ze, Duncan, Louis, &
394 Flint, 2012). However, *Bifidobacterium* (Actinobacteria) species are also well-known fiber
395 fermenters. Although Bifidobacteria have fewer GHs encoded in their genomes than
396 Bacteroidetes, they also exhibit a great versatility for the uptake and catabolism of
397 oligosaccharides. This versatility is well exemplified in genome-wide expression analyses,
398 which have disclosed a wide variety of genes appearing to respond specifically to different
399 carbon sources (Andersen, et al., 2013; O'Connell, et al., 2013). In this context, we have
400 recently described the genome response of *B. pseudocatenulatum* CECT 7765, a strain
401 isolated from breast-fed babies, during utilization of lactulose-derived oligosaccharides. An

exhaustive inventory of GH enzymes present in the genome of this species have a set of open reading frames (ORFs) that seem to control the uptake and degradation of this digestion-resistant oligosaccharide (Benitez-Paez, Moreno, Sanz, & Sanz, 2016).

Although GHs and related proteins appear to be the key traits to infer versatility of gut microbes for utilization of polysaccharides and their contribution to the production of fermentation end-products such as SCFAs, little is known about the effects of fiber fermentation on secondary metabolic pathways and the generation of other nutrients (e.g. amino acids and vitamins) and bioactive compounds. Some *in vitro* studies have reported that oligosaccharide fermentation also increases amino acid synthesis (Benitez-Paez, et al., 2016; Sulek, et al., 2014). In particular, our study revealed that the utilization of GOS by *B. pseudocatenulatum* CECT 7765, using bacteria cultures, increased the production and extracellular accumulation of branched-chain amino acids such as leucine (Benitez-Paez, et al., 2016). Additional studies are, however, needed to understand the effects of the interplay between dietary fiber and amino acid metabolism in the large intestine and fully understand the metabolites resulting from the activity of the gut microbiota and their potential consequences on health beyond the well-known SCFAs.

3. Effects of dietary fiber on metabolic health mediated by gut microbiota

There is a wealth of human intervention studies with dietary fibers, but only a few of them have assessed the relationship between microbiota-induced changes and endpoints related to physiological functions and metabolism. Further studies are also needed that directly assess the effects of fiber-induced microbiota changes on metabolic outcomes, for example via fecal transplantation or via inoculation of specific bacterial consortia from humans into animal models. Consequently, there is still a large degree of uncertainty about to what

extent the effects attributed to dietary fibers on metabolic health are mediated by gut microbiota in humans, and which are the key species involved. Nonetheless, considerable mechanistic data are available from other animal study approaches, as summarized below.

3.1. Gut barrier integrity, metabolic endotoxemia and inflammation

Obesity and particularly the intake of a high-fat diet (HFD) are thought to lead to a leaky gut and metabolic endotoxemia (increased serum LPS levels) in animal models and to some extent in humans. This is assumed contributing to the low-grade chronic inflammation leading to metabolic dysfunction and disease (metabolic syndrome and T2D). In fact, LPS is a potently inflammatory bacterial antigen linked to common metabolic diseases (Conlon & Bird, 2015). LPS is an endotoxin consisting of three parts; lipid A, the oligosaccharide core and the O-antigen, with the lipid A causing endotoxicity. LPS is normally present in the human gut (≥1 g) and under normal conditions it does not cause negative health effects. In healthy humans the normal/low plasma concentration of LPS is 1-200 pg/ml, but increased levels have been found in subjects with obesity and diabetes (Erridge, Attina, Spickett, & Webb, 2007; Moreira, Texeira, Ferreira, Peluzio Mdo, & Alfenas Rde, 2012). LPS binds to TLR4 via CD14 on, for example, the membrane surface of immune cells leading to activation of genes that codify pro-inflammatory cytokines (e.g. TNF-α and IL-6) involved in metabolic inflammation. Experimental models of obesity have shown prebiotic-induced increases in bifidobacteria and *Akkermansia* spp. associated with reduced endotoxemia and systemic inflammation (Cani, et al., 2007; Schneeberger, et al., 2015). These effects can be partly explained by the ability of those bacteria to ferment glycans leading to SCFA production and promoting local decrease of pH, which may modulate gut microbiota composition and inhibit the growth of enterobacteria, which may be a source of

LPS (Delzenne, Neyrinck, & Cani, 2013; Everard, et al., 2013). This effect could also be related to the role of SCFAs in strengthening the gut barrier function, which also reduces LPS translocation via different mechanisms, including modulation of expression and localization of tight-junction proteins, induction of endocrine peptide production (GLP-2) and modification of the intestinal levels of endocannabinoids (Everard, et al., 2013).

SCFAs also play an anti-inflammatory role by regulating the size and function of the colonic regulatory T cells (Treg), specifically inducing Foxp3+IL-10–producing Tregs (Smith, et al., 2013). SCFAs may also interact with peroxisome proliferator-activated receptor (PPAR) γ, thereby inhibiting pro-inflammatory signal transduction pathways (e.g. nuclear factor-kappa B [NF-κB]) leading to reduction of downstream cytokine/chemokine production (IL-6, IL-8, and MCP-1) in intestinal epithelial cells and metabolic tissues (e.g. adipose tissue) (Mastrofrancesco, et al., 2014). Activation of PPARγ also seems to be crucial in orchestrating Treg accumulation and function in the adipose tissue, which play an important role in preventing inflammation and insulin resistance (Cipolletta, Cohen, Spiegelman, Benoist, & Mathis, 2015). Butyrate as well as other SCFAs, protects against the liver inflammation process associated with steatosis by inhibiting the NF-κB activation and downregulating expression of TLR4 receptor (Mattace Raso, et al., 2013). The molecular mechanisms underlying SCFA modulation of NF-κB activity have recently been disclosed as related to JNK and p38 kinases, which control NF-κB activity (Haghikia, et al., 2015). However, we cannot discard additional mechanisms to control NF-κB function involving acetylation/deacetylation of histones and the RelA (p65) monomer itself (Davie, 2003; Glozak, Sengupta, Zhang, & Seto, 2005).

3.2. Enteroendocrine secretion and appetite

In obese animals fed inulin-type fructans, there is an increase in plasma anorexigenic peptides (peptide YY and glucagon-like peptide - GLP-1) and a decrease in the orexigenic peptide ghrelin, which increases satiety (reviewed in (Delzenne, et al., 2013)). In addition, supplementation with fructans in HFD-fed mice modulates neuronal activation within the arcuate nucleus, which can help to control food intake (Anastasovska, et al., 2012). These effects on anorexigenic peptide secretion could be mediated by interactions of SCFAs with G-protein receptors such as FFAR2 (GPR41) and FFAR3 (GPR43), which could explain induction of satiety and increased insulin sensitivity (Blaut, 2014). Also in humans, prebiotic interventions with fructans have led to increases in anorexigenic peptides and/or decreases in orexigenic (ghrelin) peptides (Cani, Joly, Horsmans, & Delzenne, 2006; Cani, et al., 2009; Parnell & Reimer, 2009; Verhoef, Meyer, & Westerterp, 2011), but effects on satiety have not always been consistent (Peters, Boers, Haddeman, Melnikov, & Qvyjt, 2009).

3.3. Adiposity, lipid and glucose metabolism

Reduced adiposity in rodents due to dietary supplementation with inulin-type fructans or AX has also been attributed to the role of SCFAs in modulating PPARγ expression via interaction with the G-protein coupled receptor protein FFAR3 (Delzenne, Neyrinck, Backhed, & Cani, 2011). Interestingly, den Besten and co-workers found that SCFAs decrease PPARγ expression, thus promoting activity of the uncoupling protein 2 (UCP2) and, thereby, stimulating oxidative metabolism in liver and adipose tissue, insulin sensitivity and weight loss (den Besten, et al., 2015). Studies with inulin-type fructans have also shown they can decrease hepatic accumulation of triglycerides and/or cholesterol in

497 liver tissue. These effects have been associated with a decrease in sterol-response-element-
498 binding protein-dependent cholesterogenesis, lipogenesis, or changes in PPARα-driven
499 fatty acid oxidation (reviewed in (Delzenne, et al., 2013)). The majority of studies show
500 prebiotic administration also leads to improved fasting or postprandial glycemia due to the
501 very low digestion rates of prebiotics compared with digestible carbohydrates (for review
502 see (Roberfroid, et al., 2010)). In addition, SCFA-stimulation of GLP-1 secretion can also
503 mediate an improvement in glucose metabolism, reducing obesity-related hepatic insulin
504 resistance.
505 In humans, intervention studies with fructans have reported modest effects on body weight
506 and fat mass in obese adults, but simultaneous changes in microbiota were not considered
507 to have any correlation (Genta, et al., 2009; Parnell & Reimer, 2009). Nevertheless, there
508 are also reports of a lack of effect on body weight in obese children (Liber & Szajewska,
509 2014). On the other hand, a rapid improvement in glucose tolerance has been observed for
510 individuals consuming WG barley the night prior to analysis. These results were thought to
511 be caused by the high amount of soluble dietary fiber and resistant starch contained in
512 barley kernels, which facilitated bacterial fermentation in the colon overnight and produced
513 significantly higher levels of SCFAs. This was indirectly measured from breath H_2
514 excretion (Nilsson, Granfeldt, Ostman, Preston, & Bjorck, 2006). Moreover, recent results
515 of this dietary intervention model indicate that the fiber-associated improvement of glucose
516 metabolism is also associated with an increase in *Prevotella* spp. (Kovatcheva-Datchary, et
517 al., 2015).
518
519 ***4. Impact of dietary fat on gut microbiota and associated metabolic endpoints***

520 Globally, an increase in dietary fat content is usually paralleled with a decrease in
521 carbohydrates, including dietary fiber content, thus making it difficult to attribute the
522 observed changes, at physiology or gut microbiota levels, exclusively to one of the
523 macronutrients whose proportion is being increased. Consequently, a decreased abundance
524 of butyrate-producing bacteria and lower fecal SCFA excretion following a HFD is most
525 likely caused by a decrease in dietary carbohydrate intake. Therefore, major conclusions
526 derived from future animal or human studies including HFD interventions must be
527 addressed carefully in order to consider confounding effects regarding the proportions and
528 energetics or other macronutrients administrated.
529
530 *4.1. Evidence from animal studies*
531 The role of gut microbiota in HFD-induced obesity was suggested through animal
532 experiments involving germ-free mice fed a HFD, which were protected from obesity
533 compared to conventionally raised mice (Rabot, et al., 2010), thus highlighting the role of
534 microbiota in HFD-induced obesity. Furthermore, a study in mice by Hildebrandt and
535 coworkers showed that changes in the gut microbiota composition were caused by dietary
536 fat content rather than the degree of obesity, suggesting that fat directly impacts on
537 microbiota regardless of the metabolic phenotype (Hildebrandt, et al., 2009). Gut
538 microbiota transferred to germ-free mice from conventionally raised mice resulted in
539 weight gain and a higher relative abundance of Firmicutes and a lower abundance of
540 Bacteroidetes when mice were fed a HFD compared to a low-fat chow diet from 16 weeks
541 of age (Turnbaugh, Backhed, Fulton, & Gordon, 2008). Although differences established at
542 phylum level are of limited value since each phylum comprise many different species
543 which may potentially play many different functions, a common trait for HFD-feding

544 seems to be that it increases the Firmicutes:Bacteroidetes ratio (de Wit, et al., 2012;
545 Hildebrandt, et al., 2009; Lam, et al., 2012; Turnbaugh, et al., 2008), although there is not
546 complete consistency across studies (Lecomte, et al., 2015); this would also be due to
547 experimental and environmental differences. A recent 16-week study in mice fed a HFD
548 reports that the abundance of *Akkermansia muciniphila* was progressively and drastically
549 decreased while other groups including *Bifidobacterium* spp. and *Lactobacillus* spp.
550 showed a transient decrease. In contrast the abundance of *Roseburia* spp. and *Bilophila*
551 *wadsworthia* increased after 12 and 16 weeks upon HFD, respectively (Schneeberger, et al.,
552 2015). Interesting, *B.wadsworthia* have been linked to insulin resistance and inflammation
553 in humans (Brahe, et al., 2015).
554 Animal studies have revealed different mechanisms by which HFDcould exert adverse
555 effects, partly mediated by the microbiota, on the host metabolic phenotype. For example,
556 diets rich in saturated fat may contribute to inflammation, a hallmark of metabolic
557 dysfunction leading to metabolic syndrome and T2D, by promoting the expansion of
558 pathobionts, reducing the proportion of protective bacteria, and promoting a leaky gut that
559 in turn facilitates the translocation of bacterial products (e.g. LPS) causing immune
560 activation (Caesar, et al., 2015; Delzenne, et al., 2011; Devkota, et al., 2012). In a recent
561 study, HFD-induced microbiota changes were correlated with obesity-related inflammatory
562 and metabolic biomarkers (Schneeberger, et al., 2015). *Akkermansia muciniphila* was the
563 species showing the clearest inverse associations with inflammatory markers in the adipose
564 tissue and also with biochemical/hormonal parameters in circulation (i.e., insulin, glucose,
565 triglycerides and leptin).
566 However, as the majority of the dietary fat is absorbed in the small intestine and does not
567 serve as an energy source for gut microbes, the effect of fat on gut microbiota must be

partly mediated by indirect mechanisms. Increased fat intake also leads to increases in fat quantities and of BAs reaching the colon, and particularly the concentration and composition of BAs modulates the gut microbiota exerting antimicrobial effects (Islam, et al., 2011; Ridlon, Kang, Hylemon, & Bajaj, 2014). Primary BAs (e.g. cholic acid [CA] and chenodeoxycholic acid [CDCA] in humans and beta-muricholic acid [β-MCA] in mice) are sterol compounds synthesized from cholesterol in the liver, conjugated with taurine and glycine, and then secreted into the small intestine to emulsify lipids to facilitate their digestion and absorption. The majority of BAs are reabsorbed (enterohepatic recycling), but as increased fat intake leads to increased BA secretion, theoretically more BAs will escape enterohepatic recycling, and hence reach the large intestine. During the transit to the large intestine, primary BAs undergo deconjugation, oxidation of hydroxyl groups at C-3, C-7, andC-12, and 7α/β-dehydroxylation reactions mediated by intestinal bacterial enzymes, yielding secondary BAs such as deoxycholic acid (DCA), lithocholic acid (LCA), and β-muri-deoxycholicacid. Bacterial bile salt hydrolases (BSH), e.g. produced by *Clostridium* spp, catalyze the first reaction on secondary BAs and this is a step necessary for the subsequent7α/β-dehydroxylation (Degirolamo, Rainaldi, Bovenga, Murzilli, & Moschetta, 2014). Overall, the amount and composition of BAs are strongly influenced by gut microbiota and *vice versa,* and BA biotransformation has important biological consequences due to their role in dietary lipid absorption and as signaling molecules, modulating cholesterol and triglyceride metabolism and glucose and energy homeostasis (Degirolamo, et al., 2014; Staels & Prawitt, 2013). Secondary BAs have strong antimicrobial activity (e.g. damage of the bacterial cell membrane by interaction with phospholipids) due to their amphipathic properties. For example, DCA has 10 times the bactericidal activity of CA (Islam, et al., 2011), therefore an increase in the proportion of

592 secondary BAs following HFD very likely affects the microbiota composition. A rat study,
593 evaluating the effect of adding CA at different doses compared with controls (no CA
594 added), demonstrated adose-dependent increase of fecal BA and DCA (Islam, et al., 2011).
595 Furthermore, a dose-dependent decrease in fecal SCFA concentration was observed along
596 with a reduction in total bacterial count and an increase in Firmicutes at the expense of
597 primarily Bacteroidetes.
598 Dietary saturated fat compared to poly-unsaturated fatty acids (PUFAs) was also reported
599 to favor taurine conjugation of hepatic BAs, which caused an expansion of δ-
600 Proteobacteria-type pathobionts, in particularly *B. wadsworthia* which is a sulfite-reducing
601 bacterium exerting a cytotoxic effect on epithelial cells and activating Th1-type
602 inflammatory response (Devkota, et al., 2012).
603 Studies in rodent models of HFD-induced obesity have also shown that saturated fat
604 reduces the mucus layer, which acts as the first barrier separating the immune system from
605 microbial and antigen interactions that may activate an inflammatory response. This effect
606 was parallel to a reduction in the abundance of *Akkermansia* spp., while administration of
607 this bacterium reversed it, increasing mucus layer thickness, and thus suggesting a
608 microbiota-mediated effect (Everard, et al., 2013). Other animal studies have reported
609 correlations between HFD-induced changes in the microbiota and alterations in the
610 expression of tight junction-related proteins, and in gut permeability. In mice a HFD has
611 been shown to reduce the expression of the tight-junction-related protein zonula occludens
612 (ZO)-1 mRNA (Cani, Delzenne, Amar, & Burcelin, 2008) associated leading to increased
613 gut permeability measured by transepithelial resistance (Lam, et al., 2012). Additionally,
614 decreased transepithelial resistance (i.e. increased gut permeability) was associated a drop

615 in the abundance of *Lactobacillus* spp. and augmented abundance of *Oscillibacter* spp.
616 (Lam, et al., 2012).
617 Animal studies also show that when a HFD is supplemented with either prebiotics (Cani, et
618 al., 2007; Everard, et al., 2013; Serino, et al., 2012) or antibiotics (Cani, Bibiloni, et al.,
619 2008) the HFD-induced alterations in gut microbiota and metabolism are partially reversed,
620 indicating that gut microbiota partly mediate the consequences of HF feeding.
621 A few studies have investigated the effects of different dietary fatty acids (Lam, et al.,
622 2012; Lappi, et al., 2013; Simoes, et al., 2013). In mice, it has been shown that n-6 high fat
623 diets do not increase insulin resistance, intestinal permeability and fat accumulation to the
624 same degree as saturated fatty acid diets, which is possibly due to a lower increase in H_2S-
625 producing bacteria (Lam, et al., 2012). Likewise, lower decreases in Bacteroidetes have
626 been found under diets rich in n-3 or n-6, compared to diets rich in saturated fatty acids(T.
627 Liu, Hougen, Vollmer, & Hiebert, 2012).
628
629 *4.2. Evidence from human studies*
630 Only a few human intervention studies have investigated the effects of HFD compared to
631 low-fat diets(LFD) or the type of fat (saturated fat versus PUFAs) in relation to changes in
632 gut microbiota and the metabolic consequences. As found in animal studies, total bacterial
633 counts decrease in humans who consume a HFD (35-38 E%), compared to a LFD (23-27
634 E%) over 24 weeks (Fava, et al., 2013). Moreover, low/moderate-fat intake appears to
635 induce a higher abundance of *Bacteroides* spp. and/or *Bifidobacterium* spp., compared to
636 high-fat intake in human intervention trials (Brinkworth, Noakes, Clifton, & Bird, 2009;
637 Fava, et al., 2013). An energy-restricted HFD (58 E%), compared with an isocaloric
638 moderate-fat diet (28 E %) was shown to increase the total number of anaerobes in the

639 moderate-fat group, but not in the high-fat group, but the ratio between anaerobe:aerobe
640 remained unchanged in each group (Brinkworth, et al., 2009). Additionally, a study
641 comparing high-fat and moderate-fat *ad libitum* diets (66 E% vs. 35 E%) over 4 weeks did
642 not report any effect on the gut microbiota in terms of total bacterial count; however, the
643 methodology used to study microbiota abundance was based on a limited number of
644 species(Duncan, et al., 2007).
645 As stated above , an increase in the intake of dietary fat is usually at expenses of a decrease
646 in that of simple or complex carbohydrates, making it difficult to attribute the observed
647 effectexclusively to one of the macronutrients. O'Keefe and coworkers (O'Keefe, et al.,
648 2015) compared the effects on gut microbiota in a cross-over study with a 2-week diet
649 period administering either African- or American-food. The switch from a rural African to
650 an American-diet (52% fat, 21% carbohydrate, 27% protein, and 12% fiber) decreased the
651 abundance of butyrate-producing bacteria and the production of acetate, propionate and
652 butyrate (O'Keefe, et al., 2015). Similarly, Duncan and coworkers observeda higher
653 abundance of *Roseburia* and *Eubacterium* and higher fecal excretion of butyrate in humans
654 following a moderate fat diet compared to high-fat intake, with these changes in the gut
655 microbiota and derived metabolites being positively correlated with carbohydrate intake
656 (Duncan, et al., 2007).
657 O'Keefe and coworkers also measured BA excretion and observed that the high-fat diet of
658 Americans was associated with increased expression of microbial genes coding for the
659 enzyme related to converting primary BAs to secondary BAs, whereas a dietary switch to a
660 lower-fat diet reduced the abundance of these bacteria. Furthermore, excretion of the
661 secondary BAs LCA and DCA was increased by the HFD. Also short-term consumption of
662 diets composed entirely of animal (rich in fat and protein) or plant products (rich in fiber)

663 can rapidly alter gut microbial composition (David, et al., 2014). An animal-based diet
664 increased the abundance of bile-tolerant microorganisms, including *Alistipes*, *Bilophila*, and
665 *Bacteroides* species. By contrast this diet decreased the abundance of Firmicutes, including
666 genus and species specialized in the utilization of polysaccharides (*Roseburia, Eubacterium*
667 *rectale*, and *Ruminococcus bromii*). Furthermore, the animal-based diet increased the
668 abundance of *B. wadsworthia* and secondary BAs. These findings support the observations
669 in rodent models comparing diets rich in PUFA or saturated fat (D. L. Gibson, et al., 2015;
670 Schneeberger, et al., 2015), suggesting similar mechanisms of action and similar metabolic
671 effects.
672 The relationship between PUFAs and the microbiota are even less well understood. A
673 recent study in women with obesity and metabolic syndrome who consumed inulin-type
674 fructans for 3 months reported that PUFA-derived metabolites were associated with
675 *Bifidobacterium* spp., *Eubacterium ventriosum*, and *Lactobacillus* spp., and negatively
676 correlated with serum cholesterol (Druart, et al., 2014). However, another human
677 intervention study found that supplementation with n-3 fatty acids (180 mg EPA and 120
678 mg DHA) for 6 weeks did not induce changes in the gut microbiota although it decreased
679 insulin resistance and CRP (Rajkumar, et al., 2014). Unfortunately, amelioration of these
680 metabolic parameters could not be directly associated with one specific fatty acid since
681 only a mixture was tested. Therefore, further studies are needed to gain greater
682 understanding of how the quality of dietary fat influences gut microbiota composition and
683 function, and potential mediated effects on metabolism in humans.
684

685 ## Concluding remarks

Fiber is an instrumental dietary component that can be used to remodel gut microbiota composition and function to potentiate the beneficial effects of healthy diets on body weight management and metabolism. However, efforts are still needed to identify the optimal functional partnership between key bacterial species and types of fibers, considering the specificities of the individual's microbiota. Fermentation of dietary fiber generates SCFAs, which presumably articulate beneficial effects in the context of obesity; yet many other secondary metabolic products resulting from diet-microbe interactions have yet to be discovered. Gut microbiota appears to contribute to the adverse consequences of high-fat diets on the metabolic phenotype, aggravating the associated low-grade inflammation and increasing energy absorption; however, further studies are needed to understand the potential effects of the quality of dietary fat on the gut microbiota and secondary metabolic process, such as those involving bile acids and their signaling roles. Additional efforts must be conducted to identify the specific components of the gut microbiota, at species and strain level, influenced by different types of dietary fibers and fats and to understand their roles and mechanisms of action in humans to facilitate the use of this information in nutritional practice. This ambitious goal is expected to be accomplished by developing translational research approaches that integrate controlled dietary interventions in humans, combining functional omics technologies and physiological/clinical endpoints, and mechanistic studies in experimental models colonized with specific dietary-driven human microbiotas.

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Figure Legends

Figure 1. The bacterial butyrate synthesis pathways (adapted from (Vital, et al., 2014)). Vital and coworkers have reconstructed four different pathways for butyrate synthesis through and an extensive metagenomic approach. Protein names and major substrates are shown across the different biosynthetic pathways. Genes/proteins responsible of the last step of butyrate production, and frequently used as biomarkers for gut microbiota studies, are highlighted in red. They are known as: 4Hbt, butyryl-CoA:4-hydroxybutyrate CoA transferase; But, butyryl-CoA:acetate CoA transferase; Ato, butyryl-CoA:acetoacetate CoA transferase (α, β subunits); and Buk, butyrate kinase.

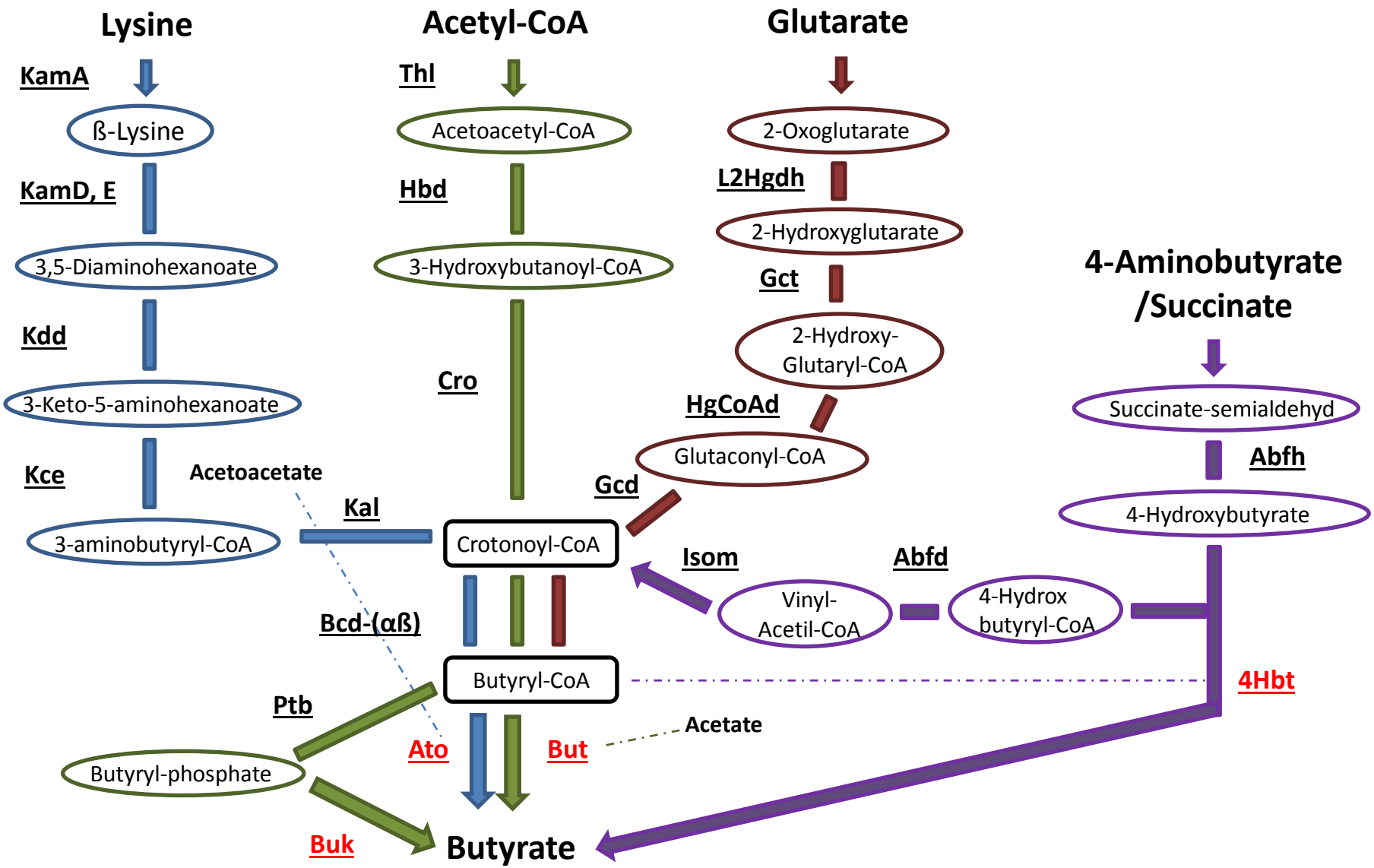
Lysine
KamA
ß-Lysine
KamD, E
3,5-Diaminohexanoate
Kdd
3-Keto-5-aminohexanoate
Kce
3-aminobutyryl-CoA
Acetoacetate
Kal
Acetyl-CoA
Thl
Acetoacetyl-CoA
Hbd
3-Hydroxybutanoyl-CoA
Cro
Crotonoyl-CoA
Bcd-(αß)
Butyryl-CoA
Ptb
Butyryl-phosphate
Ato
But
Acetate
Buk
Butyrate
Glutarate
2-Oxoglutarate
L2Hgdh
2-Hydroxyglutarate
Gct
2-Hydroxy-
Glutaryl-CoA
HgCoAd
Glutaconyl-CoA
Gcd
Isom
Vinyl-
Acetil-CoA
Abfd
4-Hydrox
butyryl-CoA
4-Aminobutyrate
/Succinate
Succinate-semialdehyd
Abfh
4-Hydroxybutyrate
4Hbt

Table 1. Summary of dietary fiber interventional studies with gut microbiota assessments in humans.

Fiber	Study Design	Subjects	Time	Gender	Population	Effects on gut microbiota[1]	Reference
Maize-derived WG cereal	DB, R, PC, CO	32	3 weeks	Females (21) Males (11)	European UK	↑*Bifidobacterium*	(Carvalho-Wells, et al., 2010)
WG wheat cereal	DB, R, PC, CO	31	3 weeks	Females (16) Males (15)	European UK	↑*Bifidobacterium, Lactobacillus*	(Costabile, et al., 2008)
WG barley	R, CO	28	4 weeks	Females (17) Males (11)	USA	↑*Blautia,Bifidobacterium, Roseburia, Dialister* ↔*Dialister*- plasma IL-6levels ↔*Eubacterium*- plasmaglucose/insulin	(Martinez, et al., 2013)
Inulin	DB, R, PC, CO	32	4 weeks	Females	European Switzerland	↑*Bifidobacterium*	(Petry, et al., 2012)
Inulin (Agave)	DB, R, PC, CO	29	3 weeks	NA	USA	↑*Bifidobacterium* ↓*Desulfovibrio* ↔*Faecalibacterium* - fecal butyrate	(Holscher, et al., 2015)
Inulin / FOS	DB, R, PC	31	8 weeks	Females	European Spain	↑*Bifidobacterium, Lactobacillus*	(Garcia-Peris, et al., 2012)
Inulin-type fructans	DB, R, PC	30	12weeks	Females	European Belgium	↑*Bifidobacterium, Faecalibacteriumprausnitzii* ↓*Bacteroides, Propionibacterium* ↔*Bifidobacterium* - plasma LPS levels ↔*Faecailbacterium* - plasma LPS levels ↔*Bacteroides* - Fat mass	(Salazar, et al., 2015)
Inulin / Oligofructose	DB, R, PC	22	12 days (mean)	Females (9) Males (13)	European UK	↓*Faecalibacterium, Bacteroides, Prevotella*	(Majid, et al., 2014)
Inulin / Oligofructose	DB, PC	30	12 weeks	Females (44)	European Belgium	↑*Bifidobacterium, Faecalibacteriumprausnitzii*	(Dewulf, et al., 2013)
Inulin / Oligofructose	DB, R, PC	252	16weeks	Females (123) Males (129)	European Spain	↑*Bifidobacterium*	(Closa-Monasterolo, et al., 2013)
B-GOS	DB, R, PC, CO	45	6 weeks	Females (29) Males (16)	European UK	↑*Bifidobacterium* ↓*Clostridium histolyticum, Desulfovibrio, Bacteroides*	(Vulevic, et al., 2013)
GOS	DB, R, PC, CO	31	3 weeks	Females	European The Netherlands	↑*Bifidobacterium*	(Whisner, et al., 2013)
GOS	DB, R, PC	163	>16 weeks	NA	European Italy	↑*Bifidobacterium*	(Giovannini, et al., 2014)
XOS	R, PC	22	3 weeks	Females (7) Males (15)	Taiwan	↑*Bifidobacterium*	(Chung, et al., 2007)
AXOS	R, PC, CO	20	3 weeks	Females (14) Males (6)	European Belgium	↑*Bifidobacterium*	(Cloetens, et al., 2010)
AXOS	DB, R, PC, CO	63	3 weeks	Females (30) Males (33)	European Belgium	↑*Bifidobacterium*	(Francois, et al., 2012)
AXOS	DB, R, PC, CO	65	3 weeks	Females (35) Males (30)	USA	↑*Bifidobacterium*	(Maki, et al., 2012)
RS3	R, CO	14	3 weeks	NA	European Scotland	↑*Ruminococcusbromii, Eubacteriumrectale*	(Walker, et al., 2011)

RS2, RS4	DB, CO	10	3 weeks	Females (5) Males (5)	USA	↑ *Bifidobacterium adolescentis, Eubacteriumrectale, Ruminococcusbromii, Parabacteroides distasonis* ↓ *Faecalibacteriumprautsnitzii, Doreaformicigenerans*	(Martinez, et al., 2010)
RS	R, CO	46	4 weeks	Females (30) Males (16)	Australia	↑*Ruminococcusbromii*	(Abell, et al., 2008)

1 Gut microbiota changes expressed in terms of abundance. ↑ indicates higher proportions of a determined bacterial genus after intervention, and ↓ indicates the inverse effect. ↔ indicates direct correlations among bacterial abundance and metabolic parameters studied, being negative or positive, respectively.DB= Double-blind; Single-Blind = SB; R= randomized; PC = Placebo-controlled; CO = Cross-over; NA = No information was explicitly available for gender distribution into the intervention groups.