Gut microbiota role in dietary protein metabolism and health-related outcomes: the two sides of the coin

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Abstract

Background

Human gut bacteria can synthesize proteinogenic amino acids and produce a range of metabolites via protein fermentation, some known to exert beneficial or harmful physiological effects on the host. However, the effects of the type and amount of dietary protein consumed on these metabolic processes, as well as the effects of the microbiota-derived amino acids and related metabolites on the host health are still predominantly unknown.

Scope and Approach

This review provides an up-to-date description of the dominant pathways/genes involved in amino acid metabolism in gut bacteria, and provides an inventory of metabolic intermediates derived from bacterial protein fermentation that may affect human health. Advances in understanding bacterial protein fermentation pathways and metabolites generated at a global level via the implementation of 'omics' technologies are reviewed. Finally, the impact of dietary protein intake and high-protein diets on human health is discussed.

Key Findings and Conclusions

The intestinal microbiota is able to synthesize amino acids, but the net result of amino acid production and utilization, according to dietary patterns still needs to be determined. The amount of ingested dietary protein appears to modify both the diversity and composition of the intestinal microbiota as well as the luminal environment of the intestinal epithelium and peripheral tissues. The understanding of the consequences of such changes on the host physiology and pathophysiology is still in an early stage but major progress is expected in the near future with the investigation of host-microbe omics profiles from well-controlled human intervention studies.

Key words: Microbiota, microbiome, protein metabolism, high-protein diet

1. Introduction

The dietary protein consumption level in humans is vastly different according to food availability and cultural dietary habits (Wu, et al., 2014). Although insufficient protein consumption remains a persistent problem in the developing world, the average daily protein intake in countries from Western Europe and the United States of America is generally higher than the recommended dietary intake of 0.83 g protein•kg^{-1}•day^{-1} for adults (EFSA Panel on Dietetic Products, 2012; Rand, Pellett, & Young, 2003). In individuals consuming a high-protein (HP) diet as a way to reduce their body weight, the protein consumption generally consists of approximately two to three times the recommended dietary intake; and can even represent five times this latter value (Pesta & Samuel, 2014). Such diets have been shown to increase satiety, modify lipid metabolism, and facilitate short- and medium-term weight reduction (Westerterp-Plantenga, Nieuwenhuizen, Tome, Soenen, & Westerterp, 2009). Although a reduction of body weight in overweight and obese individuals is obviously associated with favorable outcomes in terms of health (Papillard-Marechal, et al., 2012), such dietary modification are also associated with potentially deleterious effects in both healthy situations in the long-term and in some pathological situations, notably in kidney diseases (Juraschek, Appel, Anderson, & Miller, 2013) and in inflammatory bowel diseases (Jowett, et al., 2004).

Besides host physiological factors, recent evidence demonstrates that human gut microbiota in the small and large intestine also plays a role in host dietary protein metabolism. The interplay between host and gut microbial metabolism is complex, with microbes utilizing and even competing for dietary and endogenous proteins. Fermentation of amino acids by gut bacteria produces metabolites that can affect host protein/amino acid uptake (transport) and metabolism, as well as affect host cell

physiology (Davila, et al., 2013). Bacteria can also synthesize amino acids, which can be provided to the host (Metges, 2000). However, the net result of amino acid synthesis and degradation remains largely to be determined along with the role of the gut microbiota for the management of whole body nitrogen metabolism (Neis, Dejong, & Rensen, 2015). Such knowledge is important since it will yield information regarding the role of the microbiota in the utilization of amino acids from dietary origin in different physiological and pathological situations, as well as the role of the microbiota in the production of metabolites that could be available for the host and impact host metabolism and other physiological functions.

Despite the relatively rapid transit of the luminal content in the small intestine, part of the amino acid pool released from proteins through the action of pancreatic enzymes can be used by the host enterocytes (Davila, et al., 2013) as well as by the small intestinal microbiota (Dai, Zhang, Wu, & Zhu, 2010). Protein digestion in the mammalian digestive tract is a very efficient process, being generally equal to or even higher than 90% (Bos, et al., 2005). In the large intestine, where the microbiota concentration is much higher and the transit time is longer than in the small intestine, the remaining protein is broken down to peptides and amino acids via extracellular bacterial proteases and peptidases (Macfarlane, Cummings, & Allison, 1986). In contrast to the small intestine, however, the amino acids generated cannot be absorbed to any significant extent by the large intestine epithelium, except during the neonatal period in mammals (Darragh, Cranwell, & Moughan, 1994). Gut bacterial fermentation of amino acids thus results in an accumulation of various metabolic end-products in the luminal content, some of these metabolites being largely absorbed through the large intestinal epithelial cells, while others are released in feces in large amounts (Davila, et al., 2013). Several bacterial metabolites have also been shown to be active on colonic

epithelial cells, which, as detailed below, depending on their luminal concentrations, can exert beneficial or deleterious effects.

Bacterial metabolites which are not fully metabolized/detoxified by the colonic epithelial cells during their transcellular journey from the intestinal lumen to the bloodstream may reach the liver through the portal vein and then peripheral tissues where they can exert some biological effects, notably on kidney functions.

Investigations into the effects of microbially-derived metabolites on human health and the interaction of the microbiota with the human host have previously been limited due to the complexity of interactions between these two systems. The rapid advance of 'omics' technologies are beginning to expand our understanding of the relationships between the human host and gut microbiota by allowing a global analysis of the flow of host- and microbially-produced metabolites and genes involved in specific biochemical pathways (Qin, et al., 2010; Sridharan, et al., 2014). A thorough characterization and understanding of the bacterial pathways involved in amino acid metabolism and their derivatives is required for precise interpretation and prediction of dietary protein effects on the host health. Currently, a comprehensive review of those bacterial genes and metabolic routes is lacking.

This review merges up-to-date genomic information regarding amino acid-related metabolism in gut bacteria with their potential effects on human health. A description of the dominant pathways for bacterial amino acid biosynthesis as well as for amino acid degradation into metabolites that may play different roles in human health is provided. Moreover, information on the enzymes and homologous genes involved in these pathways as deduced from the KEGG database (Kanehisa, et al., 2014) is given. We then discuss the recent advances in understanding the effects of different dietary strategies (i.e. high-fat diet and HP diet) on the human gut microbiome and its role in

151 protein/amino acid metabolism based on metagenomic and metabolomic studies.
152 Finally, we analyzed how this metabolic activity, notably in terms of bacterial
153 metabolite production, may be responsible for the effects of dietary protein intake levels
154 on health-related outcomes in both physiological and pathological situations as well as
155 underline research areas that need new developments.
156
157
158 **2. Bacterial synthesis of amino acids**
159 The effects of *de novo* production of amino acids by microbes on whole-body fluxes
160 and human health are still not clearly understood. Bacterial production of amino acids
161 that are accessible to the host may be useful to compensate indispensable amino acid
162 deficiency in low quality protein diets. However, bacterially-produced amino acids
163 could also have detrimental consequences in conditions such as insulin resistance in
164 type 2 diabetes where systemic concentrations of amino acids such as aromatic and
165 branched-chain amino acids are elevated (Neis, et al., 2015). A deeper understanding of
166 the effects of microbially-produced amino acids on host health is warranted, as well as a
167 revision of the biosynthetic pathways of amino acids in bacteria which is provided here.
168
169 **2.1. Pathways/genes involved in *de novo* biosynthesis of amino acids**
170 Due to the high metabolic cost of synthesizing amino acids, the carbon backbone of
171 all amino acids originates from common metabolic intermediates involved in processes
172 such as the tricarboxylic acid cycle, the pentose phosphate pathway and glycolysis
173 (Berg, Tymoczko, & Stryer, 2002). Among these intermediates, α-ketoglutarate plays a
174 central role in amino acid biosynthesis through its conversion to glutamate, as well as
175 its participation in the biosynthetic pathways of other amino acids. Amino acids can be

grouped into families according to common starting products or use of common enzymes for synthesis. These consist of the following families: glutamate, serine, aspartate, pyruvate, and aromatic amino acid families, as well as several unique pathways for individual amino acids (Umbarger, 1978). It is important to note that an overwhelming amount of the literature on bacterial metabolism has historically been focused on a few bacterial taxa, namely *Escherichia coli* and *Salmonella typhimurium*, and to a lesser extent *Bacillus subtilis* and recently *Corynebacterium glutamicum*, thus creating a potential bias towards mechanisms found in these organisms. Although many of these pathways are conserved across bacterial lineages including those inhabiting the intestine, diversity is found among different bacterial species at both the species and strain level. For example, whole genome analysis has revealed that the common gut bacterium *Clostridium perfringens* lacks numerous amino acid biosynthetic genes for glutamate, arginine, histidine, lysine, methionine, serine, threonine, aromatic and branched-chain amino acids (Shimizu, et al., 2002), while other *Clostridium* spp., such as *Clostridium acetobutylicum,* has a complete set of genes for amino acid biosynthesis (Nolling, et al., 2001). The gut bacterium *Lactobacillus johnsonii* also appears incapable of carrying out *de novo* biosynthesis of almost all amino acids due to a lack of complete biosynthetic pathways, and exhibits an apparent dependence on exogenous host amino acids/peptides for protein synthesis (Pridmore, et al., 2004). Other animal and human intestinal bacteria, including *Campylobacter jejuni*, *Helicobacter pylori*, *Enterococcus faecalis* and *Streptococcus agalactiae* have also lost certain amino acid biosynthetic pathways (Yu, Walker, Liu, & Zhang, 2009), suggesting a dependence on availability of exogenous peptides/amino acid sources. Furthermore, the sole presence of genes within a genome does not confirm their functionality. Genes for the biosynthesis of all 20 standard amino acids have been identified in the common gut

bacterium *Lactococcus lactis*, although supplemented sources of certain amino acids (isoleucine, valine, leucine, histidine, methionine, and glutamic acid) are also required for growth since genes in these biosynthetic pathways have been demonstrated to be non-functional due to point mutations (Bolotin, et al., 2001; Godon, et al., 1993). This may also be the case with another gut bacterium *Staphylococcus aureus*, as two distinct strains exhibited auxotrophy for different amino acids, despite the presence of complete sets of genes for biosynthetic pathways of these essential amino acids in both strains (Kuroda, et al., 2001). Given these different scenarios, it is likely that alternative intestinal bacterial co-metabolic pathways and regulation strategies remain to be discovered.

Glutamate Family (glutamate, glutamine, proline, arginine)

Glutamate and glutamine are both key nitrogen/amino group donors for amino acid synthesis and provide the major entry points of ammonia into bacterial metabolism (H. Shimizu & Hirasawa, 2007). These enzyme pathways are particularly important for some gut bacteria with a reliance on ammonia as a nitrogen source, such as many *Bacteroides* spp., which cannot replace ammonia with other nitrogen sources such as amino acids, peptides, urea or nitrate (reviewed in Fischbach & Sonnenburg, 2011). Glutamate is one of the most important central metabolites in all bacteria providing a link between carbon and nitrogen metabolism. Most enteric bacteria have two primary pathways for the synthesis of glutamate (Fig. 1a) involving either the enzyme glutamate dehydrogenase (GDH) or glutamine oxoglutarate aminotransferase (also called GOGAT or glutamate synthase) (Reitzer & Magasanik, 1987; H. Shimizu & Hirasawa, 2007). Preferential use of either pathway depends on both the energy state and intracellular ammonium concentrations in the cells. GDH is preferentially utilized for glutamate

226 synthesis when both energy and carbon sources are limited for the cell but ammonium
227 and phosphate are present in excess, while the GOGAT pathway is preferred when the
228 cell is not under energy limitation since this pathway requires the expenditure of ATP
229 (Helling, 1994). However, exceptions to this strategy are seen in the common gut
230 bacterium *Bacteroides fragilis* which contains 2 distinct glutamate dehydrogenase
231 enzymes: a dual cofactor NAD(P)H-dependent (gene = *gdhB*) enzyme whose activity
232 depends on high ammonia concentrations, and a NADH-specific enzyme whose activity
233 depends on high peptide concentrations in which ammonia has no effect on activity
234 (Abrahams & Abratt, 1998; Yamamoto, Saito, & Ishimoto, 1987). In the Gram-positive
235 bacterium *Bacillus subtilis*, glutamate is exclusively synthesized by reductive amination
236 of α-ketoglutarate by the enzyme glutamate synthase encoded by the *gltAB* operon
237 (Belitsky, 2002).
238 Glutamine is synthesized by the single reaction of glutamine synthetase (GS; EC
239 6.3.1.2) in which ammonia is added to glutamate through ATP hydrolysis (Fig. 1a).
240 Three distinct types of glutamine synthetases have been identified in bacteria: GSI is
241 found in eubacteria and archaea including the ruminal *R. albus*, GSII is present only in
242 eukaryotes and several soil bacteria: *Rhizobium*, *Frankia*, and *Streptomyces*, while
243 GSIII has only been found in several unrelated bacteria *Bacteroides fragilis*, *Rhizobium*
244 *leguminosarum*, and *Butyrivibrio fibrisolvens*, and *R. albus* (Brown, Masuchi, Robb, &
245 Doolittle, 1994; Kim, Henriksen, Cann, & Mackie, 2014).
246 Several pathways exist for bacterial proline biosynthesis, however the most common
247 pathway found in many groups involves a four-step process starting with the
248 phosphorylation of glutamate (Fig. 1a) (reviewed in (Fichman, et al., 2014). Genomic
249 analysis of proline biosynthesis has revealed that many eubacterial and archael species
250 contain only one recognizable gene from this pathway (i.e. Δ1-pyrroline-5-carboxylate

reductase) while other species lack all genes from this pathway, suggesting that either these bacteria are dependent on exogenous proline sources for growth or that alternative pathways may exist that may not yet be elucidated (Fichman, et al., 2014).

Biosynthesis of arginine involves an eight-step process starting with the acetylation of the glutamate (Fig. 1a). This step occurs through the so-called "classical" pathway initially described for *E. coli* via the enzyme N-acetylglutamate synthase (NAGS) and encoded by the gene *argA* (reviewed in (Xu, Labedan, & Glansdorff, 2007). In many bacterial species, this step can also be achieved by an ornithine *N*-acetyltransferase (OAT) with dual functional activities (E.C. 2.3.1.35 / 2.3.1.1) that can also synthesize acetylglutamate *de novo* from acetyl-CoA and glutamate (Marc, et al., 2000; Xu, et al., 2007).

Serine Family (serine, glycine, cysteine)

Serine is an important metabolite and precursor to multiple amino acids in bacteria (glycine, cysteine, tryptophan). It is also a precursor for the metabolism of sphingolipids, folate, methane, sulfur, cyanoamino acid, and pyruvate, and also participates in the biosynthesis of purines and pyrimidines (Stolz, et al., 2007; Umbarger, 1978). Furthermore, L-serine plays a fundamental role in stabilizing blood sugar concentration in the liver (Remesy, Fafournoux, & Demigne, 1983). Synthesis of serine is a three-step process starting with the glycolytic intermediate 3-phosphoglycerate (Fig. 1b) (Peters-Wendisch, et al., 2005). Glycine is generated from serine by the single enzyme serine hydroxymethyltransferase (Fig. 1b) that catalyzes the reversible conversion of serine and tetrahydrofolate to glycine and 5,10-methylene tetrahydrofolate, which is a key intermediate in the biosynthesis of purine, thymidine, choline, glutathione and methionine (Trivedi, et al., 2002). In enterobacteria and

Bacillus subtilis, the synthesis of cysteine is a two-step reaction (Fig. 1b) that involves the substrates coenzyme A and hydrogen sulfide (H_2S) (Kredich, 1996). *Bifidobacterium longum* lacks the genes for the final step with cysteine synthase and may use alternative pathways with several enzymes such as succinylhomoserine and the sulfur-containing compounds H_2S or methanethiol produced by other colonic bacteria (Schell, et al., 2002). Several strains of *Bifidobacterium bifidum* have further displayed cysteine auxotrophy (Ferrario, et al., 2015), indicating a dependence of exogenous sources of this amino acid.

Aspartate Family (aspartate, asparagine, lysine, threonine, methionine)

Aspartate is an important precursor for the biosynthesis of numerous amino acids. It is synthesized from the transfer of an amino group from glutamate to oxaloacetate via the enzyme aspartate transaminase (Fig. 2). Asparagine is commonly made by one of two distinct asparagine synthetases in bacteria in which one enzyme utilizes ammonia while the other synthetase carries out a transamination reaction from glutamine to aspartate (Fig. 2) (Min, Pelaschier, Graham, Tumbula-Hansen, & Soll, 2002). Alternate pathways exist in some gut bacteria, such as *B. longum*, which lack both types of asparagine synthetases and likely use an asparaginyl-tRNA-dependent route (Schell, et al., 2002).

Two main pathways exist for lysine biosynthesis in bacteria, the diaminopimelic acid (DAP) and aminoadipic acid (AAA) pathways. The DAP pathway is used by most bacteria and some archaea and utilizes aspartate and pyruvate as starting material with meso-2-6-diaminopimelic acid as an intermediate (Fig. 2) (Patte, 1996). Four variations of this pathway have been identified in bacteria: the succinylase (most common) (Fig. 2), acetylase, aminotransferase, and dehydrogenase pathways (Liu, White, & Whitman,

2010). No organism is known to possess both DAP and AAA pathways (Liu, et al., 2010). In *E. coli*, the first step in the DAP pathway employs three distinct aspartate kinase isozymes (ThrA, MetL, and LysC), each specific to one of three different biochemical pathways under regulation from lysine, methionine, and threonine (Vitreschak, Lyubetskaya, Shirshin, Gelfand, & Lyubetsky, 2004).

Synthesis of threonine shares the first two steps of the biosynthetic pathway of lysine (Fig. 2). In addition to the conversion of aspartate to 4-phospho-L-aspartate, ThrA and MetL isozymes also contain a homoserine dehydrogenase (Hom) domain that carries out the third step in the biosynthetic pathway in which aspartate 4-semialdehyde is converted to homoserine (Fig. 2) (Vitreschak, et al., 2004). Methionine shares the first three steps of the biosynthetic pathway of threonine (Fig. 2); after which several different pathways are utilized by different bacteria to attach a sulfur group to O-Succinyl-L-homoserine via either L-cysteine (Fig. 2) or incorporate inorganic sulfur with O-acetylhomoserine to form homocysteine (Rodionov, Vitreschak, Mironov, & Gelfand, 2004).

Pyruvate Family (isoleucine, valine, leucine, alanine)

The branched-chain amino acids (BCAA), valine, leucine and isoleucine share common biosynthetic pathways all stemming from intermediates from pyruvate metabolism. These pathways are so similar that many of the same enzymes are shared for biosynthesis of all these amino acids (Fig. 3). In *E. coli* and *S. enterica* serovar *Typhimurium*, three different isoenzymes of the enzyme acetolactate synthase (AHAS; EC 2.2.1.6;) are made up of a large and small subunit encoded by the respective genes *ilvIH*, *ilvBN*, and *ilvGM* (Umbarger, 1996). Isoleucine biosynthesis starts with the conversion of threonine to 2-oxobutanoate via the enzyme threonine deaminase and

326 then uses the same enzymes as those described for valine biosynthesis (Fig. 3). Leucine
327 biosynthesis uses the last intermediate from the valine pathway, 2-oxoisovalerate, to
328 carry out the initial reaction (Fig. 3) (Pátek, 2007). Leucine has also been demonstrated
329 to be biosynthesized via the precursor SCFA isovalerate via carboxylation in the gut
330 bacteria *Bacteroides fragilis* and *Prevotella ruminicola* (previously *Bacteroides*
331 *ruminicola*) (Allison, Baetz, & Wiegel, 1984). Both of these bacteria also preferentially
332 utilize 2-methylbutyrate as a precursor for isoleucine biosynthesis instead of carrying
333 out *de novo* synthesis starting from glucose (Allison, et al., 1984). Several pathways
334 exist for alanine biosynthesis, with starting precursors such as pyruvate and aspartate
335 among the most common (Fig. 3). L-alanine is mainly synthesized from pyruvate and
336 glutamate via an alanine transaminase (Oikawa, 2007).
337
338 **Aromatic Family (phenylalanine, tyrosine, tryptophan)**
339 Biosynthesis of aromatic amino acids typically follows the shikimate pathway and
340 starts with the condensation of the glycolytic intermediate phosphoenolpyruvate and the
341 pentose phosphate pathway intermediate erythrose 4-phosphate, via the enzyme 3-
342 Deoxy-D-arabinoheptulosonate 7-phosphate (DAHP) synthase (Fig. 4) (Sprenger,
343 2007). In *E. coli*, three distinct isoenzymes (called AroF, AroG and AroH) are involved
344 in this step and each is specifically feed-back inhibited by the terminal end products
345 tyrosine, phenylalanine and tryptophan, respectively (Sprenger, 2007). A single non-
346 homologous DAHP synthase (AroA) is found in the Gram-positive *B. subtilis* (Panina,
347 Vitreschak, Mironov, & Gelfand, 2003). The pathway proceeds for 6 steps to the
348 common intermediate chorismate, which serves as a branchpoint to the individual
349 pathways for phenylalanine, tryptophan and tyrosine (Fig. 4).

Alternative pathways exist for synthesis of each respective aromatic amino acid in different bacterial species, but commonalities within the pathways remain. The first reaction for both phenylalanine and tyrosine uses the enzyme chorismate mutase to convert chorismate to prephenate. In *E. coli*, chorismate mutases are bifunctional enzymes which can participate in the first two steps of both phenylalanine and tyrosine biosynthesis from chorismate (Fig. 4) (Sprenger, 2007). In *E. coli*, both pathways then use either the enzyme aromatic amino acid aminotransferase or aspartate aminotransferase (EC 2.6.1.1) to catalyze the transamination reaction into each respective α-keto acid using glutamate as the amino group donor (Pittard, 1996) (Fig. 4). Aromatic amino acid aminotransferase can also participate in an alternative pathway that catalyzes the conversion of prephenate to L-arogenate which can be further converted to either phenylalanine or tyrosine using prephenate dehydratase or cyclohexadieny/prephenate dehydrogenase, respectively. Instead of *de novo* synthesis of phenylalanine, several gut bacteria (*B. fragilis* and *P. ruminicola*) preferentially use phenylacetate as a precursor for this amino acid (Allison, et al., 1984).

Tryptophan biosynthesis is five-step pathway from chorismate (Fig. 4) in which glutamine or ammonia is used in the first step with anthranilate synthase (Nichols, 1996; Pittard, 1996). In the final step, serine is used as the amino group donor for tryptophan synthase (Sprenger, 2007). The tryptophan biosynthesis pathway is quite conserved in different bacteria, although differences in gene order and the enzymatic reactions carried out by separate or fused enzyme units exist in different bacteria (reviewed in Xie, Keyhani, Bonner, & Jensen, 2003). For example, all of the genes in the biosynthetic pathway for tryptophan have been identified in the gut bacterium *B. longum* except for TrpF (phosphoribosylanthranilate isomerase), indicating a replacement of this gene with an unidentified homolog (Schell, et al., 2002).

Histidine

Histidine biosynthesis is a complex ten-step enzymatic pathway (Fig. 5) encoded by eight different genes, three of which (*hisD, hisB,* and *hisI*) encode bifunctional enzymes (reviewed in (Alifano, et al., 1996). Most of the work on the histidine biosynthetic pathway has mainly been studied in *E. coli* and *Salmonella typhimurium* and more recently in the industrially important *Corynebacterium glutamicum*, demonstrating large conservation along with some differences in the biochemical pathways between the different species (Kulis-Horn, Persicke, & Kalinowski, 2014). Whole genome studies in bacteria identified in the gut such as *L. lactis* confirm the presence of all the genes of this pathway (Bolotin, et al., 2001).

3. Overview of uptake systems and catabolism of proteins/amino acids in gut bacteria

3.1. Protein hydrolysis and peptide/amino acid transporters

Initial steps of bacterial protein catabolism include the extracellular hydrolysis of proteins via different bacterial proteases. According to the MEROPS peptidase database (Rawlings, Waller, Barrett, & Bateman, 2014), bacteria contain a highly diverse number of different proteases, present in many common gut microbiota species such as *Clostridium spp. Bacteroides* spp*., Lactobacillus* spp., etc. containing up to hundreds of different identified proteases. Some bacteria, such as lactic acid bacteria, have developed sophisticated proteolytic systems to compensate their reduced capabilities for amino acid biosynthesis (Pessione, 2012). Proteolytic systems in lactic acid bacteria

consist of either extracellular or membrane-bound proteases (principally PrtP and CEP) that degrade proteins into oligopeptides, followed by their incorporation into the cell via peptide transporters (Opp, Dpp, Dtp, for oligopeptide, dipeptide, and di- and tripeptide, respectively) and finally numerous intracellular peptidases that degrade the peptides into short peptides and amino acids (reviewed in Liu, Bayjanov, Renckens, Nauta, & Siezen, 2010; Steiner, Naider, & Becker, 1995).

Amino acids and their derivatives are imported and exported from the bacterial cell via transmembrane proteins comprising ATP-dependent ABC transporters, several families of channel proteins, or secondary carriers relying on proton-motive force, sodium-ion motive force, solute-solute exchange, or uniport (Saier, 2000). Efflux systems for some amino acids such as lysine, arginine, threonine, cysteine, leucine, isoleucine, and valine are well-known in the bacteria *E. coli* and *Clostridium glutamicum* (Eggeling & Sahm, 2003), whereas to date no export systems for histidine have been described in any organism (Kulis-Horn, et al., 2014).

3.2. Transamination, deamination and decarboxylation

Bacteria may directly incorporate available amino acids as substrates for protein biosynthesis or may carry out catabolic reactions to use them as energy sources or to produce other metabolites. Under aerobic conditions, bacteria typically convert proteinogenic α-amino acids to α-oxo acids (aka. α-ketoacids) or saturated fatty acids via transamination or deamination, which are further oxidized as energy sources in the tricarboxylic acid (TCA) cycle. However, in the absence of oxygen or other suitable electron acceptors, only strict or facultative anaerobic bacteria in the gut, such as Clostridia and Fusobacteria, are capable of utilizing amino acids as energy sources, thus fermenting amino acids to short-chain fatty acids (SCFAs), molecular hydrogen (H_2),

carbon dioxide (CO_2), and ammonia, with minor products of H_2S, methylmercaptane, phenols, alcohols and organic acids (Davila, et al., 2013; J. Kim, Hetzel, Boiangiu, & Buckel, 2004). Several mechanisms for α-amino acid degradation exist for anaerobic bacteria, which includes the well-known Stickland reaction found in many proteolytic Clostridia which involves the coupled oxidation and reduction of two respective amino acids to organic acids. Other fermentation pathways found in various Clostridia as well as *Fusobacterium* spp. and *Acidaminococcus* spp. involve single amino acids that act as electron donors as well as acceptors (Fischbach & Sonnenburg, 2011; J. Kim, et al., 2004). The genus *Clostridium* contains unique amino acid degradation pathways, such as B12-dependent aminomutases, selenium containing oxidoreductases and oxygen-sensitive 2-hydroxyacyl-CoA dehydratases (Fonknechten, et al., 2010).

Amino acids can also be metabolized through decarboxylation reactions ultimately yielding amines and polyamines as products. Factors such as pH can influence the activity of deaminases and decarboxylases, ultimately affecting the accumulation of specific end products. Furthermore, many complex amino acids can undergo a series of metabolic reactions that produce a large variety of structurally-related metabolic end-products (Davila, et al., 2013).

Amino acid utilization may be achieved in a preferential manner, as Fonknechten et al. (2010) demonstrated that *Clostridium sticklandii* preferentially used threonine, arginine and serine for carbon and energy sources, but hardly utilized glutamate, aspartate and aromatic amino acids, even though these catabolic pathways are found in this organism. Furthermore, lysine degradation, which is a process that provides a major energy source, was only observed in stationary growth phases (Fonknechten, et al., 2010).

3.3. Factors influencing protein fermentation:

Many different factors may influence protein fermentation in the gut, such as substrate availability, transit time, pH and osmolarity. The ratio of available carbohydrates:protein determines substrate utilization by the gut microbiota (Smith & Macfarlane, 1996), and in humans, it has been shown that availability of complex carbohydrates lowers protein fermentation (Birkett, Muir, Phillips, Jones, & O'Dea, 1996; De Preter, et al., 2008; Geboes, et al., 2006). When the main energy sources (i.e. fermentable carbohydrates) for microbiota are abundant, nitrogenous substrates can be used for biosynthetic (anabolic) process and bacterial growth. On the contrary, proteins are catabolized by bacteria when energy is scarce, leading to the production of amino acid-derived end products. Due to high carbohydrate fermentation in the proximal colon, there is a progressive decrease of carbohydrate availability in the distal colon, resulting in higher protein fermentation (Macfarlane, Gibson, & Cummings, 1992). Long transit time and elevated pH are also associated with high levels of protein fermentation (Cummings, Hill, Bone, Branch, & Jenkins, 1979; Macfarlane, Cummings, Macfarlane, & Gibson, 1989). Therefore, dietary carbohydrate and protein ratios may strongly influence the metabolic pathways activated in the large intestine and flow of metabolites generated.

4. Key intermediate products from bacterial protein/amino acid catabolism and effects on host physiology/health

Fermentation of amino acids derived from endogenous luminal and dietary protein by intestinal microbiota produces numerous metabolites with suspected or established

474 effects on host intestinal physiology, liver and peripheral tissues. Relevant examples of
475 such metabolites are described below.
476
477 **4.1. Microbially-produced compounds with neuroactive properties**
478 Recent evidence has shown that bacteria isolated from the mammalian gut have the
479 capacity to synthesize neuroactive compounds including neurotransmitters, many of
480 which result from the catabolism of amino acids. These compounds include GABA
481 (produced by *Lactobacillus* spp., *Bifidobacterium* spp., and *Lactococus lactis*);
482 norepinephrine (produced by *Escherichia* spp. and *Bacillus* spp.); dopamine (produced
483 by *Bacillus* spp.); histamine (produced by numerous bacterial genera); and serotonin
484 (produced by *Streptococcus* spp., *Escherichia* spp. and *Enterococcus* spp.) (Sanders, et
485 al., 1998; Wall, Ross, & Stanton, 2014). In addition, bacteria have been demonstrated to
486 produce other neuroactive compounds from amino acid degradation such as nitric oxide
487 and the biogenic amines tryptamine and phenethylamine. It is worth noting that for most
488 of these bacterial metabolites, the understanding of their precise effects on the intestine
489 and peripheral tissues remains in its infancy.
490 γ-aminobutyrate (GABA), known as an inhibitory neurotransmitter, is microbially-
491 produced by the decarboxylation of glutamate via the enzyme glutamate decarboxylase
492 (Table 1). This enzyme makes up part of the glutamate decarboxylase (GAD) system
493 found in several bacterial genera, which is implicated in acid tolerance by maintaining
494 intracellular pH homeostasis through proton consumption (Feehily & Karatzas, 2013).
495 Other factors in addition to acidic stress have been demonstrated to activate the GAD
496 system, including sodium, polyamines and hypoxia (Feehily & Karatzas, 2013). GABA
497 is subsequently exported from the cell via antiporters that import glutamate, or it
498 remains in the cells and is metabolized to succinate via the GABA shunt pathway

(Feehily & Karatzas, 2013; Karatzas, Brennan, Heavin, Morrissey, & O'Byrne, 2010). Glutamate/GABA antiporters are found in numerous Gram-negative (*Escherichia, Shigella, Brucella*) and Gram-positive genera (*Listeria, Lactobacillus, Lactococcus, Clostridium, Bifidobacterium*) (Feehily & Karatzas, 2013). An *in vivo* study in mice also revealed that chronic administration of *Lactobacillus rhamnosus (JB-1)* induced changes in mRNA of GABA receptors B1b and A2, as well as reduced anxiety- and depression-related behavior (Bravo, et al., 2011), although the direct production of GABA by gut bacteria was not investigated. *In vivo* studies are required to evaluate whether gut microbiota derived GABA is active on the host.

Serotonin, a neurotransmitter involved in numerous processes including behavior, learning, appetite and glucose homeostasis, is produced in the human brain but also notably in intestinal enteroendocrine cells (El-Merahbi, Loffler, Mayer, & Sumara, 2015). The key role of serotonin in the gut-brain axis has been extensively reviewed, indicating that peripherally it is involved in modulation of the gut immune system, gastrointestinal secretions, motility and visceral sensitivity and centrally in mood and cognition (O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015). In humans, serotonin is produced from tryptophan degradation in a two-step process via the enzymes tryptophan 5-monooxygenase (tryptophan hyroxylase 1) and aromatic-L-amino-acid decarboxylase (Table 1). It has been experimentally demonstrated *in vitro* that serotonin can also be produced by several bacterial genera isolated from the human gut, including *Streptococcus*, *Escherichia*, *Enterococcus*, *Lactococcus* and *Lactobacillus* (Wall, et al., 2014). However, the full mechanism of direct serotonin production by bacteria has not been clearly established. Analysis of the serotonin biosynthetic pathway in bacteria via the KEGG database reveals that several bacterial genera contain homologous genes for encoding aromatic-L-amino-acid decarboxylase used in the second step of this pathway

(Kanehisa, et al., 2014), but no homologous genes have presently been found for tryptophan 5-monooxygenase. Bacteria can also directly interact with the host to induce the production of serotonin by the host, with experimentally demonstrated effects on host physiology (Yano, et al., 2015). A comparison between germ-free mice and colonized mice (with either mouse or human gut microbiota) has shown that microbiota increased colonic expression of tryptophan hydroxylase 1, the rate limiting enzyme for mucosal serotonin synthesis, and thereby serotonin production, likely acting through SCFAs (Reigstad, et al., 2015).

Histamine and its receptors were first described as part of the immune and gastrointestinal systems, but their presence in the central nervous system and implication in behavior and energy homeostasis is gaining increasing attention (Baronio, et al., 2014). Production of histamine from the decarboxylation of the amino acid histidine has been well demonstrated in numerous Gram-positive and Gram-negative bacterial strains (Landete, De las Rivas, Marcobal, & Munoz, 2008). Gram-positive bacteria, such as lactic acid bacteria, use a pyruvoyl-dependent histidine decarboxylase (EC 4.1.1.22) (Table 1), whereas Gram-negative bacteria use a pyridoxal phosphate-dependent histidine decarboxylase (Landete, De las Rivas, et al., 2008). In *Lactobacillus* spp. histamine is readily exported from the cell using electrogenic histidine/histamine antiporters (Molenaar, Bosscher, ten Brink, Driessen, & Konings, 1993). Bacterial histamine production can exert both positive and negative effects on human health. Recently, Thomas et al. (2012) demonstrated that histamine derived from a human gut isolate of the species *Lactobacillus reuteri* suppresses pro-inflammatory TNF-α production in isolated Toll-like receptor 2-activated human monocytoid cells, indicating a potential beneficial effect of microbially-derived compounds for inflammatory conditions. However, histamine production by bacteria has also been

549 implicated in food poisoning related to the ingestion of fish fermented by histamine-
550 producing bacteria (scombrotoxin) (Bjornsdottir-Butler, Green, Bolton, & McClellan-
551 Green, 2015).
552 Tyrosine is a precursor for several hormones including the thyroid hormones
553 thyroxine (3,5,3',5'-tetraiodothyronine) and triiodothyronine, as well as a precursor to L-
554 dihydroxyphenylalanine (L-DOPA) which is in itself a precursor to the catecholamine
555 neurotransmitters dopamine, norepinephrine and epinephrine. Bacterial tyrosinases (EC
556 1.14.18.1) (Table 1), which catalyze the conversion of tyrosine to L-DOPA, are widely
557 found in many bacterial genera (Claus & Decker, 2006). Although *Escherichia* spp. and
558 *Bacillus* spp. have been reported to produce norepinephrine (Wall, et al., 2014),
559 enzymes necessary for production of the catecholamines norepinephrine and
560 epinephrine have only been identified to date in animals, suggesting some mode of
561 cooperation between host and gut microbes in the catecholamine biosynthetic process.
562 However, catecholamine stress hormones have been demonstrated to promote both
563 bacterial growth and virulence (Freestone, 2013). Some opportunistic pathogens
564 inhabiting the intestinal tract (*Escherichia coli*, *Staphylococcus aureus*, etc.) are
565 equipped with catechol siderophore uptake systems that facilitate the import Fe(III)-
566 catecholamine complexes and, thereby, their growth under iron-restricted conditions
567 (Beasley, Marolda, Cheung, Buac, & Heinrichs, 2011).
568 Tyramine is a biogenic amine capable of causing food poisoning if consumed at high
569 levels and is involved in hypertension and migraine syndromes (Marcobal, De las
570 Rivas, Landete, Tabera, & Munoz, 2012; Millichap & Yee, 2003). Tyramine is
571 produced by the decarboxylation of tyrosine by the bacterial enzyme tyrosine
572 decarboxylase (Table 1), which has been identified in Gram-positive bacteria such as
573 lactic acid bacteria, but putative tyrosine decarboxylases in Gram-negative bacteria

574 remain to be characterized (Marcobal, et al., 2012). Tyramine is exported from bacterial
575 cells via a tyrosine-tyramine antiporter (Linares, Fernandez, Martin, & Alvarez, 2009).
576 In Gram-positive bacteria, high concentrations of tyrosine and acidic pH conditions
577 have been demonstrated to increase the transcription of tyrosine decarboxylase and the
578 tyrosine-tyramine antiporter (Linares, et al., 2009; Marcobal, et al., 2012). Gut
579 microbiota derived tyramine has been shown to increase circulating serotonin levels by
580 elevating its synthesis by enteroendocrine cells (Yano, et al., 2015) although this
581 mechanism has not been defined.
582 Nitric oxide (NO) is produced from the amino acid arginine by many bacterial
583 species via the enzyme nitric oxide synthase (NOS) (Table 1), although only several
584 bacterial homologs of mammalian NOSs have been identified to date (Sudhamsu &
585 Crane, 2009). Little is known about the extent that microbially-produced NO impacts
586 the human host, notably at the intestinal mucosa level, although it is known that NO is
587 involved in immune defense mechanisms and exerts cytotoxic effects at high
588 concentrations.
589 Tryptamine is a β-arylamine neurotransmitter that has numerous biological roles
590 including a ligand for the sigma-2 receptor, a trace amine-associated receptor that
591 increases the inhibitory responses of cells to serotonin, and an inducer of serotonin
592 release from enteroendocrine cells which can modulate gastrointestinal motility and
593 may play a role in the pathology of inflammatory bowel diseases (Williams, et al., 2014
594 and references therein). Recently it was demonstrated that tryptamine is produced by
595 *Clostridium sporogenes* by the decarboxylation of tryptophan via a Trp decarboxylase
596 (Table 1) (Williams, et al., 2014). Williams et al. (2014) also used a phylogenic-
597 informed screening of other decarboxylases from the gut microbiota to show that a

second Trp decarboxylase appears in the Firmicute *Ruminococcus gnavus*, and similar enzymes were found in at least 10% of the human population.

Phenethylamine is a trace amine that acts as a neurotransmitter and, depending on the concentrations, is associated with specific psychological disorders (Irsfeld, Spadafore, & Pruss, 2013) and can affect satiety and mood (Pessione, 2012). Phenethylamine is produced in bacteria by the decarboxylation of phenylalanine (Diaz, Ferrandez, Prieto, & Garcia, 2001) via the enzyme aromatic-L-amino-acid decarboxylase (Table 1). Potential physiological effects of microbially-derived phenethylamine on host health have yet to be elucidated.

4.2. Short- and branched-chain fatty acids

The main short-chain fatty acids (SCFAs) produced in the large intestine by the mammalian gut microbiota are acetate, butyrate and propionate (Rechkemmer, Ronnau, & von Engelhardt, 1988). Although it is well known that dietary substrates for SCFA production are mainly fibers and resistant starches (Laparra & Sanz, 2010), isolated colonic bacteria growing *in vitro* on proteins as the only available carbon source have been demonstrated to produce SCFAs as well as branched-chain fatty acids (BCFAs) (Neis, et al., 2015). Bacterial fermentation of glycine, alanine, threonine, glutamate, lysine and aspartate can produce acetate; threonine, glutamate and lysine can produce butyrate, and alanine and threonine can produce propionate (Davila, et al., 2013). Nonetheless, the molecular pathways involved in production of these SCFAs are not well-defined.

Branched-chain fatty acids (BCFAs), namely isobutyrate, 2-methylbutyrate and isovalerate, are derived from branched-chain amino acids and are present at much lower concentrations in the large intestine luminal content (Liu, et al., 2014). Although a full

623 description of bacterial synthesis of BCFA from amino acids is beyond the scope of this
624 article, many Gram-positive bacteria use the primers isovaleryl-CoA, isobutyryl-CoA,
625 and 2-methylbutyryl-CoA derived from valine, isoleucine, and leucine to produce
626 BCFA using a specialized branched-chain-keto acid dehydrogenase complex (Cronan &
627 Thomas, 2009). The relative proportions of SCFAs and BCFAs depend on numerous
628 factors including the type of protein degraded as well as the available concentrations of
629 carbohydrates (Macfarlane & Macfarlane, 2012).
630 The SCFAs acetate, propionate and butyrate produced via bacterial fermentation of
631 carbohydrates and/or amino acids have been proposed to exert various physiological
632 effects, which have been recently reviewed (Hamer, et al., 2008). Briefly, for instance,
633 butyrate provides energy to colonocytes and regulates cell proliferation and
634 differentiation as well as the transcription of numerous genes involved in mucin
635 production and hormone secretion (i.e. PYY, GLP-1, GLP-2) that influence gut
636 integrity and transit, appetite and glucose metabolism (Daly, Cuff, Fung, & Shirazi-
637 Beechey, 2005). Butyrate also plays an anti-inflammatory role via different
638 mechanisms, including inhibition of LPS-mediated inflammatory cytokine secretion by
639 intestinal epithelial cells and other immune cells and via induction of colonic regulatory
640 T cells partly by an epigenetic modification of the forkhead box-P3 promoter
641 (Furusawa, et al., 2013). Propionate and acetate undergo partial oxidation in
642 colonocytes or can travel to the liver via the portal vein where they serve as substrates
643 for gluconeogenesis and lipogenesis (Tremaroli & Backhed, 2012). Acetate is also a
644 substrate for cholesterol synthesis, whereas propionate can decrease cholesterol
645 synthesis (Demigne, et al., 1995). In addition, SCFAs are ligands for G protein-coupled
646 receptors, namely Gpr41 and Gpr43, which are expressed in enteroendocrine L-cells of
647 the distal small intestine and colon (Reigstad, et al., 2015). Gpr41 deficiency has been

shown to be associated with reduced expression of peptide YY (PYY), a gut hormone involved in satiety and gut motility (Samuel, et al., 2008). Direct administration of SCFAs in the colon increase PYY plasma level, and *in vitro* administration on primary colonic crypts also increase the release of PYY, suggesting a direct effect of SCFA on secretion (Chambers, et al., 2014; Cherbut, et al., 1998). Butyrate can also bind the GPR109a receptor expressed by intestinal macrophages and dendritic cells, thus activating production of the anti-inflammatory cytokine Il-10 (Pabst & Bernhardt, 2010). BCFAs have also been shown to regulate electrolyte absorption and secretion (Musch, Bookstein, Xie, Sellin, & Chang, 2001). It has been proposed that SCFAs could exert effects beyond the gut via their interactions with Gpr expressed in different tissues and cellular types (e.g. adipocytes, pancreatic cells, neuronal cells) and thereby regulate metabolism by inducing energy expenditure and mitochondrial function (Gao, et al., 2009). Whether these effects could be mediated by the SCFAs generated by the gut microbiota requires further investigation.

4.3. Sulfur-containing bacterial metabolites

Fecal S-containing substances are a complex mixture of bacterial metabolites including methanethiol and the well-studied H_2S, which is found at millimolar concentrations in the human colonic luminal contents and in feces (Macfarlane, et al., 1992). In addition to production of H_2S via reduction of inorganic sulfate by intestinal sulfate reducing bacteria or microbial catabolism of intestinal sulfomucins, sulfide is produced by intestinal microbiota activity through fermentation of several S-containing amino acids of both dietary and endogenous origins (Blachier, et al., 2010). H_2S is notably produced as a byproduct by the bacterial fermentation of cysteine via cysteine desulfhydrases, cystathione-B-lyase and tryptophanase (Table 1) (Awano, Wada, Mori,

673 Nakamori, & Takagi, 2005). Fermentation of methionine yields the sulfur-containing
674 compound methanethiol (Davila, et al., 2013). Due to its lipophilic activity, H_2S
675 penetrates biological membranes (Reiffenstein, Hulbert, & Roth, 1992) and, when
676 present in excess, inhibits mitochondrial cytochrome c oxidase activity reversibly with a
677 binding constant similar to cyanide (Leschelle, et al., 2005). At lower concentrations,
678 H_2S is able to increase cellular respiration and ATP production by means of the
679 mitochondrial sulfide-oxidizing unit (Bouillaud & Blachier, 2011). Although several
680 lines of evidence suggest that excessive concentrations of sulfide in the intestinal
681 luminal content may be implicated in the etiology of ulcerative colitis (Pitcher &
682 Cummings, 1996) and in the risk of relapse after an inflammatory episode (Jowett, et
683 al., 2004), there is also evidence that endogenously-formed low levels of H_2S in
684 intestinal mucosa may participate in the resolution of mucosal inflammation (Flannigan,
685 et al., 2015). Experiments from colonocyte incubation and intra-colonic instillation
686 with millimolar concentrations of NaHS, used as a H_2S donor, indicate that hydrogen
687 sulfide reversibly inhibits colonocyte oxygen consumption and increases the expression
688 of hypoxia-inducible factor 1 alpha (HIF-1alpha) together with several inflammation-
689 related genes, namely inducible nitric oxide synthase (iNOS) and interleukin 6 (Il-6)
690 (Beaumont, et al., 2016). Importantly, it has been demonstrated that endogenously
691 produced H_2S maintains colon cancer cellular bioenergetics supporting colonic tumor
692 growth (Szabo, et al., 2013).
693
694 **4.4. Aromatic compounds**
695 In addition to neuroactive compounds, the catabolism of aromatic amino acids
696 (phenylalanine, tyrosine and tryptophan) by the microbiota also produces phenolic and
697 indolic compounds (Nyangale, Mottram, & Gibson, 2012). Recent support for a role of

gut microbiota in the production of various aromatic amino acid metabolites has recently been demonstrated by comparing metabolites between specific pathogen-free (SPF) mice and germ-free (GF) mice (Sridharan, et al., 2014). However, bacterial metabolism of aromatic compounds largely depends on the type of carbon availability, as fermentable carbohydrates largely inhibit aromatic amino acid fermentation (Smith & Macfarlane, 1996).

Phenol is produced from the conversion of tyrosine via the enzyme tyrosine phenol-lyase (Table 1). Optimal conditions for production of phenol by anaerobic bacteria in the human large intestine include a near-neutral pH of 6.8 and availability of free amino acids as opposed to peptides (Smith & Macfarlane, 1997). The types of intestinal bacteria may also play a significant role in production of phenol as aerobic bacteria tended to produce phenol from tyrosine degradation *in vitro* while anaerobic bacteria produced *p*-cresol (Bone, Tamm, & Hill, 1976). Phenol has been shown to decrease the integrity of the barrier function of colonocytes *in vitro* (Hughes, Kurth, McGilligan, McGlynn, & Rowland, 2008) and impaired the viability of human colonic epithelial cells at concentrations higher than 1.25 mM (Pedersen, Brynskov, & Saermark, 2002).

Formation of *p*-cresol (or 4 methylphenol) by microbes begins with the two-step conversion of tyrosine to 4-hydroxyphenylacetate (Meyer & Hostetter, 2012), although the genes involved in this conversion are unknown (Table 1) (Dawson, et al., 2011). In the final step, the bacterium *Clostridium difficile* uses the enzyme *p*-hydroxyphenylacetate decarboxylase (genes *hpdB, hpaC, hpdA*) to convert 4-hydroxyphenylacetate to *p*-cresol (Selmer & Andrei, 2001; Yu, Blaser, Andrei, Pierik, & Selmer, 2006). The *hpdBCA* operon is rarely found in gut microflora other than *Clostridium* spp. (Dawson, et al., 2011), and therefore it is unknown to what extent, if any, other gut genera play a role in *p*-cresol formation. Production of *p*-cresol is

723 stimulated by Fe(III) and competitive growth conditions in *Clostridium* spp. (Doerner,
724 Mason, Kridelbaugh, & Loughrin, 2009; Selmer & Andrei, 2001). The *p*-cresol
725 concentration in human feces averages approximately 0.4 mM (Gostner, et al., 2006;
726 Lecerf, et al., 2012). These compounds are absorbed from the intestinal lumen to the
727 portal bloodstream through colonocytes, metabolized in the liver and finally excreted by
728 the kidneys with more than 90% of urinary phenolic compounds being recovered as *p*-
729 cresol (Hughes, Magee, & Bingham, 2000). At millimolar concentrations, *p*-cresol
730 inhibits human colonocyte proliferation and cell respiration and increases superoxide
731 production (Andriamihaja, et al., 2015). Most importantly, in this latter study, *p*-cresol
732 was found to be genotoxic towards human colonocytes. *p*-cresol can be conjugated in
733 the colonic epithelium and in the liver, generating *p*-glucuronide and cresyl sufate,
734 (Evenepoel, Meijers, Bammens, & Verbeke, 2009). Phenolic compound sulphation has
735 been shown to be impaired in the mucosa of ulcerative colitis patients (Ramakrishna,
736 Roberts-Thomson, Pannall, & Roediger, 1991). Interestingly, *p*-cresyl sulfate promotes
737 insulin resistance in chronic kidney disease (CKD) patients (Koppe, et al., 2013) and is
738 found at elevated concentrations in the urine of autism patients (Heinken & Thiele,
739 2015). It can also cause damage in renal tubular cells by induction of oxidative stress
740 through activation of NADPH oxidase (Watanabe, et al., 2013).
741 Indole is produced from tryptophan via the enzyme tryptophanase (Table 1), which is
742 found only in microbes (Meyer & Hostetter, 2012). After absorption through the
743 intestinal epithelium, indole is transported to the liver where it undergoes hydroxylation
744 by the host to 3-hydroxy-indole and finally sulfonated to indoxyl sulfate, a uremic
745 toxin, via a sulfotransferase (Meijers & Evenepoel, 2011; Wikoff, et al., 2009). In
746 contrast, indole also has a potential beneficial effect on host intestinal epithelial cells, as
747 treatment of human enterocytes with indole was found to result in increased expression

748 of genes involved in the mucosal barrier functions and was associated with a reduction
749 of inflammatory parameters (Bansal, Alaniz, Wood, & Jayaraman, 2010). Secretion of
750 the incretin GLP-1 from enteroendocrine cells is increased during short exposures to
751 indole via interference of voltage-gated K^+ channels, but reduced over long periods of
752 exposure to indole via slowing ATP production by blocking NADH dehydrogenase
753 (Chimerel, et al., 2014). Besides its role in glucose metabolism through increasing
754 insulin secretion, GLP-1 is involved in inhibiting gastric secretion and motility; these
755 phenomenons being associated with increased satiety (Steinert, Beglinger, & Langhans,
756 2015). The indole derivative indole-3-aldehyde was also shown to regulate gut mucosal
757 immune response through aryl hydrocarbon receptor as well as intestinal barrier
758 function and inflammation through its sensing by pregnane X receptor (Venkatesh, et
759 al., 2014; Zelante, et al., 2013).
760 Serum levels of the amino acid tryptophan itself also have a demonstrated role in
761 host health, as decreases in this amino acid have been associated with increased immune
762 activation, inflammatory diseases such as Crohn's disease, as well as cognitive deficit
763 (Gupta, et al., 2012; Widner, et al., 1999). This may be particularly detrimental in
764 elderly populations, as increased proportions of genes from gut microbiota involved in
765 aromatic amino acid metabolism (including tryptophan), as well as decreased levels of
766 tryptophan have been reported in elderly patients (Collino, et al., 2013; Rampelli, et al.,
767 2013).
768
769 **4.5. Polyamines**
770 Intestinal luminal polyamines (i.e. agmatine, putrescine, spermidine, cadaverine,
771 etc.) can originate from dietary components, microbiota metabolism (Davila, et al.,
772 2013), and endogenous origin, notably released from desquamated intestinal epithelial

773 cells. Gram-negative bacteria such as *E. coli* produce high concentrations of putrescine
774 and spermidine in minimal media (Tabor & Tabor, 1985). Numerous amines (including
775 polyamines) have been measured in the intestinal luminal contents at concentrations
776 ranging from micro- to millimolar (Osborne & Seidel, 1990). Intestinal epithelial cells
777 have the capacity to take up polyamines from the intestinal luminal contents (Blachier,
778 et al., 1992).

779 Agmatine is formed by the decarboxylation of the amino acid arginine via the
780 enzyme arginine decarboxylase (Table 1). Agmatine, which is produced and released
781 by colonic bacteria as well as by desquamated intestinal epithelial cells and ingested in
782 food, exerts inhibitory effects on colonocyte proliferation (Mayeur, et al., 2005).
783 Putrescine can be synthesized by bacteria from the amino acid arginine either through
784 the intermediate ornithine or through agmatine (Nakada & Itoh, 2003). Agmatine is
785 either directly converted to putrescine via the enzyme arginine decarboxylase, or goes
786 through a two-step process which uses the enzymes agmatine deiminase and N-
787 carbamoylputrescine amidase (Table 1). The agmatine deiminase pathway has been
788 found in several bacterial genera such as *Pseudomonas*, *Enterococcus*, *Bacillus* and
789 *Lactobacillus* (Landete, Arena, Pardo, Manca de Nadra, & Ferrer, 2008). Agmatine-
790 putrescine antiporters have been identified in bacterial genera and function by importing
791 agmatine and exporting putrescine (Polo, Gil-Ortiz, Cantin, & Rubio, 2012). Putrescine
792 synthesis has been shown to be strictly necessary for colonic epithelial cell proliferation
793 (Gamet, Cazenave, Trocheris, Denis-Pouxviel, & Murat, 1991; Mouille, Delpal,
794 Mayeur, & Blachier, 2003).

795 In many bacteria including *E. coli*, spermidine is synthesized from putrescine and
796 decarboxylated S-adenosylmethionine (SAM) via spermidine synthase (Tabor & Tabor,
797 1985). Recently, bacteria lacking spermidine synthase orthologues were discovered to

798 have an alternate pathway in which spermidine is formed by the enzymes
799 carboxynorspermidine dehydrogenase (aka carboxynorspermidine synthase) and
800 carboxynorspermidine decarboxylase via the intermediate carboxyspermidine (Lee, et
801 al., 2009). This alternative pathway of spermidine synthesis has been identified as the
802 dominant pathway in the human gut microbiota as well as diverse human pathogens and
803 is critical for growth in selected species (Hanfrey, et al., 2011). In host cells, SAM can
804 react with spermidine via another enzyme, spermine synthase, to produce spermine.
805 Prokaryotic cells do not appear to contain spermine synthase, but they can produce a
806 different tetra-amine compound, thermospermine, that has been detected in lower
807 eukaryotes and plants (Minguet, Vera-Sirera, Marina, Carbonell, & Blazquez, 2008).
808 Spermidine and spermine are polycationic amines that are involved in numerous
809 processes such as mitigating oxidative stress, and induction of autophagy to stimulate
810 cellular longevity (Eisenberg, et al., 2009; Yamamoto, et al., 2012).
811 The polyamine cadaverine is synthesized from lysine in a one-step reaction with
812 lysine decarboxylase (Table 1) and has been shown to provide an acid resistance
813 mechanism in *E. coli* (Le Gall, et al., 2011). However, the effects of cadaverine on the
814 colonic epithelial cells remain unknown.
815
816 **4.6. Ammonia**
817 Ammonia provides the source of nitrogen for all amino acids and is primarily
818 assimilated through either glutamate dehydrogenase or the glutamine
819 synthetase/GOGAT cycle. It is also used directly in the biosynthesis of various amino
820 acids including glutamate, glutamine, asparagine, valine, isoleucine, leucine,
821 phenylalanine, tyrosine and tryptophan (Figs. 1-4). Ammonia can be produced by the
822 microbial degradation of numerous amino acids to specific metabolites (i.e. arginine to

putrescine, tyrosine to phenol, tryptophan to indole, Table 1). Ammonia (taken as the sum of NH_3 and NH_4^+) is found at millimolar concentrations in the large intestine luminal content of mammals including humans (Mouille, Robert, & Blachier, 2004). In humans, the luminal ammonia concentration progressively increases from the ascending to the descending colon (Macfarlane, et al., 1992), in accordance with a higher rate of protein fermentation in the distal colon. The luminal ammonia concentration in the large intestine is primarily the net result of microbiota utilization and production through amino acid deamination, urea hydrolysis, and absorption from the luminal content to the portal blood, with the unabsorbed/unmetabolized ammonia being excreted in feces (Eklou-Lawson, et al., 2009). Although relatively large amounts of ammonia can be transferred from the intestinal lumen to the bloodstream, a part of this ammonia can be metabolized by colonocytes into citrulline and glutamine (Eklou-Lawson, et al., 2009; Mouille, et al., 2004), allowing control of the intracellular ammonia concentration in colonocytes during its transfer from the luminal content to the portal bloodstream. Ammonia inhibits mitochondrial oxygen consumption in a dose-dependent manner, leading to the concept that excessive luminal ammonia concentration behaves as a metabolic troublemaker towards colonocyte energy metabolism (Andriamihaja, et al., 2010). Accordingly, high millimolar concentrations of ammonia have been shown to markedly inhibit short-chain fatty acid oxidation in isolated colonocytes (Cremin, Fitch, & Fleming, 2003).

5. Gut microbiome features related to amino acid metabolism as a function of the subject's metabolic phenotype and the diet

847 Recent metagenomic studies have allowed a detailed examination of the metabolic
848 capacity of the mammalian gut microbiome in metabolizing nitrogenous components,
849 particularly amino acid-related compounds. An important insight from these studies
850 revealed that the gut microbiome contains a large enrichment of genes involved in
851 amino acid metabolism compared to the human genome (Gill, et al., 2006), thus
852 expanding the human metabolic capacity to form a more diverse number of metabolites.
853 In particular, the human microbiome had large enrichments of genes involved in the
854 biosynthesis of lysine, phenylalanine, tyrosine, tryptophan, valine, leucine and
855 isoleucine as well as enrichment in genes associated with the metabolism of alanine,
856 aspartate, glutamate, histidine, methionine, glycine, serine, threonine and the urea cycle,
857 with only slight to moderate enrichments of genes for the metabolism of other amino
858 acids and non-protein amino acids (Gill, et al., 2006). Qin et al. (2010) also found an
859 enrichment of genes involved in pathways such as the biosynthesis of lysine,
860 phenylalanine, tyrosine, tryptophan, valine, leucine and isoleucine in the human gut
861 microbiome compared to the host genome. Since biosynthetic pathways for essential
862 amino acids do not exist in humans, it is not surprising that gut microbiota has
863 developed a specialized set of genes and metabolic pathways for synthesizing these
864 essential nutrients to ensure its survival.
865 Metagenomic sequencing analysis has shown that the metabolic phenotype (obese
866 *versus* lean subjects) and the diet (e.g. high-fat diets, prebiotic intake, etc.) are
867 associated with shifts in both specific microbiota taxonomic groups and functions of the
868 mammalian gut microbiome, such as those involved in the metabolism of amino acids.
869 Recent studies comparing the short-term effects of animal- and plant-based diets on the
870 human gut microbiota and expression of metabolic-related genes using RNA-Seq
871 revealed an increased expression of several catabolic amino acid genes for glutamine

872 and glutamate in animal-based diets, while increased expression of biosynthetic
873 pathways for these amino acids was observed for plant diets (David, et al., 2014). David
874 et al. (2014) also found that KEGG modules and pathways involved in methionine and
875 leucine biosynthesis and cysteine metabolism were significantly associated with animal
876 diets, while histidine biosynthesis and lysine and branched-chain amino acid
877 degradation were significantly associated with plant diets.
878 In studies in rodents, Turnbaugh et al. (2006) found that KEGG pathways related to
879 lysine biosynthesis and D-alanine metabolism were significantly enriched in the pooled
880 cecal microbiome of *ob/ob* obese mice relative to the pooled lean cecal microbiome,
881 while the KEGG pathways involved in the metabolism of glutamate, glycine, serine,
882 threonine, cysteine, arginine and proline and the biosynthesis of phenylalanine, tyrosine
883 and tryptophan were depleted in the *ob/ob* mouse microbiome relative to the lean one.
884 Comparison of obese/lean sibling pairs of mice revealed that KEGG pathways involved
885 in phenylalanine, tyrosine and tryptophan biosynthesis were also depleted in the cecal
886 microbiomes of the obese mice (Turnbaugh, et al., 2006). Furthermore, using fecal
887 samples from obese and lean human twin pairs, Turnbaugh et al. (2009) revealed a
888 depletion of the KEGG pathway involved in tryptophan metabolism in obese twins
889 compared to their lean counterparts. Recent studies by Everard et al. (2014) revealed
890 that the clusters of orthologous group (COG) for amino acid transport and metabolism
891 was enriched in both high-fat diet-fed (HFD) mice and high-fat diet-fed mice treated
892 with the prebiotic oligofructose (HFD-Pre) compared to controls with and without
893 prebiotic treatment, with the HFD-Pre mice yielding the highest enrichment of all
894 groups. Nevertheless, the physiological meaning of those changes in gut microbiome
895 amino acid metabolic pathways associated with genetically (*ob/ob*) or diet-induced
896 (HFD) obesity remains unknown. Furthermore, to our knowledge there have not been

detailed metagenomic studies examining the effect of a high-protein (HP) diet in either murine models or humans.

Recent metabolomic studies have provided further evidence of the role of gut microbiota in amino acid metabolism. Zheng et al. (2011) compared metabolites produced by the host (rat) and microbiota during antibiotic administration and also after a 14-day recovery period and showed that the recovered mammalian gut microbiota alters the host's systemic metabolism in terms of production of short chain fatty acids, tryptophan and tyrosine and possibly indole-melatonin. Metabolomic analyses have also recently suggested that individual amino acids may play key roles in diet-related diseases. For example, high concentrations of branched-chain amino acids (BCAA) and their respective metabolites have been linked with obesity and type 2 diabetes, while aromatic amino acids and high ratios of glutamate/glutamine have also been associated with type 2 diabetes (reviewed in Heinken & Thiele, 2015). Although the association of BCAA and other amino acids with insulin resistance and type 2 diabetes has been well-known for decades, Newgard et al. (2009) recently performed metabolomic profiling of obese and lean humans to show that the addition of branched-chain amino acids (valine, leucine, isoleucine) and two aromatic acids (phenylalanine and tyrosine) to a high-fat diet contributes to the development of obesity-associated insulin resistance. Wang et al. (2011) also carried out metabolic profiling to investigate whether specific metabolites could predict diabetes development and discovered that the same five branched-chain and aromatic acids were strongly associated with the onset of diabetes, thus suggesting a potential role of amino acid metabolism in the pathogenesis of diet-related diabetes. Although the direct role of the gut microbiota in production of these amino acids has not yet been clearly defined *in vivo*, theoretically the gut bacteria have the capacity to

921 synthesize all of these essential amino acids and, thereby, play a role in those
922 conditions. However, more work is needed to elucidate this connection.
923 In the case of increased dietary protein intake, the amount of undigested protein that
924 is transferred to the large intestine markedly increases (Chacko & Cummings, 1988).
925 Consequently, more substrate is available for bacterial amino acid catabolism. However,
926 the consequences of this increased availability is not well described at a metabolic level
927 as only a few studies have only partially examined microbial-derived metabolite profiles
928 in HP diets by a non-targeted analytical approach in which all potential metabolites are
929 analyzed. Russell et al. (2011) demonstrated that HP diets with low and moderate
930 carbohydrate intake in humans displayed increased branched-chain fatty acids,
931 phenylacetic acid and *N*-nitroso compounds compared to a weight-maintenance diet
932 with moderate protein levels at the beginning of the intervention study. Reduced
933 proportions of butyrate and antioxidant phenolic acids were also detected in the HP low-
934 carbohydrate diets (Russell, et al., 2011) likely as a result of the low carbohydrate
935 intake. Those changes in fecal metabolic profiles were associated with a decrease in
936 proportions of the known butyrate-producing bacteria *Roseburia*/*Eubacterium rectale*
937 (Russell, et al., 2011). Other studies in humans found that H_2S fecal concentration was
938 increased in humans fed a meat-rich diet (Magee, Richardson, Hughes, & Cummings,
939 2000), and increased levels of phenol, *p*-cresol and phenylacetate have been detected in
940 urine and feces of individuals receiving HP diets (Cummings, et al., 1979; Geypens, et
941 al., 1997; Russell, et al., 2011). Increasing the amount of alimentary protein also results
942 in an increase of the luminal and fecal ammonia concentration in humans (Geypens, et
943 al., 1997). HP diets in humans also caused elevations in plasma levels and urinary
944 excretion of indoxyl sulfate and urinary excretion of indoxyl glucuronide, kynurenic
945 acid and quinolinic acid (Poesen, et al., 2015). Although several of these compounds are

946 strictly produced only by gut microbiota, no study has effectively examined the direct
947 role of the gut microbiota in the production of these metabolites.
948 In murine models, HP diets have been shown to modify the composition and the
949 diversity of the colonic microbiota in rats, with associated changes in the total amount
950 of ammonia, SCFAs, H_2S, branched-chain fatty acids, ethanol and several organic acids
951 (L- and D-lactate, succinate) in the colonic luminal content (Liu, et al., 2014). Although
952 this experiment did not determine the direct role of microbiota composition and
953 increased substrate availability in the changes observed after HP diet ingestion, it
954 appears that both parameters are likely to be involved in such changes. A metabolomic
955 analysis comparing a HP, low-carbohydrate (HPLC) diet to a moderate
956 protein/carbohydrate (MPMC) diet in a rat model revealed that the HPLC diet induced
957 weight loss and reduced adipose weight, and the plasma metabolites glucose, insulin,
958 triglyceride, linoleate, palmitate, α-glycerophosphate and pyroglutamic acid and caused
959 a significant increase in several plasma metabolites (i.e. urea, pyruvate, α-tocopherol, 2-
960 oxoisocaproate, and β-hydroxybutyrate) (Mu, Yang, Luo, & Zhu, 2015). In the plasma
961 of mice, increases in tryptophan and other aromatic acid-derived metabolites such as
962 indole-3-acetic acid, p-cresyl glucuronide, phenyl sulfate and phenylacetic acid were
963 detected in HP diets (Poesen, et al., 2015). In the urinary metabolite profile, the HPLC
964 urinary metabolite profile showed an increase in the fatty acids palmitate and stearate
965 and a reduction of pantothenate and the TCA cycle intermediates citrate, 2-ketoglutarate
966 and malate (Mu, et al., 2015). In addition to murine models, study designs in humans
967 still need to be refined to integrate data from complementary functional omics-
968 technologies to progress in the understanding of the flow of metabolites between the
969 microbiota and the host derived from dietary protein and their physiological
970 consequences on the metabolic phenotype.

6. Impact of protein intake levels on health-related outcomes: possible contribution of gut microbiota metabolic pathways

Some of the metabolites produced by the microbiota from amino acids have been shown to be active on numerous host functions as illustrated above. These results suggest that some of the effects of HP diets on host metabolism and physiology may involve some of these metabolites and, therefore, microbiota-mediated metabolic pathways. In the following sections, the effects of high dietary protein intake observed in human and animal studies are summarized and suggest mechanisms that might implicate the gut bacteria-derived metabolites (Fig. 6).

6.1. HP diet-induced satiety

Two recent meta-analyses of clinical trials concluded that, when compared to normoproteic (NP) diet, a HP diet modestly reduces body weight, blood pressure, fat mass and triglyceride levels while sparing fat-free mass (Santesso, et al., 2012; Wycherley, Moran, Clifton, Noakes, & Brinkworth, 2012). Those effects are mainly attributed to dietary protein induced satiety (Westerterp-Plantenga, et al., 2009). In humans, HP diet-induced satiety is associated with an increase of PYY plasma levels (Batterham, et al., 2006). Implication of gut microbiota in those mechanisms, however, has not been investigated yet. Nevertheless, SCFAs, indole, tryptamine and tyramine are compounds produced by the gut microbiota from amino acid precursors and are candidates for mediating the effects of HP diet on enteroendocrine cell hormone secretion with an impact on satiety (Figure 6).

6.2. HP diet effects on the intestinal mucosa

In the rat model, HP diet consumption for 15 days resulted in a marked reduction in the height of the colonocyte brush-border when compared with control animals receiving a NP/hyperglucidic isocaloric diet (Andriamihaja, et al., 2010). This morphological change could be related to lower energy efficiency in HP rat colonocytes due to proton leaks in the mitochondrial inner membrane (Andriamihaja, et al., 2010). A reduction of the mucosal myeloperoxidase activity (representative of neutrophil infiltration) together with a down-regulation of mucosal T_{helper} cytokines was measured in the ileum of rats receiving a HP diet (Lan, et al., 2015). This decreased inflammatory status was associated with a hyperplasia of mucus-producing cells concomitant with an increased expression of Muc2 at both the gene and protein levels (Lan, et al., 2015). A HP diet also induced DNA damages in rat colonocytes, in association with an increase in the bacterially-derived genotoxic metabolite *p*-cresol (Toden, Bird, Topping, & Conlon, 2005).

In an intervention study comparing volunteers receiving either HP diets or low protein isocaloric diet for two weeks, no evidence was found for a role of protein fermentation in gut toxicity in healthy human subjects despite identification of several metabolites in fecal water with presumably cytotoxic and genotoxic effects towards colonic epithelial cells (Windey, et al., 2012). SCFA, H_2S, *p*-cresol and ammonia are metabolites produced from amino acids that could interfere with colonocyte energy metabolism and mucin secretion and may mediate the effects of HP diet on the intestinal mucosa. These metabolites could partly result from the activity of gut microbiota (Figure 6). However, the consequences of a HP intake on large intestine mucosal health have never been directly investigated in humans.

6.2.3. HP diet effects in inflammatory bowel diseases

SCFAs, H_2S, indolic compounds, serotonin and histamine are all amino acid-derived bacterial metabolites which are likely to interfere with the process of mucosal inflammation due to their effects on the mucosal immune response and on the maintaining of the epithelial barrier function (Figure 6). As reviewed by Halmos and Gibson (2015), only a few studies have examined the impact of the level of protein consumption on the risk of inflammatory bowel disease or risk of relapse in diagnosed patients. Jowett et al. (2004) reported an increased risk of relapse over 1 year in ulcerative colitis patients with the highest meat and protein consumption. A HP diet was also associated with an increased risk of incident inflammatory bowel diseases (Jantchou, Morois, Clavel-Chapelon, Boutron-Ruault, & Carbonnel, 2010), while Shoda et al. (1996) demonstrated that increased consumption of animal protein was related to increased incidence of Crohn's disease. However, another study did not find any association between elevated protein intake and development of ulcerative colitis (Hart, et al., 2008).

6.2.4. HP diet effects in colorectal cancer

Experimental studies with animal models of chemically-induced colonic preneoplasic and neoplasic lesions have shown that dietary protein can influence the colonic carcinogenesis process, depending on their quantity and quality (McIntosh & Le Leu, 2001). The production of amino-acid derived genotoxic metabolites produced by the microbiota (e.g. *p*-cresol) could be implicated in this process. However, the complexity of the Western diet makes the identification of alimentary compounds that impact the risk of colorectal cancer a difficult task. In this context, it has been proposed that the positive association between high consumption of red and processed meat and colorectal cancer found in some epidemiological studies, may result from both the

composition of meat (e.g. heme and protein) and from compounds generated by the cooking process (Kim, Coelho, & Blachier, 2013). In support of this hypothesis, a recent study has shown that hydrogen sulfide, which is produced by the gut microbiota from L-cysteine released from dietary and endogenous protein, can drive mucin denaturation and possibly increase the access of heme (a cytotoxic and genotoxic compound) to colonic epithelial cells (Ijssennagger, et al., 2015).

6.3. HP diet effects on kidney function

It was concluded from a systematic review that indoxyl sulfate and *p*-cresyl sulfate are toxic for kidneys notably through reactive-oxygen species (ROS) generation (Vanholder, Schepers, Pletinck, Nagler, & Glorieux, 2014). Moreover, it has been proposed that *p*-cresol level in the blood is an indicator of CKD severity (Bammens, Evenepoel, Keuleers, Verbeke, & Vanrenterghem, 2006). Recently, it has been shown that CKD alters gut microbiota composition (Vaziri, Wong, et al., 2013) together with an increased bacterial metabolic capacity for nitrogen utilization, as evidenced by the increased abundance of urease, indole- and *p*-cresol-forming enzymes in CKD patients (Wong, et al., 2014). Indeed, *p*-cresyl sulfate (or glucuronide) and indoxyl sulfate are known conjugates of the gut microbiota-produced metabolites *p*-cresol and indole, respectively, and are elevated in the serum of chronic kidney diseases (CKD) patients (Vanholder & Glorieux, 2015). Furthermore, the elevated uremia in CKD induces an important diffusion of urea into the intestinal lumen, and it has been shown that elevated urea concentration impairs intestinal barrier function and induces inflammation in the digestive tract, most likely through excessive ammonia production by bacterial ureases (Vaziri, Yuan, & Norris, 2013), raising the view that CKD may impact gut health. Those events may play a causative role in the establishment of CKD-associated

dysbiosis. Moreover, CKD patients generally have a lower fiber intake, prolonged transit time and protein malabsorption, all of which favor protein fermentation in the gut (Evenepoel, et al., 2009).

Moderate protein intake (0.6-0.8 g protein/kg/day) has been demonstrated to be beneficial for CKD patients (Fouque, Pelletier, Mafra, & Chauveau, 2011). Reduced renal exposure to gut microbiota amino acid-derived metabolites through control of protein intake may contribute to better health outcomes. In healthy subjects, HP diets increase glomerular filtration rate, but the long-term consequences for kidney health remains unclear (Marckmann, Osther, Pedersen, & Jespersen, 2015). Thus, renal consequences of increased colonic protein fermentation should be further studied in healthy subjects.

6.4. Strategies to limit toxicity associated with the control of protein fermentation

Increased fermentable carbohydrate intake is one of the nutritional strategies that may help to limit adverse effects of protein fermentation, as demonstrated in an intervention study which has shown that dietary resistant starch lowers excretion of ammonia and phenols (Birkett, et al., 1996). In healthy subjects, *p*-cresyl sulfate and indoxyl sulfate levels are markedly lower in vegetarians than in omnivores, likely because of a higher fiber intake and lower protein intake (Patel, Luo, Plummer, Hostetter, & Meyer, 2012). In rats, supplementation with resistant starch protects against HP diet-induced DNA damages in colonocytes in association with decreased excretion of *p*-cresol (Toden, Belobrajdic, Bird, Topping, & Conlon, 2010). Resistant carbohydrate supplementation also attenuates renal injury possibly through a decreased production of amino acid-derived metabolites by the microbiota (Vaziri, et al., 2014). In CKD patients, improved disease markers (including lower *p*-cresyl sulfate) were

observed after resistant starch supplementation (Vanholder & Glorieux, 2015). Unfortunately, CKD patients are advised to restrict their fruit and vegetable intake to avoid potassium overload, thus excluding one of the main fiber sources. In that context, the use of fibers may be an efficient tool to reduce protein fermentation (De Preter, Hamer, Windey, & Verbeke, 2011). This has been demonstrated in human studies with isotope-labeled biomarkers showing that fiber (lactulose) efficiently lowered ammonia and *p*-cresol production by the gut microbiota (De Preter, et al., 2004).

Consumption of specific probiotic bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium* has also been shown to decrease urinary *p*-cresol although to a lesser extent than with prebiotics, and with no effect on ammonia (De Preter, et al., 2004; Wutzke, Lotz, & Zipprich, 2010). In CKD patients, some probiotic bacteria used to reduce the formation of gut-derived toxins have been investigated, but the level of evidence remains low (Rossi, et al., 2014). Combining pre- and probiotics (known as "synbiotics") has also been considered an attractive approach, but requires further clinical investigation (De Preter, et al., 2007).

Other strategies such as elementary nutrition (free amino acid supplementation) or the use of substrates that bind deleterious compounds derived from amino-acid fermentation (e.g. zinc that binds H_2S) have been proposed to minimize their effects in medical conditions (e.g. CKD patients) (Mimoun, et al., 2012), but are out of the scope of this review.

7. Conclusion and perspectives.

Dietary protein metabolism is the result of the interplay between host and gut bacterial metabolic pathways but their respective roles and contributions to host

physiology and metabolic health remain undefined. Several gut microbiota-derived amino acid metabolites may theoretically have both beneficial and deleterious physiological consequences on host cells and tissues, but so far their effects have only been tested individually (i.e. in un-physiological conditions). The use of global-scale omics technologies in tightly-controlled intervention studies will help to identify the specific gut microbial metabolic pathways activated in response to dietary protein and to disentangle the flow of metabolites between the host and the gut microbiota and their contribution to host physiology. Moreover, since human diets consist of a wide range of different protein sources (plant and animal) with different characteristics (digestibility, amino acid composition); these differences also need to be taken into consideration for interpretation of metadata obtained in the context of a varied diet. These future studies will represent an important and necessary step for a better understanding of the complex interplay between diet, microbiota and host metabolism and physiology and will contribute to informing microbiome-based dietary recommendations.

Acknowledgements

This works is supported by the European Union's Seventh Framework Program under the grant agreement no 613979 (MyNewGut).

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Figure Captions

Figure 1: Prokaryotic biosynthetic enzymatic pathways of (A) glutamate and (B) serine amino acid families. Enzymes with associated gene names compiled from the KEGG database are provided for each step in reaction pathways. Gene names include orthologous and paralogous gene groups among different bacterial taxa. Intermediate metabolites (yellow boxes) from the most common reaction pathways are provided for each end product amino acid (blue boxes) from each enzymatic family.

Figure 2: Prokaryotic biosynthetic enzymatic pathways of the aspartic acid amino acid family. Enzymes with associated gene names compiled from the KEGG database are provided for each step in reaction pathways. Gene names include orthologous and paralogous gene groups among different bacterial taxa. Intermediate metabolites (yellow boxes) from the most common reaction pathways are provided for each end product amino acid (blue boxes) from each enzymatic family.

Figure 3: Prokaryotic biosynthetic enzymatic pathways of the pyruvate amino acid family. Enzymes with associated gene names compiled from the KEGG database are provided for each step in reaction pathways. Gene names include orthologous and paralogous gene groups among different bacterial taxa. Intermediate metabolites (yellow boxes) from the most common reaction pathways are provided for each end product amino acid (blue boxes) from each enzymatic family.

Figure 4: Prokaryotic biosynthetic enzymatic pathways of the aromatic acid amino acid family. Enzymes with associated gene names compiled from the KEGG database are provided for each step in reaction pathways. Gene names include orthologous and

paralogous gene groups among different bacterial taxa. Intermediate metabolites (yellow boxes) from the most common reaction pathways are provided for each end product amino acid (blue boxes) from each enzymatic family.

Figure 5: Prokaryotic biosynthetic enzymatic pathway of histidine. Enzymes with associated gene names compiled from the KEGG database are provided for each step in reaction pathways. Gene names include orthologous and paralogous gene groups among different bacterial taxa. Intermediate metabolites (yellow boxes) from the most common reaction pathways are provided for each end product amino acid (blue boxes) from each enzymatic family.

Figure 6: High protein diet effects with potential involvement of gut microbiota metabolism. Left side: Peptides are degraded by the intestinal microbiota which release amino acid derived metabolites in the lumen. Gut bacteria can also produce amino acids. Right side: Host-functions affected by a high-protein diet demonstrated in human and animal studies (capital letters and bold). High protein diets increase the amount of protein available for fermentation by gut microbiota, which may mediate some of the effects of high protein diets on host physiology. Candidate bacterial amino acid derived metabolites for each effect of high protein diet are proposed. *(Illustrations are from Servier Medical Art)*

Table 1: Gut microbiota-produced metabolites from amino acid catabolism that have potential effects on host physiology and health. Genetic pathways for each metabolite are described along with enzymes and respective genes for each step in metabolic pathways. Gene names identified in the bacterium *E. coli* are highlighted in

1947 bold. Gene names include orthologous and paralogous gene groups among different
1948 bacterial taxa. All gene names are compiled from KEGG database except where noted.
1949 Amino acid abbreviations refer to glutamate (Glu); tryptophan (Tryp); histidine (His);
1950 tyrosine (Tyr); arginine (Arg); phenylalanine (Phe); cysteine (Cys); methionine (Met);
1951 lysine (Lys).

Metabolite	Amino Acid	Step	Substrate(s)	Product(s)	Enzyme (Enzyme Commision Number)	Gene(s)
Neuroactive compounds						
GABA	Glut	1	L-Glutamate	γ-Aminobutanoate, CO_2	Glutamate decarboxylase (4.1.1.15)	***gadA, gadB***
Serotonin (5-hydroxytryptamine)	Tryp	1	L-Tryptophan, Tetrahydrobiopterin, O_2	5-Hydroxy-L-tryptophan, Dihydrobiopterin, H_2O	Tryptophan 5-monooxygenase (1.14.16.4)	No genes found in prokaryotes
		2	5-Hydroxy-L-tryptophan	Serotonin, CO_2	Aromatic-L-amino-acid decarboxylase (4.1.1.28)	*ddc, dcd, dcd1*
Histamine (pathway 1)	His	1	L-Histidine	Histamine, CO_2	Histidine decarboxylase (4.1.1.22)	*hdc, hdcA, angH, pmsA, basG, dchS, vlmD*
Histamine (pathway 2)	His	1	L-Histidine	Histamine, CO_2	Aromatic-L-amino-acid decarboxylase (4.1.1.28)	*ddc, dcd1*
L-DOPA	Tyr	1	L-Tyrosine, O_2	3,4-Dihydroxy-L-phenylalanine, (DOPA), H_2O	Tyrosinase (1.14.18.1)	*melO, melC1, melC2*
		2	2 N(ω)-Hydroxyarginine, NADH, H^+,	2 Nitric oxide, 2 L-Citrulline,	Nitric-oxide synthase	*nos, nosA, yflM, rplB,*

			O_2	NAD^+, 2 H_2O	(1.14.13.165)	*sdaAB, txtD*
Tyramine (pathway 1)	Tyr	1	L-Tyrosine	Tyramine, CO_2	Tyrosine decarboxylase (4.1.1.25)	*tyrDC, tdcA, mfnA, mfmA, gadD, gadB*
Tyramine (pathway 2)	Tyr	1	L-Tyrosine	Tyramine, CO_2	Aromatic-L-amino-acid decarboxylase (4.1.1.28)	*ddc, dcd, dcd1*
Nitric Oxide	Arg	1	L-Arginine, NADH (or NADPH), H^+, O_2	N(ω)-Hydroxyarginine, NAD^+ (or $NADP^+$), H_2O	Nitric-oxide synthase (1.14.13.165)	*nos, nosA, yflM, rplB, sdaAB, txtD*
Tryptamine	Tryp	1	L-Tryptophan	Tryptamine, CO_2	Aromatic-L-amino-acid decarboxylase (4.1.1.28)	*dcd, dcd1, ddc*
Phenylethylamine	Phe	1	L-Phenylalanine	Phenethylamine, CO_2	Aromatic-L-amino-acid decarboxylase (4.1.1.28)	*dcd, dcd1, ddc, tyrDC*
Sulfide-containing metabolites						
H_2S (pathway 1)	Cys	1	L-Cysteine, H_2O	H_2S, Pyruvate, Ammonia	Cysteine desulfhydrase (4.4.1.1)	*cysA, metB, metC, yrhB, mccB,*
H_2S (pathway 2)	Cys	1	L-Cysteine, H_2O	H_2S, Pyruvate, Ammonia	Cystathionine beta-lyase	***metC**, metB, **malY**, **patB***

					(4.4.1.8)	
Methanethiol	Met	1	L-Methionine, H_2O	Methanethiol, Ammonia, 2-Oxobutanoate	Methionine-gamma-lyase (4.4.1.11)	*mdeA, megL, metB, metZ*
Aromatic compounds						
Phenol	Tyr	1	L-Tyrosine, H_2O	Phenol, Pyruvate, Ammonia	Tyrosine phenol-lyase (4.1.99.2)	*tpl*
p-cresol	Tyr	1	L-Tyrosine	unknown	unknown	Pathway unknown (Dawson et al., 2011)
		2	unknown	unknown	unknown	
		3	4-hydroxyphenylacetate, H^+	4-methylphenol (*p*-cresol), CO_2	4-hydroxyphenylacetate decarboxylase (4.1.1.83)	*hpdB, hpdC, hpdA* (Yu et al., 2006)
Indole	Tryp	1	L-Tryptophan, H_2O	Indole, Pyruvate, Ammonia	Tryptophanase (4.1.99.1)	***tnaA***
Polyamines						
Agmatine	Arg	1	L-Arginine	Agmatine, CO_2	Arginine decarboxylase (4.1.1.19)	***speA***, ***adiA***, *cad, pdaD, aaxB*
Putrescine (pathway 1)	Arg	1	L-Arginine	Agmatine, CO_2	Arginine decarboxylase	***speA***, ***adiA***, *cad, pdaD,*

					(4.1.1.19)	*aaxB*
		2	Agmatine, H_2O	Putrescine, Urea	Agmatinase (3.5.3.11)	***speB***, *speB1, speB2, pah, gbh*
Putrescine (pathway 2)	Arg	1	L-Arginine	Agmatine, CO_2	Arginine decarboxylase	***speA***, ***adiA***, *cad, pdaD, aaxB*
					(4.1.1.19)	
		2	Agmatine, H_2O	N-Carbamoylputrescine, Ammonia	Agmatine deiminase (3.5.3.12)	*aguA, aguA1, aguA2*
		3	N-Carbamoylputrescine, H_2O	Putrescine, CO_2, Ammonia	N-carbamoylputrescine amidase (3.5.1.53)	*aguB*
Putrescine (pathway 3)	Arg	1	L-Arginine, H_2O	L-Ornithine, Urea	Arginase (3.5.3.1)	*rocF*
		2	L-Ornithine	Putrescine, CO_2	Ornithine decarboxylase (4.1.1.17)	***speC***, ***speF***
Spermidine (pathway 1)	Arg	1	S-Adenosylmethioninamine, putrescine	5'-Methylthioadenosine, Spermidine	Spermidine synthase (2.5.1.16)	***speE***
Spermidine (pathway 2)	Arg	1	L-aspartate 4-semialdehyde, putrescine, NADPH, H^+	carboxyspermidine, H_2O, $NADP^+$	Carboxynorspermidine synthase (1.5.1.43)	*cansdh*
		2	carboxyspermidine	spermidine, CO_2	Carboxynorspermidine decarboxylase (4.1.1.96)	*nspC, cansdc*

	Spermine	Arg	1	S-Adenosylmethioninamine, Spermidine	5'-Methylthioadenosine, Spermine	Spermine synthase (2.5.1.22)	SMS (not found in prokaryotes)
	Cadaverine	Lys	1	L-Lysine	Cadaverine, CO_2	Lysine decarboxylase (4.1.1.18)	***ldcC, cadA,*** *speA, cad*

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