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1 # Colonic bacterial metabolites and human health

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13 **Abstract (100-120 words)**

14 The influence of the microbial–mammalian metabolic axis is becoming increasingly important for
15 human health. Bacterial fermentation of carbohydrates and proteins produces short-chain fatty
16 acids (SCFA) and a range of other metabolites including those from aromatic amino acid (AAA)
17 fermentation. SCFA influence host health as energy sources and via multiple signalling mechanisms.
18 Bacterial transformation of fibre-related phytochemicals is associated with a reduced incidence of
19 several chronic diseases. The 'gut–liver axis' is an emerging area of study. Microbial deconjugation of
20 xenobiotics and release of aromatic moieties into the colon can have a wide range of physiological
21 consequences. In addition, the role of the gut microbiota in choline deficiency in non-alcoholic fatty
22 liver disease and insulin resistance is receiving increased attention.

23

24

25 **Highlights:**

26 - *Diet-driven changes in microbially-produced SCFA can influence health via signalling*
27 - *Gut microbiota mediates the release and transformation of many bioactive phenolics*
28 - *Gut microbiota degrades dietary choline to methylamines*
29 - *Interactions between the microbiota, inflammasomes and host influence liver disease*

30

31

32 **Abbreviations:** SCFA, short-chain fatty acids; CHO, carbohydrate; FFAR, free fatty acid receptor; WL,
33 weight loss; NSP, non-starch polysaccharide 'fibre'; AAA, aromatic amino acids; NAFLD, non-alcoholic
34 fatty liver disease; NASH, non-alcoholic steatohepatitis; HMS, hepatic macrovesicular steatosis; PC,

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35 phosphatidylcholine; PEMT, phosphatidylethanolamine-*N*-methyltransferase; SNP, single nucleotide
36 polymorphism; TMA, trimethylamine; TMAO, trimethylamine-*N*-oxide.

37 Introduction

38 The human large intestine is colonised by dense microbial communities that utilise both diet- and
39 host-derived energy sources for growth, predominantly through fermentative metabolism. This
40 highly diverse community has the capacity to perform an extraordinary range of biochemical
41 transformations that go well beyond those encoded by the host genome, and these activities exert
42 an important influence upon many aspects of human health. Metabolites formed by the gut
43 microbiota are largely determined by the composition of the diet and the pattern of food intake, and
44 it is now clear that the species composition of the colonic microbiota is itself altered by the diet
45 [1*,2, 3**]. This review will consider selected examples where recent progress has been made in
46 understanding the links between diet, gut microbial activity and metabolites relevant to health.
47

48 Bacterial metabolites derived from the fermentation of plant-derived
49 carbohydrates and their impact on the host

50

51 Many carbohydrates (CHOs) present in plant-derived foods are digested slowly, if at all, in the small
52 intestine, making them available for microbial fermentation in the large intestine. Intake of starch
53 that is resistant to digestion in the small intestine (resistant starch) can have benefits for metabolic
54 health [4] and results in changes in the gut microbiota [1*]. Recent work also shows a beneficial
55 influence of whole grain intake upon inflammation, again with concomitant changes in the gut
56 microbiota [5*]. Diet-induced changes in the metabolic activity of the gut microbiota are thought
57 likely to mediate these effects.
58

59 Hexose and pentose sugars are fermented by isolated human colonic bacteria via pathways leading
60 to the formation of acetate, succinate, propionate, butyrate, formate, lactate, ethanol, hydrogen
61 and CO_2, depending on the strain and species. Butyrate formation occurs in certain Firmicutes
62 bacteria, either via butyrate kinase (in many *Clostridium* and *Coprococcus* species) or via butyryl
63 CoA:acetate CoA transferase [6]. The latter pathway is found in the numerically predominant
64 butyrate-producing species of *Roseburia*, *Eubacterium rectale, E. hallii* and *Faecalibacterium*
65 *prausnitzii*, and involves the net uptake of external acetate [7]. Acetate is produced by most
66 anaerobes, including acetogens that are able to perform reductive acetogenesis from formate or
67 hydrogen plus CO_2. Producers of succinate and propionate largely belong to the phylum

68 Bacteroidetes, but also include some Firmicutes. Lactate can be formed by many groups, but is
69 generally converted into acetate, propionate or butyrate by a subset of lactate-utilizing species [8].
70 Formation of the gases hydrogen and CO_2 varies widely between species in pure culture; in the
71 mixed community these products are partially converted to acetate, methane or hydrogen sulfide
72 [9]. The net outcome of all of these complex cross-feeding interactions for a typical healthy
73 microbiota is that, in faecal samples, acetate is the dominant short-chain fatty acid (SCFA) detected
74 (typically 40 – 70 mM) followed by propionate and butyrate (each 10 - 30 mM) [10]. While
75 alternative products such as ethanol, succinate and lactate are normally found at lower
76 concentrations, they can accumulate in some circumstances and a link has been proposed between
77 endogenous alcohol formation and non-alcoholic steatohepatitis [11].
78
79 At these concentrations, SCFA have a major impact on the large intestinal environment and on
80 absorption from the lumen. While butyrate is largely utilised by the gut epithelium, and propionate
81 is largely metabolised in the liver, acetate is the SCFA that reaches the highest concentrations in
82 plasma [10]. There is increasing evidence that acetate plays an important role in controlling
83 inflammation and in combating pathogen invasion [12,13]. Acetate and lactate were also found
84 recently to influence cyclin gene expression and epithelial cell proliferation in a pH-dependent
85 manner *in vitro* [14]. The importance of butyrate as an energy source for epithelial cells has long
86 been recognised, but its role in regulating inflammation, cellular differentiation and apoptosis, and
87 in helping to prevent colorectal cancer, is still emerging [15]. Interestingly, butyrate was recently
88 found to be the most potent SCFA in activating the AP-1 signalling pathway in epithelial cell lines
89 [16]. Interactions have been recognised between SCFA and the host cell receptors FFAR2 and FFAR3
90 that might influence satiety, protect against diet-induced obesity and improve insulin sensitivity,
91 with propionate considered to have a potentially important role [17,18]. In view of this it is
92 important to understand how diet and microbiota composition can influence relative, as well as
93 total, SCFA production. Studies in obese subjects on weight loss diets demonstrate that dietary
94 intake of CHO has a major impact on faecal SCFA concentrations [19,20**] presumably reflecting
95 decreased fermentation in the colon (Fig. 1). More surprising, however, is that butyrate per cent
96 responded disproportionately, an effect that correlates with a marked decrease in the *Roseburia-E.*
97 *rectale* group of butyrate-producing bacteria [19]. This may be explained by the greater dependence
98 of this group, compared with other members of the microbiota, on intake of resistant dietary CHOs,
99 and provides evidence that SCFA relative production rates are responsive to diet composition. An
100 inverse relationship has been noted between faecal pH and butyrate concentration *in vivo* [21]; this

101 is likely to reflect the great competitive ability of some butyrate-producers at the reduced pH arising
102 from active fermentation in the proximal colon [22].

103 Decreased numbers of butyrate-producing bacteria, especially *Faecalibacterium prausnitzii*, have
104 been noted in patients suffering from Crohn's disease. This species exerts anti-inflammatory effects
105 that appear to involve soluble factors in addition to butyrate [23]. Interestingly, *F. prausnitzii* was
106 recently shown to diminish the impact of the acetate-producing species *B . thetaiotaomicron* on
107 mucus production and goblet cell development in a gnotobiotic rodent model [24].
108

109 Formation and metabolism of aromatic compounds

110

111 Fibre-related phytochemicals

112 It is suggested that the inverse relationship between the intake of fibre-rich diets and the incidence
113 of several chronic diseases is mediated in part by the gut microbiota. Microbial release of
114 phytochemical metabolites may be a contributing factor and most widely studied for disease
115 prevention are the aromatic metabolites produced by the phenylpropanoid pathway [25,26].
116 Increasing the fibre content of the diet from 8.8 to 14 g day^{-1} in a human volunteer study resulted in
117 significantly increasing certain phenolic acids and their derivatives in the gut, specifically ferulic acid,
118 4-hydroxy-3-methoxyphenylpropionic acid and 3-hydroxyphenylpropionic acid [20]. Ferulic acid,
119 which is found extensively bound to plant polysaccharides, can be released and metabolised by the
120 gut microbiota [20,27] (Fig. 2). Indeed, the major esters of other phenolic acids such as caffeic acid
121 (chlorogenic and caftaric acid) are also rapidly de-esterified by human faecal microbiota [28]. It
122 appears that the gut microbiota can effectively de-esterify compounds, whether the conjugate is
123 quinic acid, tartaric acid or a sugar moiety to release the aglycone for further metabolism. Gut
124 bacteria can also effectively hydrogenate the α,β-unsaturated bond present on the side chain of
125 phenolic acids [27] and the extent to which this occurs appears to be dependent on additional
126 dietary factors, with high-protein diets decreasing the efficiency of this transformation [20]. Site-
127 specific dehydroxylation and demethylation of the phenolic hydroxyl present in phenolic acids has
128 also been observed [20, 27]. The resultant microbial products of ferulic acid metabolism had
129 differing effects on prostanoid production *in vitro* suggesting that the microbial transformation of
130 dietary compounds will have important consequences for inflammation [27,29].
131

132 Aromatic amino acid metabolites

133 Protein metabolism is a major alternative mechanism for production of aromatic metabolites [30] as
134 observed in recent human dietary interventions involving carefully controlled intakes of CHO and

135 protein [20]. Until recently, the major metabolites of aromatic amino acid (AAA) fermentation were
136 considered to be phenol, *p*-cresol and indole, with *p*-cresol suggested to be a product of phenol
137 catabolism. It has now been demonstrated that a much wider metabolic pathway of metabolism
138 exists for all three AAAs [31]. In particular, phenylacetic acid, 4-hydroxyphenylacetic acid and indole-
139 3-acetic acid were found to be major (de-aminated and chain-shortened) products of phenylalanine,
140 tyrosine and tryptophan, respectively [31]. Bacteria capable of producing these products could
141 effectively metabolise all three AAA substrates. These included *Bacteroides thetaiotaomicron, B.*
142 *eggerthii, B. ovatus, B. fragilis, Parabacteroides distasonis* and the Gram-positive bacteria
143 *Clostridium bartlettii* and *Eubacterium hallii*. Bacterial species that did not substantially produce
144 these de-aminated and chain-shortened products were identified. These included *Megamonas*
145 *hypermegale, Roseburia intestinalis, Ruminococcus obeum, Eubacterium rectale* and
146 *Faecalibacterium prausnitzii*, but strains of these species often produced higher amounts of benzoic
147 acid, 4-hydroxybenzoic acid and indole-3-carboxylic acid and oxidation products including
148 phenylpyruvic acid, phenyllactic acid, 4-hydroxyphenyllactic acid, indole-3-pyruvic acid and indole-3-
149 lactic acid. Given that certain species of gut bacteria can metabolise all three AAAs by specific
150 mechanisms, it is likely that other structural forms of amino acids can undergo these molecular
151 transformations. This will give rise to a range of novel metabolites, which require to be investigated
152 to assess their potential to affect human health.
153
154 It is clear that macronutrient balance influences not only the composition of the gut microbiota but
155 also the availability of aromatic metabolites. Certain metabolites such as SCFA and phenyl
156 metabolites can be produced by bacterial metabolism of both CHO and protein in the large intestine,
157 whereas certain branched-chain fatty acids and nitrogen-containing metabolites are considered to
158 be derived from protein metabolism alone. There is a positive association between animal protein
159 consumption (specifically red and processed meat) and colorectal cancer [32]. Evidence is also
160 beginning to emerge that the concentrations of aromatic gut metabolites in the systemic circulation
161 plays a role in vascular health and [33].
162

163 **Enterohepatic circulation and β-glucuronidase**

164 Many diet-derived aromatic compounds, including drugs, are treated as xenobiotics and are
165 conjugated in the liver followed by release into the intestine via the bile. One of the main
166 mechanisms for conjugation is glucuronidation, but it has been known for some time that bacterial
167 β-glucuronidases in the large intestine tend to cleave these conjugates, thus releasing the aromatic
168 moiety and making it available again for re-absorption. The *gus* gene from *Escherichia coli* was
169 originally identified as encoding this activity. A recent survey used degenerate *gus* primers to detect

170 related genes among the faecal microbiota from 10 healthy volunteers; this showed a highly uneven
171 distribution with 60 % of sequences accounted for by only 4 operational taxonomic units, while in
172 total 96 % of sequences came from Firmicutes and 3 % from *E. coli* [34]. It seems likely that this
173 activity is associated with enzymes involved in degrading plant polysaccharides. The contribution of
174 a second putative β-glucuronidase gene identified from metagenomic libraries [35] has still to be
175 fully established [34].
176

177 The 'gut–liver' axis, dietary amines, the intestinal microbiota and the 178 methylamines' pathway

179 The 'gut–liver' axis

180 Given the exposure of the liver to intestinal-derived catabolites and the microbiota to biliary/waste
181 products, the 'gut–liver axis' is receiving great attention with respect to host health and its potential
182 to affect systemic host processes [36]. A recent study has nicely demonstrated the direct
183 involvement of the gut microbiota in the development of obesity-independent non-alcoholic fatty
184 liver disease (NAFLD), and the microbiota's influence on whole body glucose homeostasis and liver
185 lipid metabolism [37**]. Germ-free mice inoculated with intestinal microbiota from a mouse that
186 developed hyperglycaemia and had a high plasma concentration of pro-inflammatory cytokines after
187 being fed a high-fat diet developed hepatic macrovesicular steatosis (HMS) after high-fat feeding,
188 with increased expression of hepatic genes involved in *de-novo* lipogenesis and lipid uptake (SREBP,
189 ChREBP, acetyl-CoA carboxylase 1 and CD36) observed. In comparison, germ-free mice inoculated
190 with faeces from a mouse that was normoglycaemic and had a lower level of systemic inflammation
191 after being fed a high-fat diet developed low-level steatosis on the same diet [37**]. Differences
192 were observed in the faecal microbiota of the two groups of mice: *Lachnospiraceae* and *Barnesiella*
193 (*Porphyromonadaceae*) sequences were significantly overrepresented in the HMS mice, while the
194 low-level steatosis mice had an increased number of sequences related to *Bacteroides vulgatus*.
195 Concentrations of isobutyrate and isovalerate, branched-chain amino acids resulting from the
196 bacterial fermentation of valine and leucine, respectively, were significantly higher in the caecum of
197 the HMS mice. In addition, these animals had significantly higher fasting glycaemia, fasting
198 insulinaemia, homeostasis model assessment—insulin resistance index and leptinaemia, and higher
199 plasma concentrations of aspartate aminotransferase than the animals that developed low-level
200 steatosis. Taken together, these results demonstrate that the gut microbiota constitutes an
201 environmental factor driving the progression of NAFLD [37**].
202

203 Although both groups of animals were fed the same diet in the Le Roy study [37**], it is well known
204 that the intestinal microbiota can influence the 'gut–liver axis' and the development of NAFLD (and
205 other diseases) by microbial utilization of dietary methylamines.
206

207 **Choline deficiency and NAFLD**

208 Choline is an essential nutrient of the vitamin B complex with numerous roles in the body: acting as
209 a methyl donor in biochemical reactions, as a precursor for the biosynthesis of phospholipids
210 [phosphatidylcholine (PC), lysophosphatidylcholine, choline plasmalogen and sphingomyelin], of
211 acetylcholine and of lipoproteins, and in homocysteine reduction [38,39,40]. The main fate of
212 choline in the body is its incorporation into PC via the Kennedy pathway [41].
213

214 Exogenous choline is derived from either dietary choline or, more commonly, PC from plant and
215 animal material [38,39,42]. Foods high in choline include meat and dairy products, fish, soybeans,
216 nuts and whole grains, with PC added to a number of foods as an emulsifier [43]. Endogenous
217 sources of choline, in the form of PC, include biliary lipids, exfoliated epithelial cells and intestinal
218 bacteria [44,45]. *De novo* synthesis of choline occurs via a reaction catalysed by
219 phosphatidylethanolamine-*N*-methyltransferase (PEMT) [41].
220

221 The intestinal microbiota plays a role in the catabolism of choline in humans and rodents
222 [46,47,48,49,], with trimethylamine (TMA), acetate and ethanol the products of fermentation [50].
223 Choline degradation by the human intestinal microbiota is temporally stable [47]. TMA produced by
224 intestinal bacteria from choline is absorbed by colonic cells and converted to trimethylamine-*N*-
225 oxide (TMAO) by flavin mono-oxygenase enzymes [51], demethylated into dimethylamine and
226 (mono)methylamine in the liver, or excreted in the urine. The methylamine pathway is a typical
227 example of microbial–mammalian co-metabolism [52,53] (Figure 3).
228

229 Knowledge pertaining to those members of the intestinal microbiota responsible for producing TMA
230 from choline is sparse. *In silico* predictions have suggested that several members of the human
231 intestinal microbiota (including *Clostridium*, *Anaerococcus*, *Collinsella*, *Desulfitobacterium*, *Klebsiella*,
232 *Escherichia*, *Providencia*, *Yokenella* and *Proteus* spp.) have the ability to degrade choline to TMA via
233 choline TMA-lyase [54**]. In addition to the aforementioned species, many more members of the
234 human intestinal microbiota may be able to degrade choline to TMA using the same mechanism
235 and/or via an alternative pathway(s).
236

237

238 Choline-deficient diets in humans (≤50 mg day^{-1}) and rodents are known to lead to NAFLD, non-
239 alcoholic steatohepatitis (NASH) and hepatic damage [39,55]. To combat these and other
240 complications (e.g. infertility, renal haemorrhage and hypertension), the Food and Nutrition Board of
241 the Institute of Medicine of America recommends an adequate intake of choline for men is 550 mg
242 per day and for women 425 mg per day [38,43]. Reduced or delayed urinary excretion of
243 TMAO/TMA is specific to hepatic disease, and it has been suggested dysbiosis of the intestinal
244 microbiota in patients with hepatobiliary diseases may delay/decrease conversion of choline to TMA
245 and subsequent urinary excretion of TMAO/TMA [47,48]. Analyses of urinary metabolites produced
246 by mice fed high-fat diets led to the proposals that microbial utilization, and subsequent reduced
247 availability, of dietary choline contributes to the development of NAFLD [56] and insulin resistance
248 [57]. The only study to date comparing the faecal microbiotas of healthy and NAFLD individuals
249 found no difference in their compositions [58]. However, studies in rodents have shown that
250 probiotic [59] and antibiotic administration [60**] can offer protection against the onset of NAFLD.
251 The role for dietary choline in NAFLD can be explained by the bioavailability of free choline to form
252 lipoproteins in the liver (in particular, VLDL), which allows the export of free fatty acids from this
253 organ. If the gut microbiome converts excessive amounts of dietary choline into TMA, this leads to
254 reduced choline bioavailability and, therefore, NAFLD [57].

255

256 Recent work has demonstrated that changes in choline levels in a standardized diet modulate the
257 faecal microbiota and can lead to the development of fatty liver in human subjects [61**]. Fifteen
258 females (BMI 15–34) on a 2-week in-patient study were fed a standardized diet in which choline
259 levels were manipulated. *Gammaproteobacteria* were seemingly inhibited by high levels of dietary
260 choline, and negatively correlated with the per cent change in liver fat/spleen fat ratios.
261 *Erysipelotrichi* sequence numbers were positively correlated with the per cent change in liver
262 fat/spleen fat ratios. This led to the suggestion that baseline levels of these taxa may predict the
263 susceptibility of an individual to fatty liver disease from a choline-deficient diet [61**]. Combining
264 *PEMT* promoter SNP rs12325817 phenotype, *Gammaproteobacteria* and *Erysipelotrichi* data proved
265 a powerful method for predicting the physiological effects of choline deficiency, and led the
266 researchers to hypothesize that those with the wild-type version SNP in the *PEMT* gene were better
267 able to produce PC endogenously and were less affected by the composition of their intestinal
268 microbiota in relation to the effects of choline deficiency.

269

270 Interactions between the intestinal microbiota, inflammasomes and NAFLD are known to occur.
271 Deficiencies of the NLRP3 and NLRP6 inflammasomes positively regulated NAFLD progression in mice
272 harbouring a colitogenic intestinal microbiota [60**]. Switching the animals to a choline-deficient
273 diet modulated the faecal microbiota, particularly representation of members of the families
274 *Porphyromondaceae*, *Erysipelotrichaceae* and *Prevotellaceae*. Modulation of the intestinal
275 microbiota by the choline-deficient diet was thought to promote a TLR4/TLR9 signalling cascade in
276 the liver that led to enhanced hepatic tumour necrosis factor expression that drove progression to
277 NASH in susceptible animals.
278

279 **Microbial metabolism of phosphatidylcholine and L-carnitine is associated with cardiovascular**
280 **disease**

281 Choline present in dietary PC is degraded by intestinal bacteria, but is more resistant to degradation
282 than free choline [49,62]. The intestinal microbiota of mice is able to catabolise choline from dietary
283 PC via an unknown mechanism, which led to the proposal of a linear pathway PC → choline → TMA
284 → TMAO [63*]. It is known that human intestinal bacteria (bacteroides, bifidobacteria and clostridia)
285 are able to degrade PC with the release of choline [62].
286
287

288 Following the association of methylamines with murine insulin-resistance phenotypes [57], Wang *et*
289 *al.* [63*] proposed a link between degradation of dietary PC, the intestinal microbiota and TMAO in
290 cardiovascular disease. This hypothesis was tested further in a study in which humans were given a
291 PC challenge and their plasma levels of TMAO were measured before and after suppression of the
292 intestinal microbiota with antibiotics [64**]. Time-dependent increases in plasma TMAO levels were
293 observed at the first challenge, with TMAO production suppressed after antibiotic administration.
294 Removal of antibiotics, and 'release' of the microbiota, reinstated TMAO plasma levels post-PC
295 challenge, demonstrating the role of the microbiota in increasing circulating levels of TMAO derived
296 from PC. The authors also examined the relationship between fasting TMAO levels in 4007 patients
297 undergoing elective coronary angiography and the occurrence of major cardiovascular events
298 (death, heart attack or stroke) over a three-year follow-up period. An increased fasting plasma level
299 of TMAO was associated with experiencing a major cardiovascular event [64**].
300

301 The relationship between TMAO produced from dietary methylamines and cardiovascular disease
302 has recently been extended to include L-carnitine, a compound abundant in red meat [65**].
303 Antibiotic-induced suppression of the microbiota of humans led to almost-complete absence of

304 TMAO from plasma and urine after L-carnitine challenge. In addition, it was shown that omnivorous
305 humans have far higher circulating levels of TMAO in their plasma than their vegan and vegetarian
306 counterparts after L-carnitine supplementation, with negligible TMAO formation in vegans post-
307 carnitine challenge. Vegetarians and vegans have higher plasma levels of carnitine compared with
308 their omnivorous counterparts, though it is not known if this is due to reduced microbial metabolism
309 of carnitine to TMA by the intestinal microbiota of the non-omnivores. This suggests that the human
310 intestinal microbiota can be modulated by dietary means with respect to how it processes dietary
311 methylamines.

312

313 High levels of plasma carnitine were associated with cardiovascular disease but only in those
314 patients with accompanying high levels of plasma TMAO in a cohort of 2595 patients undergoing
315 cardiac evaluation [64**]. Using an *Apoe*$^{-/-}$ mouse model, Koeth *et al.* [65**] demonstrated that
316 atherosclerosis plaque formation during carnitine supplementation was microbiota-dependent,
317 being directly related to the presence of bacterially-derived TMAO/TMA in plasma. TMAO is
318 currently thought to induce atherosclerosis by promoting macrophage cholesterol accumulation by
319 increasing cell surface expression of CD36 and scavenger receptor A, pro-atherogenic scavenger
320 receptors [63*,65**], and by repressing reverse cholesterol transport and several bile acid
321 transporters in the liver [65**].

322

323 **Conclusion**

324 Microbial–mammalian co-metabolism is shaping human health in many ways. In this review, we
325 have covered recent findings on SCFA, AAA and methylamine metabolism and their consequences
326 on human health and disease, which are illustrating particularly well this metabolic symbiosis. With
327 the constant refinement of metagenomics and metabolomics, further insights will become available
328 from cohort studies, bearing promises for personalised nutrition and healthcare in the future.

329

330

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335

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[

Figure legends

Figure 1. Impact of reduced CHO weight loss (WL) diets in male obese volunteers on fecal SCFA concentrations. Data are from two separate dietary cross-over studies that are reported in [19] (study 1) and [20**] (study 2): M – weight maintenance diet (360-400 g day^{-1} CHO, 22-28 NSP), HPMC – high protein, moderate CHO WL diet (164-182 g day^{-1} CHO, 12-13 NSP), HPLC – high protein, low CHO WL diet (23-24 g day^{-1} CHO, 6-9 NSP). In addition to the evident decrease in total SCFA, both studies detected a significant decrease in per cent butyrate among SCFA, while in study 2 the per cent of minor SCFA (valerate, isobutyrate, isovalerate) that were derived from amino acid fermentation increased, reflecting the higher protein intake on the WL diets.

Figure 2. Concentration of fibre-derived ferulic acid and its major metabolites measured in faecal samples following high protein dietary interventions. Metabolite 1 = 4-hydroxy-3-methoxyphenylpropionic acid, Metabolite 2 = 3,4-dihydroxyphenylpropionic acid, Metabolite 3 = 3-hydroxyphenylpropionic acid. M = maintenance diet (fibre content 22 g day^{-1}), HPMC = high protein moderate CHO diet (fibre content 14 g day^{-1}) and HPLC = high protein low CHO diet (fibre content 8.8 g day^{-1}). Ferulic acid = 4-hydroxy-3-methoxycinnamic acid. Data are given as mean ± standard deviation (*n* = 8 volunteers). Statistical data were calculated as a one-way ANOVA to compare diet

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566 with blocking for volunteer and, where significant, are given for comparison between M and HPLC
567 diets. Adapted from [20**].
568
569 **Figure 3.** The methylamines' pathway and the microbial–mammalian metabolic axis. TMA is derived
570 from microbial degradation of choline, a dietary component that can also be obtained by cleavage of
571 dietary PC, and of L-carnitine. TMA is absorbed by the host to be *N*-oxidised into TMAO by FMO3 and
572 demethylated into DMA and MMA by cytochrome P450s (CYP) in the liver during first-pass
573 metabolism. Circulating TMAO can reach other cell types, such as arterial epithelial cells and
574 macrophages, leading to atherosclerosis-associated inflammation. PC, synthesized from choline
575 through the Kennedy (CDP-choline) pathway, is essential for exporting fatty acids from the liver to
576 other storage tissues; reduced choline bioavailability leads to lower levels of PC being formed and to
577 NAFLD. PEMT converts phosphatidylethanolamine (PE) into PC, using *S*-adenosylmethionine as a
578 methyl donor, and a polymorphism in *PEMT* has been associated with a higher risk of developing
579 NAFLD. When there is sufficient choline in the diet, the Kennedy pathway is responsible for
580 maintaining PC synthesis, with the PEMT pathway contributing ~30 % of the hepatic PC. When
581 choline is at low levels in the diet, the PEMT pathway is essential for maintaining the supply of PC in
582 the liver. Adapted from [41,57,63**, 64**,65**].
583