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# Advancing human gut microbiota research by considering gut transit time

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## ABSTRACT

Accumulating evidence indicates that gut transit time is a key factor in shaping the gut microbiota composition and activity, which are linked to human health. Both population-wide and small-scale studies have identified transit time as a top covariate contributing to the large interindividual variation in the faecal microbiota composition. Despite this, transit time is still rarely being considered in the field of the human gut microbiome. Here, we review the latest research describing how and why whole gut and segmental transit times vary substantially between and within individuals, and how variations in gut transit time impact the gut microbiota composition, diversity and metabolism. Furthermore, we discuss the mechanisms by which the gut microbiota may causally affect gut motility. We argue that by taking into account the interindividual and intraindividual differences in gut transit time, we can advance our understanding of diet–microbiota interactions and disease-related microbiome signatures, since these may often be confounded by transient or persistent alterations in transit time. Altogether, a better understanding of the complex, bidirectional interactions between the gut microbiota and transit time is required to better understand gut microbiome variations in health and disease.

## INTRODUCTION

The human gastrointestinal tract (GIT) is densely populated by microbes, which play an important role in a broad range of physiological processes from the digestion of complex polysaccharides to the regulation of neural signalling.<sup>1</sup> The composition and metabolism of the adult gut microbial communities are affected by a combination of factors including diet,<sup>2,3</sup> demographics,<sup>4,5</sup> use of medication,<sup>6</sup> health status<sup>7</sup> and environmental components shaping the gut environment.<sup>8</sup> Among these environmental components, gut transit time, that is, the time it takes foods to travel through the GIT, appears to be a major driver of gut microbiome variation.<sup>9–12</sup> Gut transit time varies markedly between and within individuals<sup>13–15</sup> and has been associated with gut microbial diversity, composition, and metabolism.<sup>9–12, 16–18</sup> The anatomical segments of the GIT (ie, stomach, small intestine and colon) have segment-specific transit time, affecting the composition of the residing gut microbes.<sup>12</sup> Although this knowledge is well established, differences in transit time and pH within and between individuals have largely been neglected when investigating person-specific gut microbiota signatures. Here, we review

## Key messages

- ⇒ Gut transit time varies considerably between and within individuals, and explains large proportions of the gut microbiota compositional variation between people.
- ⇒ Gut transit time affects substrate availability in the colon affecting the trade-off between saccharolytic and proteolytic fermentation.
- ⇒ The gut microbiota can via production of metabolites stimulate gut motility thus affecting the transit time
- ⇒ Many disease-related microbiome signatures may be confounded by alterations in gut transit time.
- ⇒ By considering interindividual and intraindividual differences in transit time in human studies, diet–microbiota interactions and disease-related microbiome signatures may be better elucidated.

and discuss the role of gut transit time as a key determinant of the gut microbial composition and metabolism as well as of many diet–microbiota interactions relevant to human health (figure 1). We discuss the implications of altered gut transit time in health and disease, and provide an overview of the currently available methods for assessment of gut transit time in humans.

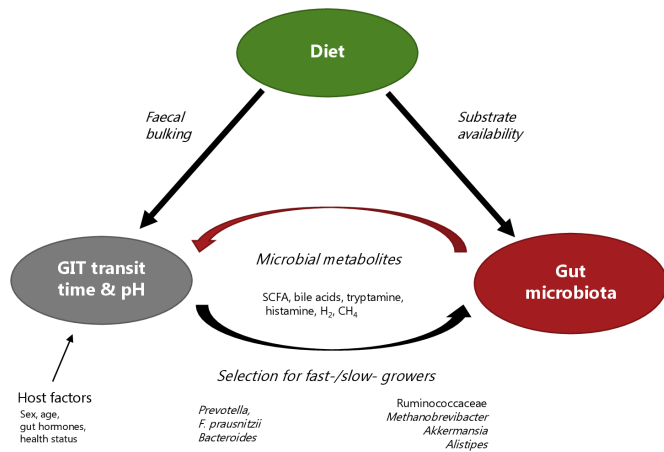
## TRANSIT TIME THROUGHOUT THE GIT Interindividual and intraindividual variation in transit time

In healthy populations, whole gut transit time (WGTT) varies substantially between individuals<sup>13, 15</sup> with a median WGTT of approximately 28 hours.<sup>11, 19</sup> Segment-specific transit times are commonly referred to as gastric emptying time (GET), small intestinal transit time (SITT) and colonic transit time (CTT). GET is the time it takes for food to empty from the stomach and enter the small intestine in a form of semiliquid chyme.<sup>20</sup> SITT is the duration time of the passage of the chyme from the duodenum (i.e. the proximal small intestine) until the ileocaecal region, and similarly, CTT corresponds to the duration time of the chyme's passage from the caecum until the egestion in a form of stool.<sup>21</sup> For GET of solids, a transit time coefficient of variation of 24.5% has been reported between individuals.<sup>22</sup> Several human studies have identified large interindividual

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**Figure 1** Illustration of the complex interplay between diet, gut microbiota and gut transit time. Diet can directly affect gastrointestinal motility, especially dietary fibre and osmotically active foods can increase the faecal bulk and thereby accelerate gut transit time. Diet can also affect gut transit time by dictating the substrate availability to the gut microbiota. As a result, the gut microbiota produces metabolites such as short-chain fatty acids (SCFA), secondary bile acids, tryptamine, histamine,  $H_2$  or  $CH_4$ . These microbial-derived metabolites can stimulate gastrointestinal motility and thereby impact gut transit time. In addition, gut transit time affects the gut microbial composition and metabolism and consequently the gut environment (eg, pH). The relation between the gut microbiota and the gut transit time is therefore bidirectional. In addition, host factors including gut hormones, gender, age, health status and physical activity also affect the gut transit time. GIT, gastrointestinal tract.

variation in SITT with a median of approximately 5 hours (range of 2–7.5 hours).<sup>21 23–25</sup> Compared with the small intestine, transit through the colon is much slower with a median of 21 hours.<sup>19</sup> Consequently, large interindividual variations are often seen in CTT with the minimum and the maximum reported transit times of 0.1–46 hours for the proximal colon, 0.3–80 hours for the distal colon and 1–134 hours for the rectosigmoid colon<sup>19 26–29</sup> (figure 2).

Gut transit time also varies within individuals over time.<sup>13 14 16</sup> For example, repeated measurements of CTT using radio-opaque markers within eight healthy subjects over a period of several months showed that each subject exhibited a wide range of CTT with a mean coefficient of variation of 25%.<sup>30</sup> Furthermore, a recent study showed that the percentage of the faecal water content, a proxy of transit time, varied from day to day in both healthy subjects and patients suffering from irritable bowel syndrome (IBS).<sup>31</sup> Similarly, intrasubject differences in SITT and CTT have been observed with tandem measurements in 10 healthy adults using the SmartPill capsule<sup>13</sup> that can directly assess WGTT and segmental transit times.<sup>32</sup>

The currently available methods for WGTT and segment-specific transit time assessment include direct methods such as the SmartPill capsule and indirect methods such as stool consistency, stool frequency and faecal water content (table 1). It is important to note that the methods provide a range of outcomes, some of which provide similar results while others may not be comparable. For example, while scintigraphy relies on calculating the geometric centre based on recorded radioactivity in the different GIT regions at certain time points,<sup>33</sup> the SmartPill capsule uses landmarks in the gastrointestinal pH to calculate the segmental transit times.<sup>34</sup>

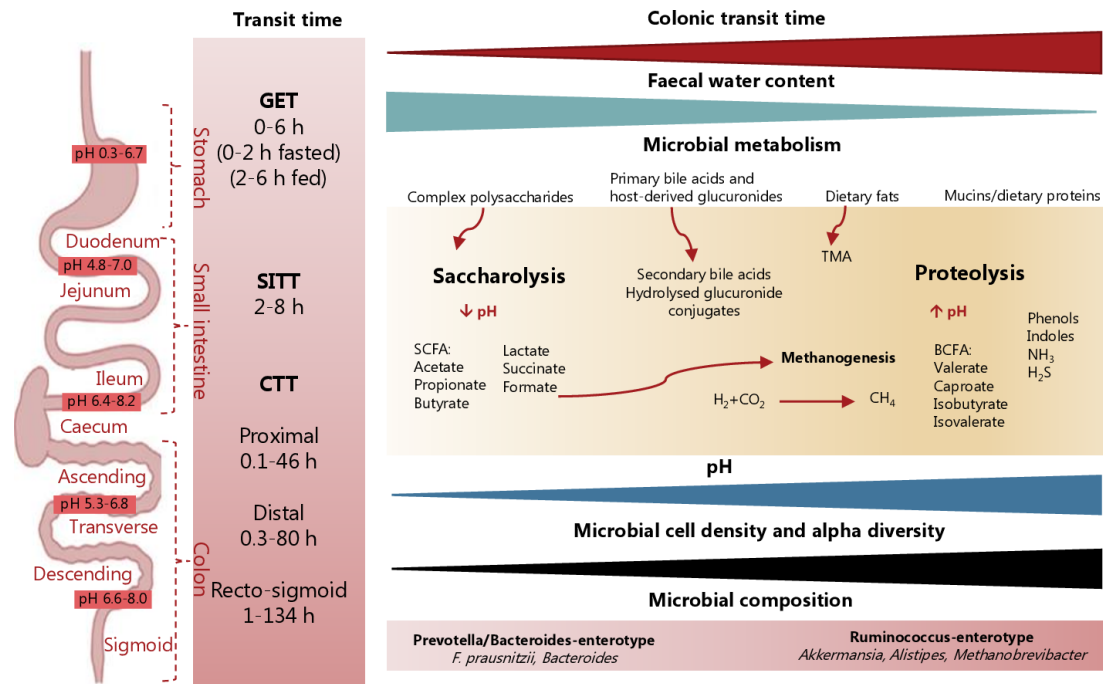
### Host and environmental factors influencing transit time

Several factors affect the intraindividual and interindividual variations in gut transit time including sex, ageing, stress, body mass index, colonic anatomy, gut hormones and diet.<sup>35</sup> Moreover, the gut microbiota and its metabolites also affect gut transit time, which is discussed in further detail below.

### Dietary impact

Dietary patterns, dietary factors such dietary fibres, as well as individual dietary ingredients, can directly affect the gut physiology via stimulation of gastrointestinal motility either independently of the gut microbiota or via gut microbiota dependent pathways, which will be discussed below. Wu *et al* showed that intestinal transit time was faster on a high-carbohydrate/low-fat diet when compared with a low-carbohydrate/high-fat diet in 10 healthy subjects.<sup>3</sup> A randomised control trial with 120 obese participants reported significantly more cases of constipation (68% vs 35%) and diarrhoea (23% vs 7%) in a ketogenic diet group compared with a low-fat diet group.<sup>36</sup> However, whether the adverse gastrointestinal outcomes in these studies were caused by the high fat, high protein or low carbohydrate content could not be concluded. Nonetheless, a high intake of fat was associated with constipation and prolonged CTT,<sup>35 37</sup> and infusion of fat into the small intestine of healthy subjects has been shown to slow gastric emptying.<sup>38</sup> In contrast, a 4-week intervention on a high-fat diet with 12 healthy men resulted in accelerated gastric emptying and orocaecal transit time when compared with a low-fat diet.<sup>39</sup> Therefore, the dietary patterns may exert different effects on the GIT depending on their composition and content.

One particularly important component of the dietary patterns is the dietary fibres. Dietary fibres affect the functionality of the GIT including the gut transit.<sup>40</sup> Different types of dietary fibres have very different physicochemical characteristics with regards to solubility, fermentability and gel-formation (viscosity). The different characteristics influence their effects on gut transit time, as reviewed in detail elsewhere.<sup>41 42</sup> Today, most studies investigating the effects of dietary fibres on the gut transit time have been limited to wheat bran and psyllium. While wheat bran consistently decreases the WGTT,<sup>43</sup> the effects of psyllium, which is minimally fermented by the gut microbiota, on WGTT are inconsistent.<sup>44</sup> It has been shown that the laxative effects of coarse wheat bran are greater than that of fine wheat bran suggesting that the particle size plays a role for the mechanical stimulation of the intestinal epithelium.<sup>45</sup> In line with this, an intervention study with powder arabinoxylan-oligosaccharides (wheat bran-based prebiotics) in 48 subjects resulted in softer stools but did not change the CTT.<sup>46</sup> Unlike wheat bran, psyllium contains a soluble type of fibre and has gel-forming properties that increase the water retention in the colon thus increasing the faecal water content and bulk.<sup>47</sup> Cellulose, which is also non-fermentable but not gel-forming, has been shown to lower faecal pH, increase the stool outputs and to decrease CTT.<sup>48</sup> Fermentable fibres such as inulin seem to alleviate constipation and improve physical discomfort.<sup>49</sup> Other human studies have shown that increased intake of cereals and fermentable wheat fibre increased stool output.<sup>50 51</sup> Altogether, dietary fibres can accelerate transit time and increase stool outputs through water retention, bulking and via fermentation-mediated effects. However, the effects on transit time depend on the particle size, solubility, fermentability and viscosity of the given fibre. Furthermore, water supplementation has been found to enhance the effect of high-fibre diet on increasing stool frequency in constipated patients,<sup>52</sup> suggesting that fluid intake is another



**Figure 2** Segmental transit time and pH throughout the gastrointestinal tract and its association with gut environment and gut microbial metabolism. The transit time varies throughout the gastrointestinal tract with substantial interindividual differences in gastric emptying time (GET), small intestinal transit time (SITT) and colonic transit time (CTT), which account for most of the whole gut transit time. The segmental transit time ranges show the minimum and maximum transit times reported for each segment. Long gut transit time has been associated with higher faecal pH, reduced faecal water content, higher microbial cell density and diversity, and a shift in microbial metabolism from saccharolysis towards proteolysis as reflected by reduced levels of short-chain fatty acids (SCFA) and increased levels of branched-chain fatty acids (BCFA). It is likely that once easy accessible carbohydrate sources become scarce in the colon, the gut microbes switch to ferment dietary and mucin-derived proteins. While saccharolysis by the gut microbiota gives rise to SCFA that are beneficial for the host and a source of energy for the colonocytes, proteolysis can lead to the accumulation of compounds such as BCFA, phenols, indoles, ammonium (NH<sub>3</sub>) and hydrogen sulphide (H<sub>2</sub>S) that are generally considered detrimental for health. Moreover, hydrogen (H<sub>2</sub>) with carbon dioxide (CO<sub>2</sub>) or formate can be converted into methane (CH<sub>4</sub>) by methanogenic archaea, which are also linked to slower transit time. In addition, the production and circulation of secondary bile acids and hydrolysis of host-derived glucuronides excreted via bile can also be affected by alterations in gut transit time. Whether microbiota-derived trimethylamine (TMA), produced from mainly choline and carnitine, is linked to transit time remains unknown. Created with Biorender.com.

important factor to consider with respect to transit time. Indeed, a handful of studies have reported an association between lower fluid intake and constipation.<sup>53</sup> However, no human intervention studies have to our knowledge investigated the relationship between water intake, bowel habits and the gut microbiome.

Finally, also individual food ingredients may affect transit time. For instance, carboxymethylcellulose, a widely used emulsifier, has been shown to act as a laxative<sup>54</sup> and was recently linked to reduced gut microbiota diversity in humans.<sup>55</sup> Another example is sorbitol, a sugar alcohol highly abundant in prunes, which can retain water molecules by osmosis, similar to some types of fibre that increase water content in the gut lumen and may lead to softer stools.<sup>56-57</sup> In addition, consumption of turmeric, a spice increasing the bile secretion, has been associated with a significantly longer transit time in gnotobiotic mice.<sup>58</sup>

### Other factors affecting transit time

Another important factor affecting the gut transit time is sex. Gut transit time is shorter in men than in women—even when adjusting for total energy intake, diet, body weight and height.<sup>59</sup> The sex difference was shown to be the most pronounced in the distal colon with women having longer transit through the transverse and descending colon,<sup>19</sup> possibly as a result of slower gut motility in women.<sup>60</sup> Moreover, from epidemiological studies, it has been shown that women are also more prone

to developing constipation.<sup>61</sup> While there is a strong evidence for gender differences in gut transit time, it remains to be determined whether it is the sex per se or differences in behaviours and habits that drive the differences in transit time. The length of the intestine is also an important factor to consider as it can vary greatly among individuals. Based on a postmortem measurement, men have been found to have a longer intestine than women, which may contribute to longer transit time in women.<sup>60-62</sup> The same study reported that the average length of the whole intestine is  $7.96 \pm 1.3$  m with a range of 3.78–13.16 m and found that the intestine's length positively correlates with body weight but not body height.<sup>62</sup> Moreover, ageing is associated with longer gut transit time<sup>63-64</sup> and it has been shown that especially transit through the right (ascending) part of the colon is correlated to age,<sup>19</sup> perhaps as a consequence of a more refined diet and/or reduced physical activity with older age. Indeed, physical activity may improve bowel habits via increased gut motility.<sup>65</sup> Furthermore, social aspects and acute stress are likely to play a role in gut transit time variations, although the contribution of these factors is difficult to assess. In animals, induced stress results in slower transit in the upper gut, while the opposite was observed in the large bowel, where stress increased motility and stool output.<sup>65</sup> Also, other exogenous factors such as medication often exert side effects on the gut transit.<sup>66</sup> Finally, host genetics has recently been shown to

**Table 1** Methods available for gut transit measurements and examples of their application in human gut microbiome research

Method	Region	Subjects	Gut microbiome and/or transit time-related findings	References	
Direct transit time measures	Radio-opaque markers	CTT	48 healthy subjects	Distal CTT was associated with increased microbial $\alpha$ -diversity, rectosigmoid CTT was negatively associated with faecal SCFA and distal CTT was negatively associated with plasma acetate	18
		WGTT	98 subjects	CTT positively associated with microbial community structure, microbial richness and microbial protein catabolism	10
			48 healthy subjects with slow WGTT*	RCT with arabinoxylan-oligosaccharide increased faecal <i>Bifidobacterium</i> and softened stool consistency without changing the WGTT	46
			14 healthy subjects†	WGTT positively correlated with urinary sulphate, faecal methanogens and negatively with total faecal SCFA, sulphate and bile salts	134
	Scintigraphy	WGTT	50 healthy and constipated patients	Colonic mucosal microbiota was not associated with CTT and was significantly different between the two groups, faecal microbiota was associated with CTT and breath methane	88
		GET SITT CTT	36 healthy and 20 patients with liver cirrhosis	SITT was negatively correlated with B:F ratio and microbial dysbiosis index	215
	SmartPill	WGTT	11 obese and 11 normal weight subjects	Shorter SITT was associated with Bact2-enterotype, longer CTT was associated with Rum-enterotype	12
		GET SITT CTT	33 healthy and 114 IBS-C patients	Colonic intraluminal pH levels were significantly lower in IBS patients compared with HC, and total faecal SCFA levels correlated negatively with CTT	216
			19 healthy and 9 constipated subjects	Rectosigmoid pH negatively correlated with <i>Bifidobacterium</i> spp and positively with <i>Coprococcus</i> spp	108
	Gas-sensing capsules	WGTT GET SITT CTT	4 healthy volunteers	SITT was slower with a diet high in fermentable fibre (~34 g/day) compared with a diet low in fermentable fibre (~22 g/day)	217
	Blue dye	WGTT	1102 subjects	Gut microbiome composition predicted WGTT, longer WGTT was linked with <i>Akkermansia muciniphila</i> , <i>Bacteroides</i> and <i>Alistipes</i> spp.	11
	Sweet corn	WGTT	31 healthy subjects	WGTT positively correlated with faecal BCFA and <i>Coprococcus</i>	107
Indirect transit time measures	Stool frequency	WGTT	69 subjects	B:F ratio and Bacteroidetes:unclassified_Ruminococcacea positively associated with stool frequency	218
			60 healthy subjects	B:F ratio was higher in a group with stool frequency of $\leq 2$ times/week compared with one time/day or one time/2 day and $\geq 2-3$ times/day	219
	Stool consistency (BSS)	CTT	53 healthy subjects	Stool consistency was positively correlated with species richness, <i>Akkermansia</i> and <i>Methanobrevibacter</i> abundances, and negatively associated with the B:F ratio,	17
			1126 subjects	BSS was associated with the B:F ratio, high BSS score positively correlated with <i>F. prausnitzii</i>	220
	Faecal water (stool moisture)	CTT	31 healthy subjects	Faecal water positively correlated with WGTT	107
			40 subjects	Stool moisture accounted for 4.3% of interindividual microbiota variation (absolute abundances)	79
			12 IBS patients and 12 controls	Association between stool consistency and microbial community structure/microbial richness	31
	Stool crosslinking	CTT	170 samples	Faecal acetate and methionine were predictive of stool consistency	221
	Breath test	Oro-caecal	14 healthy subjects	Oro-caecal transit time was positively correlated with WGTT	134

\*Transit time also measured by BSS, faecal water content and breath test.

†Transit time also measured by the breath test.

BCFA, branched-chain fatty acids; B:F, Bacteroidetes:Firmicutes; BSS, Bristol Stool Scale; CTT, colonic transit time; GET, gastric emptying time; IBS, irritable bowel syndrome; RCT, randomised controlled trial; SCFA, short-chain fatty acids; SITT, small bowel transit time; WGTT, whole gut transit time.

be involved in gut motility as 14 independent loci were found to associate with stool frequency.<sup>67</sup>

In conclusion, WGTT and segmental transit times depend on a combination of factors, including host genetics, anatomy, physiology, health status and lifestyle as well as external factors such as intake of foods, water and drugs.

### GUT TRANSIT TIME: A KEY DETERMINANT OF THE GUT MICROBIOTA

Emphasising the importance of transit time, CTT has been linked to gut microbiota diversity and composition in both population-wide analyses and small-scale studies.<sup>10 11 17 18 46 68</sup>

Moreover, stool consistency assessed by the Bristol Stool Scale (BSS),<sup>69</sup> which is a surrogate marker for CTT, has been identified as a top covariate of the faecal microbial composition of a healthy population.<sup>9</sup> Considering that CTT varies markedly between individuals and from day to day within individuals,<sup>13-16</sup> microbiota-focused investigations should take gut transit time into account. Although faecal microbial species richness has been associated with a diverse diet<sup>70</sup> and suggested as an indicator of the host health status,<sup>71 72</sup> it has also been associated with long CTT,<sup>10</sup> indicating that faecal microbial richness is strongly confounded by CTT.<sup>73</sup>

Several bacterial groups including *Akkermansia*, *Alistipes*, *Methanobrevibacter* and *Ruminococcaceae* have consistently been associated with firm stools and a long CTT.<sup>9 11 12 17</sup> Moreover, the classification of individuals according to their prevailing microbial community structure (ie, *Bacteroides-1*, *Bacteroides-2*, *Prevotella* or *Ruminococcaceae* enterotypes) is thought to be driven at least partially by gut transit time.<sup>12 74</sup> The *Prevotella* enterotype, known to prevail on fibre-rich diets,<sup>3</sup> has been associated with loose stools while the opposite has been observed for the *Ruminococcaceae* enterotype,<sup>17 75</sup> which has been characterised by increased proteolytic capacity.<sup>76</sup> In agreement herewith, a long CTT was associated with an increased prevalence of the *Ruminococcaceae* enterotype in healthy subjects<sup>12</sup> and decreased levels of *Prevotella* in constipated patients<sup>74</sup> and Parkinson's patients<sup>77</sup> that also often suffer from constipation.<sup>78</sup> Similarly, the abundance of another saccharolytic bacterium, *Eubacterium rectale*, was reduced in individuals with a long CTT.<sup>11</sup> Furthermore, one study has investigated the associations between the SITT and pH, and the faecal microbial community composition.<sup>12</sup> This study found that shorter small intestinal transit is associated with the *Bacteroides-2* enterotype,<sup>12</sup> which is characterised by a high proportion of *Bacteroides* and low microbial cell densities in stools.<sup>79</sup> Additionally, the abundances of *Bacteroides* and *Flavonifractor* were negatively correlated with small intestinal pH, supporting the idea that inter-individual variation in environmental conditions in the small intestine is linked to gut microbial composition and activity.<sup>12</sup> Several human studies have established that longer CTT is associated with an increase in distal colonic pH,<sup>80–82</sup> suggesting that gut transit time and pH are interrelated. Also in vitro experiments simulating short and long gut transit time by high or low dilution rates, respectively, have shown that dilution rate and pH have a substantial impact on the growth of several bacterial groups. For instance, *A. muciphila* was present at all pH ranges (6.0–8.0) in low dilution rates, whereas it only grew at high pH (pH > 7.0) at a high dilution rate, while *M. smithii* was only detected at a low dilution rate.<sup>83</sup> An emerging body of scientific evidence thus shows that both gut transit time and pH drive the composition of the microbial communities along the GIT (figure 2). However, studies investigating the gut microbiome in relation to gastrointestinal pH or segmental transit time are scarce, and interventional studies to provide further evidence remain to be conducted.

Today, most research on the human gut microbiota relies on stool samples. While stool samples are generally considered to be representative of the luminal colonic microbiota,<sup>84 85</sup> the faecal microbiota represent an 'end-product' of the whole gut microbial community. Thus, the relative microbial community composition in the faeces is more similar to that of the distal colon compared with the proximal colon and small intestine.<sup>86 87</sup> However, the question remains whether absolute numbers of saccharolytic bacterial groups are similar in the proximal colon and in faeces (mirroring the distal colon), and only change in terms of relative abundance due to an increase in abundance of slow-growing and proteolytic bacteria in the distal colon. Studies including sampling throughout the human GIT are needed to deduce how transit time associate with the quantitative human gut microbiome composition in the proximal and distal colon, respectively. This may be important as the state of the gut microbial community maturation (ie, life-time of the bolus in the colon) could explain a considerable fraction of intraindividual and interindividual microbiota variation in healthy individuals.<sup>73</sup> Finally, the mucosal microbial communities, which occupy the outer mucus layer of the intestinal epithelium, are distinct

from the luminal microbial communities and their composition appears to be less affected by the gut transit time.<sup>88</sup>

## GUT TRANSIT TIME AND GUT MICROBIAL METABOLISM

Transit time not only affects the gut microbiota composition but also affects the gut microbial metabolism since differences in transit time have consequences for substrate availability throughout the GIT (figure 2).

### A trade-off between saccharolytic and proteolytic fermentation

Non-digestible polysaccharides reach the caecum and the proximal colon where they undergo fermentation by the residential microbes resulting in the generation of gases (H<sub>2</sub> and CO<sub>2</sub>) and metabolites such as short-chain fatty acids (SCFA), mostly acetate, propionate and butyrate, which are generally considered beneficial for health.<sup>89</sup> However, when easy accessible carbohydrates become scarce the microbial activity shifts towards fermentation of dietary or mucosal proteins instead.<sup>90</sup> This results in the formation of potentially deleterious compounds such as phenols, indoles, ammonia or hydrogen sulphide (H<sub>2</sub>S).<sup>90</sup> The depletion of carbohydrates ultimately leads to a decrease in SCFA consequently increasing the luminal pH that creates a selective pressure on the microbial community thereby redirecting the microbial metabolism towards proteolysis.<sup>83</sup> Long CTT has been associated with reduced faecal SCFA indicating either increased absorption, lower availability of fermentable polysaccharides in the colon, and/or changed activity.<sup>18 81 91 92</sup> In a recent publication, faecal SCFA concentrations and microbial diversity clustered according to stool consistency assessed by the BSS with higher levels of SCFA detected in looser stools, reflecting a shorter colonic transit.<sup>93</sup>

Furthermore, increased CTT has also been associated with increased proteolytic fermentation in the colon in both healthy subjects<sup>10</sup> and patients with Parkinson's disease.<sup>94</sup> In Parkinson's disease, constipation and long CTT are common complications.<sup>78</sup> One study observed elevated serum levels of host-microbial coproducts derived from bacterial proteolysis (p-cresol sulphate and phenylacetylglutamine) in a cross-sectional cohort with 197 Parkinson's patients.<sup>94</sup> Furthermore, the authors also showed that bacterial taxa (*Oscillospira* and *Ruminococcus*) positively associated with these proteolytic metabolites were also positively associated with firm stools.<sup>94</sup> Likewise, elevated levels of p-cresol sulphate have been observed in autistic children<sup>95</sup> and in patients with end-stage renal disease,<sup>96</sup> patient groups who also often suffer from prolonged CTT.<sup>97 98</sup> These findings suggest that proteolytic metabolites including the host-microbial co-metabolite p-cresol sulphate might be markers of constipation and slow transit rather than indicators of disease. In support hereof, urinary levels of p-cresol sulphate, as well as phenylacetylglutamine, were also associated with longer CTT in healthy individuals, clearly indicating a shift towards microbial proteolysis with the prolonged colonic transit.<sup>10</sup> Additionally, recent evidence shows that even on a homogenous diet, urinary levels of these metabolites remain highly variable between individuals,<sup>99</sup> further emphasising that other factors than diet modulate the concentrations of these metabolites. Altogether, this suggests that intestinal transit time plays a role in the highly individual diet-microbiota responses.

Prolonged transit time has also been linked to increased urinary sulphate excretion,<sup>100</sup> elevated levels of urinary phenol and increased excretion of faecal ammonia,<sup>101</sup> another microbial by-product of protein degradation. Ammonia has been shown to

increase mucosal damage and promote colonic cancer in rats at relatively low concentrations.<sup>102 103</sup> Similarly, ammonia, as well as phenol, have been shown to disrupt tight junctions of cultured colon cells (Caco-2).<sup>104</sup> However, there is a lack of evidence from human studies to link ammonia to colon cancer. Impaired mucosal integrity and breakdown of the mucosal barrier are also caused by H<sub>2</sub>S via inhibition of butyrate oxidation by the colonocytes.<sup>105</sup> Importantly, we have previously observed a negative correlation between mucus-degradation-associated metabolites in urine and CTT, suggesting that prolonged colonic transit may also lead to enhanced degradation of the mucus layer by the microbiota<sup>10</sup> in line with observations from fibre-depleted diets.<sup>106</sup>

Elevated concentrations of branched-chain fatty acids (BCFA) are positively associated with CTT in healthy adults.<sup>107</sup> BCFA such as isobutyrate, isovalerate, or 2-methylbutyrate are products of bacterial fermentation of branched-chain amino acids and has been positively correlated with the relative abundance of *Coprococcus* and *Blautia*.<sup>107</sup> Interestingly, a study that employed the SmartPill to measure gastrointestinal pH, showed that *Coprococcus* spp. was positively associated with recto-sigmoid pH, where pH is slightly alkaline (>7) while the inverse was observed for *Bifidobacterium* spp.,<sup>108</sup> a saccharolytic species<sup>109</sup> thriving at neutral pH.<sup>110</sup> This supports the hypothesis that prolonged transit time may lead to a less acidic environment in the colon as a result of SCFA depletion and accumulation of alkaline compounds from the proteolytic processes. In line with this, in vitro studies indicate that higher concentrations of BCFA were produced at high pH and low dilution rate simulating slow luminal washout and thereby slow CTT.<sup>83</sup> This increase of intraluminal pH towards the distal colon was not observed in rural Africans<sup>111</sup> who have shorter CTT<sup>112 113</sup> and a habitual diet rich in dietary fibres, which likely provides a surplus of SCFA.<sup>114</sup> In addition, it is important to mention that microbial metabolites of dietary fats remain an underexplored area, where only a few products of microbial origin are known including some sphingolipids, endocannabinoids or trimethylamine (TMA). TMA is produced by gut microbes from methylamine-containing nutrients (eg, choline, lecithin, L-carnitine) and further processed in the liver to trimethylamine N-oxide (TMAO).<sup>115</sup> While TMAO levels have been correlated with the risk of cardiovascular events,<sup>116</sup> no study has investigated links between TMAO and gut transit time.

Taken together, colonic fermentation is in essence a trade-off between saccharolytic and proteolytic metabolism, which depends on a complex interplay between the composition of the gut microbiome, the substrate availability and colonic pH—all of which are affected by transit time. A slow colonic transit limits the carbohydrate availability in the colon, favouring bacteria that can use other sources of energy such as dietary or host-derived proteins. Moreover, the nature of the microbial products also changes the physicochemical properties of the colonic environment for example, by changing pH and thus altering the microbial composition and metabolism.

### Cross-feeding and gas metabolism

While most of the microbes residing in the colon belong to Bacteroidetes or Firmicutes phyla<sup>117</sup> including species from the classes *Bacteroidia* and *Clostridia* that possess a large variety of carbohydrate-active enzymes,<sup>118</sup> less abundant species in the colon include those using secondary products of carbohydrate fermentation (eg, hydrogen, lactate, succinate, formate or ethanol). These include hydrogen-consumers such as acetogenic

bacteria, which comprise a phylogenetically diverse group of bacteria including *Blautia hydrogenotrophica* (previously known as *R. hydrogenotrophicus*<sup>119</sup>), methanogenic archaea with the predominance of *Methanobrevibacter smithii*, and sulphate-reducing bacteria (SRB), mostly represented by *Desulfovibrio* genus.<sup>120–122</sup> Although many species can produce lactate, it does not accumulate in the colon under healthy conditions due to the presence of lactate utilisers that use lactate for growth and produce SCFA.<sup>123</sup> Lactate can be converted into propionate by *Coprococcus catus*, while *Anaerostipes* and *Anaerobutyricum* spp can convert lactate into butyrate.<sup>124</sup> Both lactate and succinate can be converted into propionate by *Veillonella* spp. Some of the other succinate utilisers include *Dialister* and species, although some *Bacteroides*, for example, *B. vulgatus* can also produce succinate.<sup>125</sup> Lactate accumulates in stools of subjects with chronic diarrhoea, especially during severe ulcerative colitis suggesting a perturbation of the balance between lactate-producers and utilisers in those patients.<sup>126 127</sup> The production of H<sub>2</sub> by the gut microbes seems to be coupled with low pH.<sup>83</sup> Given that CTT is linked to pH, CTT likely affects the H<sub>2</sub> production and competition between hydrogen-using species including acetogens, methanogens and SRB.<sup>128</sup> While acetogens can use H<sub>2</sub> (and CO<sub>2</sub>) or formate to generate acetate at low pH,<sup>129</sup> methanogens can use H<sub>2</sub> (and CO<sub>2</sub>) or formate to produce methane (CH<sub>4</sub>) and SRB can use H<sub>2</sub> or lactate to produce H<sub>2</sub>S (in a presence of sulphate) at neutral or slightly alkaline pH.<sup>122</sup> H<sub>2</sub>S and CH<sub>4</sub> have both been found in higher concentrations at low dilution rates (simulating long gut transit time) in vitro suggesting that SRB and methanogens are affected by the rate of the luminal washout. In support hereof, high breath levels of CH<sub>4</sub> as well as the faecal abundance of *M. smithii*, a slow-growing methanogen unable to degrade sugars,<sup>130</sup> have repeatedly been associated with constipation and slow CTT.<sup>9 11 12 17 130</sup> Yet, a recent study reported that breath CH<sub>4</sub> was associated with both the faecal and mucosal microbiota even after adjusting for transit time, emphasising that other factors beside transit time affect the presence of methanogens. Indeed, the abundance of hydrogen-using species is dependent on the growth and competition of hydrogen-producers, and intraluminal factors such as substrate availability and pH.<sup>83 128</sup>

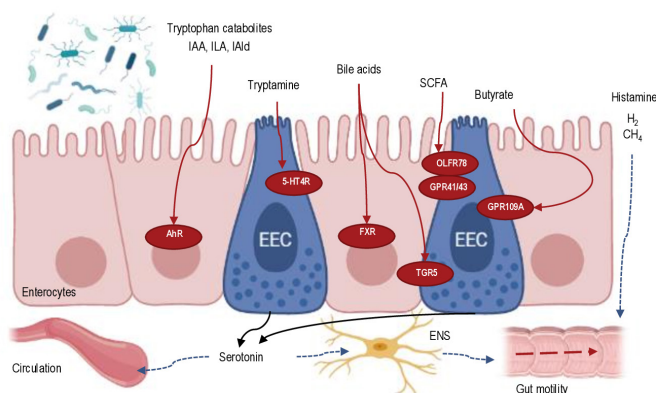
### Bile acids and enterohepatic circulation

During meals, bile is released from the gallbladder into the duodenum. Although 95% of the bile acids are reabsorbed in the terminal ileum, the rest escapes to the colon and becomes available to the colonic microbial community<sup>131</sup> that forms a large variety of secondary bile acids that upon re-absorption can re-enter the bile acid pool via the enterohepatic circulation.<sup>132</sup> Yet, the bile acid composition may be affected by CTT. Increased levels of deoxycholic acid in bile<sup>133</sup> and serum<sup>82</sup> were observed with longer colonic transit and less bile was egested via stool,<sup>134</sup> suggesting that prolonged CTT provides a longer time for the conversion into secondary bile acids and/or reabsorption of the secondary bile acids. Notably, the intestinal transit time is longer in patients with cholesterol gallbladder stones,<sup>135 136</sup> who have been shown to have higher deoxycholic acid in their bile and higher intraluminal pH in the proximal and distal colon, compared with healthy controls.<sup>82</sup> In addition, the 7 $\alpha$ -dehydroxylases, responsible for microbial conversion of primary bile acids into deoxycholic acid and lithocholic acid, are pH-sensitive and only active at pH above 6.5.<sup>137 138</sup> Since the intraluminal pH is related to CTT, interindividual differences in pH and transit time could be important for personal diet–microbiome–host interactions also concerning the microbial conversion of bile acids.

Prolonged colonic transit has also been associated with an increased concentration of circulating oestrogens,<sup>139 140</sup> which has been associated with an increased breast cancer risk in postmenopausal women.<sup>141</sup> Interestingly, a large cohort study reported that higher stool frequency, typically reflecting shorter transit time, was associated with decreased risk of breast cancer.<sup>142</sup> Steroids as well as drugs, food additives and some other dietary compounds, for example, heterocyclic amines from protein-rich diets,<sup>143</sup> undergo glucuronidation or sulphation in the liver prior to excretion to the bile. However, the glucuronide conjugates can be hydrolysed by bacterial  $\beta$ -glucuronidases in the gut, which in return increases their reabsorption and retention time in the body.<sup>144</sup> Long colonic transit may therefore increase the hydrolysis of these glucuronide-conjugates in the colon thus increasing their bioavailability.<sup>145</sup> Bacterial  $\beta$ -glucuronidases seem to be inhibited by low pH<sup>137 138</sup> and acidification of the colon (eg, by high intake of dietary fibre) may prevent accumulation of glucuronide de-conjugates. Similarly, the sulphate-conjugates excreted via bile provide substrate for the SRB that are inversely associated with transit time.<sup>92</sup> Although the relationship between transit time and the enterohepatic circulation is still not well understood, changes in transit time may alter the bile acid pool and affect enterohepatic circulation, which could have implications for both the resident gut microbes and human metabolism.

### GUT MICROBIAL METABOLISM IMPACTING GUT TRANSIT TIME

Evidently, the gut transit time is a major driver of heterogeneity of the intestinal microbial community and either directly or indirectly impacts host–microbial cometabolism.<sup>124</sup> Adding to the complexity, there is evidence that the interaction between the microbiota and the intestinal transit time is bidirectional, as the presence of microbes and their excreted compounds may affect gut motility (figure 3).<sup>146 147</sup> Germ-free mice exhibit impaired peristalsis in the GIT, which is restored by colonisation of the gut.<sup>148</sup> It has also been shown that intestinal peristalsis and colonic serotonin levels were decreased in mice that received



**Figure 3** Schematic overview of microbial-derived signalling metabolites in the intestinal epithelium and their effect on gut motility. Microbial-derived metabolites interact with various metabolite receptors expressed on enterocytes or enteroendocrine cells (EEC) and stimulate serotonin secretion from the EEC cells. The released serotonin activates the enteric neurons that promote gut motility. Other metabolites (eg, histamine) can modulate gut motility via other mechanisms. 5-HT<sub>4</sub>R, serotonin receptor-4; AhR, aryl hydrocarbon receptor; FXR, Farnesoid X receptor; IAA, indoleacetic acid; IAld, indolealdehyde; ILA, indolelactic acid; SCFA, short-chain fatty acids. Created with Biorender.com.

faecal microbiota from patients with constipation.<sup>149</sup> Moreover, administration of some probiotics has been shown to improve constipation symptoms, suggesting that presence of certain species might change gut transit.<sup>150 151</sup> One of the possible mechanisms by which microbes impact gut transit is through the host cells' recognition of bacterial molecular components by the toll-like receptors, which mediate interactions between the microbiota and the enteric neuromuscular apparatus. For example, lipopolysaccharides from the outer membrane of gram-negative bacteria impair intestinal contractility by activating oxidative stress in the mucosa.<sup>152</sup> Other mechanisms may involve the microbial-derived metabolites such as SCFA, neurotransmitter homologs and gases, which can act on the enteric neuromuscular apparatus.<sup>153</sup> SCFA can bind to G-protein coupled receptors for free fatty acids (GPR41, GPR43, OLF78, GPR109A)<sup>154</sup> and consequently stimulate the release of serotonin (5-hydroxytryptamine, 5-HT) from endocrine cells present in the colonic epithelium, a process which promotes the peristalsis via the enteric nervous system.<sup>155</sup> However, the presence of SCFA in the gut lumen also stimulates the release of gut hormones, for example, PYY<sup>156</sup> that may slow down gastrointestinal transit.<sup>157</sup> Butyrate and acetate may also affect the GIT motility through smooth muscle and myenteric neuron activation.<sup>158 159</sup> Moreover, absorption of SCFA in the colon is linked to fluid and electrolyte uptake,<sup>160</sup> which, if disrupted, can lead to altered CTT.<sup>124</sup> Recent evidence shows that secondary bile acids regulate CTT in mice with lithocholic acid inducing faster transit.<sup>161</sup> One of the mechanisms by which bile acids affect the gut transit time is via the activation of the G protein-coupled bile acid receptor 1 (TGR5) leading to increased colonic motility.<sup>162</sup> Furthermore, bile acids, both the host-derived and the microbiome-modified, can act as signalling molecules on the Farnesoid X receptor (FXR) and TGR5 receptor, which are expressed not only on epithelial cells throughout the GIT, but also outside of the GIT.<sup>163 164</sup> Through these receptors, bile acids also act as regulators of lipid, glucose and energy metabolism.<sup>165–167</sup> Furthermore, tryptophan catabolites (eg, tryptamine, indoleacetic acid, indolelactic acid or indolealdehyde)<sup>168–171</sup> may affect intestinal motility via activation of the aryl hydrocarbon receptor.<sup>172</sup> Tryptamine can activate the serotonin receptor-4 (5-HT<sub>4</sub>R)<sup>173</sup> thereby accelerating the gut transit. Histamine, produced by *M. morgani* and *L. reuteri* from histidine, has also been observed to increase colonic motility in monocolonised mice.<sup>174</sup> Finally, the gases H<sub>2</sub> and CH<sub>4</sub> are known to exert effects on the intestinal muscle contractile activity thereby affecting the gut transit time in animal models.<sup>175</sup> While the infusion of H<sub>2</sub> into the colon of guinea pigs shortened CTT, the inverse has been shown for CH<sub>4</sub>.<sup>176</sup> Altogether, the gut microbiota can modulate gastrointestinal motility via production of small molecules interacting with the host-receptors on enteroendocrine cells and other cell types such as enteric neurons.<sup>172</sup>

### THE ROLE OF GUT TRANSIT TIME IN HEALTH AND DISEASE

Sustained prolonged or shortened transit time could have consequences for host health due to the effects of gut microbial composition and metabolism.<sup>177–179</sup> Here, we discuss several areas in which transit time may play a vital role.

#### Gastrointestinal diseases

Gastrointestinal diseases such as constipation or IBS are highly prevalent worldwide. According to a recent large-scale study, more than 40% of persons worldwide suffer from at least one functional gastrointestinal disorder,<sup>180</sup> which

are disorders related to any combination of motility disturbance, visceral hypersensitivity, altered mucosal, and immune function, altered gut microbiota and altered central nervous system processing.<sup>181</sup> Here, we focus on gastrointestinal diseases that exhibit altered transit time.

Slow transit through the small bowel may result in the overgrowth of bacteria in the small intestine, a condition known as small intestinal bacterial overgrowth (SIBO). Patients with SIBO typically have high bacterial densities in the small intestine ( $>10^5$  colony forming unit (CFU)/mL) due to impaired peristalsis and insufficient washout of the bacterial mass into the colon.<sup>182</sup> Moreover, slow transit through the small intestine provides a longer time for absorption of chyme resulting in reduced flow of the chyme into the colon, which in turn also slows down the transit rate through the colon.<sup>183</sup> SIBO is prevalent among patients with IBS,<sup>184</sup> a condition characterised by abdominal pain or discomfort and associated with changes in bowel habits affecting more than a tenth of the general population.<sup>185</sup> Patients with constipation-predominant IBS (IBS-C) exhibit prolonged CTT when compared with healthy controls in all regions of the colon.<sup>186</sup> A recent study showed that IBS-C and diarrhoea-predominant IBS (IBS-D) patients have distinct faecal microbiome compositions and metabolomes. The microbiome of the patients was found to be classified according to predominant bowel habits, but not the severity of IBS symptoms.<sup>187</sup> The microbiome differences observed between the two phenotypes of IBS are thus likely to be explained by differences in transit times,<sup>188</sup> as similar compositional differences have been observed when comparing healthy individuals with long and short gut transit, respectively.<sup>11</sup>

Diarrhoea and/or constipation are also often experienced by patients with inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis, both characterised by inflammation in the gut.<sup>189</sup> It has been observed that faecal levels of secondary bile acids in IBD patients were decreased during the flare episodes but not in the remission state.<sup>190</sup> This may be explained by changes in CTT between the two states since flares are often accompanied by diarrhoea, which could limit the gut microbiota's conversion of bile acids into secondary bile acids. Nonetheless, both faecal water content and inflammatory markers were needed to predict the microbial enterotypes in IBD patients.<sup>191</sup>

Both constipation<sup>192</sup> and IBD<sup>189</sup> are risk factors for the development of colon cancer, which is the third most common cause of cancer worldwide.<sup>193</sup> Colon cancer is, among other lifestyle factors, associated with Western-type diets and prolonged gut transit time, both of which can lead to an altered bile acid pool.<sup>82 194</sup> Secondary bile acids at high physiological concentrations, especially deoxycholic acid and lithocholic acid are toxic to colonic cells as they induce apoptosis and cause DNA damage.<sup>195–198</sup> Notably, tumours often occur in the distal part of the colon,<sup>199</sup> where fermentation of complex carbohydrates is less active and the microbiota is switching to proteolysis.<sup>123 200</sup> Long CTT<sup>192 201</sup> as well as lack of fermentable dietary fibres<sup>202</sup> may lead to enhanced proteolysis in the colon, which potentially could play a role in the pathophysiology of colon cancer.<sup>203</sup> Conclusive results for the latter are scarce, but the interplay between CTT, diet and gut microbiome could be key in the prevention and management of gastrointestinal diseases.

### Diseases beyond the gut

Constipation and altered bowel habits have also been associated with neurological and metabolic diseases, and the use

of several medications.<sup>61</sup> In Parkinson's disease, constipation affects up to 80% of the patients and often precedes the onset of motor symptoms by years.<sup>204</sup> A recent meta-analysis on gut microbiota in Parkinson's patients has shown higher species richness, an increase in relative abundances of the genera *Akkermansia* and *Methanobrevibacter* as well as the family *Christensenellaceae*, depletion of butyrate producers, and low faecal SCFA when compared with healthy controls.<sup>205</sup> These changes are very similar to the associations seen between transit time and gut microbiota composition and metabolism in healthy individuals.<sup>9 11 12 17</sup> Therefore, the observed microbiome differences between Parkinson's patients and healthy controls are likely to be confounded by differences in transit time. Similar to Parkinson's disease, constipation is a common complication among patients with Alzheimer's disease<sup>78</sup> and multiple sclerosis<sup>206</sup>. Investigations of microbial compositional changes in these patients<sup>207 208</sup> could therefore be confounded by an altered gut transit time as well.

Delayed gastric emptying, as well as episodes of constipation and diarrhoea, have been reported for patients with both type 1 and type 2 diabetes mellitus.<sup>209–211</sup> Although the changes in the gut motility of these patients may be a consequence of their treatment (e.g. metformin).<sup>66</sup> In obesity, accelerated gastric emptying and changes in the transit in the small intestine, as well as both constipation and diarrhoea, have been reported.<sup>212 213</sup> Altered gut motility affects the time for nutrient absorption and may contribute to changes in hormonal responses and glucose homeostasis.<sup>107 213</sup> A recent cohort study has shown associations between stool frequency and vascular and non-vascular diseases in a Chinese population.<sup>214</sup> The authors found that 'less than three stools per week' were associated with a higher risk of ischaemic heart disease and chronic kidney disease, further suggesting a link between bowel habits and health.<sup>214</sup> These findings together suggest that gut transit time can confound investigations of microbiota composition when comparing patient groups. Whether altered transit time and bowel habits play a role in the early onset and development of diseases remains unknown.

### CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Taken together, there is convincing evidence that gut transit time varies not only between healthy individuals but also within subjects from day-to-day and that many diseases are associated with altered gut transit time. Changes in gut transit time have been associated with changes in faecal pH, faecal microbial load and composition but most importantly with diet-microbe interactions and microbial metabolism including shifts from saccharolytic to proteolytic fermentation. Since microbial-derived metabolites are important regulators of host physiology, gut transit time is likely to play a key role in host health. Although gut transit time remains largely overlooked in many gut microbiome studies, an increasing number of human studies have included WGTT or segmental transit time (SITT or CTT) and evaluated its impact on microbial composition and other target outcomes confirming the importance of this factor. By including gut transit time measurements in gut microbiome-related studies, we can advance our understanding of the links between the gut microbiome, diet and disease. Such insights may be key for the prevention, diagnosis and treatment of several diseases in the gut and beyond throughout the lifespan.

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