Age-mediated changes in the gastrointestinal tract

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Abstract

Physiological functions of the two extreme ends of the age spectrum, children (< 18 years old) and older adults (aged 65 years and over), differ from healthy young adults. This consequently affects the pharmacokinetic profiles of administered drugs, which, in turn, impacts upon clinical practice and drug therapy. The gastrointestinal milieu acts as a distinct and vital organ regulating the dissolution, absorption and metabolism of orally ingested drugs. Age-mediated alteration in the physiology and function of the gut can reshape the pharmacokinetics of certain drugs. However, our understanding of this topic is limited. This article references the gut physiology of healthy adults to capture the available evidence in the literature on the extent and nature of the changes in childhood and older age. The gut, as an organ, is examined with regards to the effect of age on luminal fluid, microbiota, transit and motility, and the intestinal mucosa. Whilst drastic developmental changes were observed in certain aspects of the gastrointestinal environment, the examination reveals significant gaps in our knowledge in the physiology and function of the developing or ageing gut. The revelation of the unknown paves the way towards a better characterization of the human gastrointestinal tract for optimized drug therapy in children and older adults.

Keywords:

Personalised medicine; paediatrics; geriatrics; aging; bioavailability; gastrointestinal tract

1. Introduction

The duration of the human life cycle and the age of the individual at death are both circumstances dependent on multiple interdisciplinary factors, ranging from living environment to nutrition, healthcare resource availability and the adoption of so-called 'lifestyle' changes such as heavy alcohol consumption, recreational drug use and cigarette smoking. The life expectancy of an individual also varies from one geographical location to another; good individual quality of life, therefore, may be reflective of a greater proportion of the population entering the elderly phase of life through undertaking of collective practices perceived as beneficial to prolongation of life (for instance, dietary, based on communal food production or availability). It has been estimated that the majority of babies born since the year 2000 in developed nations such as the United Kingdom, USA and Canada will be much more likely, for instance, to reach their 100th birthdays if the current observed increase in life expectancy for members of these populations is continued (Christensen et al., 2009). At present, the demographic most affected by this increase is by and large comprised of individuals aged 85 years and older (Wade, 2002) – to this end, it is also thought that around 18% of the US population will be accounted for by those over the age of 70 by the year 2025 (Elsner, 2002).

The natural human growing process is the cumulative effect of growth and development of different bodily organs, including the gastrointestinal tract. The gut, as with other organs, evolves and develops during gestation from a basic structure – in this case, analogous to a simple hollow tube – into a more functionalized and complex cellular system. Soon after labour, maternal support to the foetus ends, and the newborn subsequently encounters various bio-physiological challenges, such as breathing and feeding independently. Thus begins a crucial period for the functional maturity of the gut and its luminal environment with changes in digestive and transport function taking place under the influence of genetic and neurohormonal regulators that mediate gut development (Collins et al., 2006). Babies which are breastfed and those who are formula-fed harbor different bacterial species in their gut, though such differences are normally shown to dissipate after weaning at around two

years of age. This dynamic behaviour distinguishes the gut as a unique organ, whereby intra-organ – more precisely, intra-luminal – conditions are seen to be highly variable. Such variability is otherwise thought to be a consequence of the complex interplay between various factors ranging from feeding behaviour to genetics influences; gender; disease; and, somewhat inevitably, ageing.

Humans enter into adulthood as the body reaches maturity and this is followed by a downturn in advanced age. As we age, the gastrointestinal tract undergoes various morphological and functional changes, paralleling a general decline in bodily function. This involves circumstances such as delayed gastric emptying, reduced splanchnic blood flow and changes in gastrointestinal pH (Wilkinson, 1997). Such age-related changes disturb normal homeostatic mechanisms, however, and so predispose the gut to the development of certain diseases (Majumdar and Basson, 2006; Newton, 2004).

The intra-luminal environment directly influences drug bioavailability, and hence the performance of orally-administered formulations (Bai et al., 2015). The same is also true of intravenously-delivered drugs, whereby drug molecules may encounter the gut environment following hepatic diffusion or secretory processes (Arimori and Nakano, 1998). The anatomical and physiological changes, due to the development of children and the declination in advanced age alike, significantly impact upon the pharmacokinetics of certain drugs (Gidal, 2006; Roy and Varsha, 2005; Smits and Lefebvre, 1996). An understanding of the gastrointestinal environment for members of different age groups is therefore vital for the successful delivery of drugs to achieve required therapeutic outcomes in these target patient categories – broadly, the young, adult and elderly. The primary objective of this paper is to provide a general overview of the developmental changes observed in certain aspects of the gastrointestinal environment with respect to age, with particular emphasis on the lumen, microbiota, mucosa, and intestinal transit and motility. A secondary aim of this paper is to highlight the knowledge gaps in the physiology and function of the developing or ageing gut.

2. Changes in the physiology and function of the developing and ageing gut

The effects of ageing (young and elderly) on the physiology and function of the gastrointestinal tract have been investigated using various models leading to reports suggesting changes in circumstances such as gastric pH, gastrointestinal motility, serum albumin, hepatic and renal function, lean body mass and body fat when compared to younger adults influence pharmacokinetic parameters (Gidal, 2006; Roy and Varsha, 2005; Smits and Lefebvre, 1996). With age, an alteration in drug-metabolizing enzyme activity is also reported, causing a change in oral bioavailability and the plasma concentrations of drugs susceptible to first-pass metabolism (Wilkinson, 1997). However, information on age-mediated changes in gastrointestinal milieu is scarce, and there remain gaps in our knowledge. Batchelor et al., (2014) have reviewed physiological and pharmaceutical factors manipulating solubility and permeability in the paediatric population and reemphasised the current lack of appreciation of age-mediated differences in gastrointestinal tract postpartum. Moreover, age-related physiological changes in the gut have been reported to be variable mainly due to the cellular and molecular changes in the system (Saffrey, 2014). To this end, the literature presently available on human modeling has been summarized and discussed in **Table 1** below.

2.1 Changes in gastrointestinal fluid

2.1.1. Fluid volumes

Extensive studies investigating gastrointestinal water effects in humans are considerably lacking, though one publication of relevance is that by Gotch *et al* (1957), who measured the gastrointestinal water in thirteen human subjects (8 male and 5 female) post-mortem. Interestingly, the data represents elderly subjects aged 50 years and over (63 ± 9 years), with gut contents only measured up to the transverse colon. In another study, colonic mass was measured in forty six subjects (18 female, 28 male) of lower ages (48 ± 3 years) (Cummings, 1990), and Schiller et al (2005) measured the intestinal fluid volumes in even younger subjects (23-45 years). More recently, Mudie et al (2014) and Koziolek et al (2014) reported the fasted-state gastric fluid volume in young adults

comparable to Schiller et al (2005). However, it is to be noted that the measurements in older studies (Cummings, 1990; Gotch et al., 1957) were post-mortem and determined gravimetrically, whereas newer studies (Koziolek et al., 2014; Mudie et al., 2014; Schiller et al., 2005) refer to the 'free water' estimated *in-vivo* using magnetic resonance imaging. The data does not, therefore, include water bound to the gastrointestinal mass in the lumen, and so the data from older and newer studies may not be directly comparable. It is, however, noticed across different studies that gastrointestinal water varies significantly between subjects, and this inter-subject variability should be considered while planning further studies in comparing gastrointestinal fluid volumes on ageing.

Given that we know the body weight and size of the gastrointestinal tract to increase significantly from youth (childhood) to adulthood (puberty), it would be reasonable to assume that gastrointestinal mass also increases in humans from childhood to puberty (though there is no direct evidence in the literature to support this). The size and type of meal also influence the development of the gastrointestinal tract, whereas individual food choices may also adjust with age (Drewnowski and Shultz, 2001), with implications for gastrointestinal mass and luminal water content in subjects of different age groups.

2.1.2 pH

The gastric pH in neonates is neutral and drops to acidic values over the first two years of life (Bowles et al., 2010), with average gastric pH in 248 children after a 6-8 hours fast was reported as 1.37 ± 1.6, where 87 % of the children had a gastric pH of < 2.5 (Schwartz et al., 1998).

It is worth mentioning that children from 2 months to 18 years of age were included in this study and therefore average pH values may mask potential trends in gastric pH of infants (<1 mo), toddlers (<2 y), children (2-11 y) and adolescents (12-18 y). This pH data is, however, in agreement with ten different studies compiled by the authors who measured gastric pH of children (without GI symptoms who were fasted for a comparable time) in the range of 1.7 to 2.1 (Schwartz et al., 1998). The pH of 12 healthy children (5 boys and 7 girls, aged 8-14 years, median 12 y) measured after an 8-hours fast by a radio-telemetry capsule (where upon subjects continued to fast until gastric

emptying) was also comparable to that observed in adults as part of a different study from the same researchers using the pH capsule (Fallingborg et al., 1989; Fallingborg et al., 1990).

The pH in the elderly human stomach was also found to be very acidic (Hurwitz et al., 1997), though evidence was lacking to suggest that these values were different for the adult or child human. Feldman previously reviewed available evidence on gastric pH in the elderly, and suggested that the stomach of elderly subjects retains the capacity to secrete markedly high levels of gastric acid; a characteristic which does not appear to diminish with age (Feldman, 1997). Gastric and duodenal pH in 79 healthy elderly subjects (Age, 71 ± 5 years) under fasting conditions was 1.3 (range, 1.1-1.6) and 6.5 (range, 6.2-6.7), respectively. The gastric pH under fed conditions (1000 kcal meal) was increased to 4.9 (range, 3.9-5.5), but duodenal pH was unaffected (6.5, range 5.4-6.7). Notably, the rate of return of postprandially-raised gastric pH to acidic pH was comparatively slower in elderly than in young subjects (**Figure 1**). In turn, different behaviours in gastric pH were observed in the elderly subjects: the first being the typical behaviour of low fasting pH which was postprandially increased and followed by a spontaneous decrease. Around 11% of subjects were found to be achlorhydric (pH > 5 in fasted stomach), and in 45 % of these subjects, the median pH remained higher than 5.0 postprandially (Russell et al., 1993). However, in spite of the rate of gastric acid secretion being similar in older and younger individuals, the incidence of achlorhydria (characterized by consistent acid hyposecretion) is approximately 10-20 % among elderly patients compared to only <1 % in younger subjects (Gidal, 2007). It is estimated that in the USA alone, approximately 22 % of the population suffer from hypochlorhydria (characterized by intermittent acid secretion) and achlordydria; potentially compromising the oral bioavailability of certain drugs including Vitamin B12 (Russell, 1997). Elevated gastric pH was also shown to result in the compromised absorption of the weakly basic drug dipyridamole in elderly achlorhydric subjects (Russell et al., 1994).

The information about the distal gut pH in the elderly is scarce; a number of studies have suggested faecal pH in elderly human subjects was within the range of 6.57±0.10 (Bouhnik et al., 2007) and

6.97±0.75 (Chung et al., 2007), which is not too dissimilar from those values identified in adults and children (Fallingborg et al., 1989; Fallingborg et al., 1990).

2.1.3 Buffer capacity

Information on the buffer capacity of the healthy human gastrointestinal fluids due to ageing is equally scarce. The buffer capacity of gastric aspirates obtained from human volunteers (aged 20-32 years) was 14 mmol/L/ΔpH 30 minutes post-ingestion of a liquid meal (Ensure® Plus), which then increased to 28 mmol/L/ΔpH 210 minutes postprandially (Kalantzi et al., 2006). This was also related to the buffer capacity of the administered liquid meal itself (Ensure® Plus, 24 mmol/L/ΔpH). The buffer capacity of the duodenal aspirates varied between 18 and 30 mmol/L/ΔpH during 30 to 210 minutes post meal ingestion (Kalantzi et al., 2006). However, it should be borne in mind that Kalantzi and colleagues measured buffer capacity in whole gastric and duodenal aspirates; whereas the type and calorific value of the food ingested during these experiments is thought to be crucial. Little is known about the buffer capacity in the distal small intestine, however there is a report by Fadda et al (2010), where buffer capacity of human jejunal and ileal fluids were reported as 3.2 and 6.4 mM/L/ΔpH respectively, where jejunal fluids were aspirated from healthy volunteers and ileal fluids were obtained from patients undergoing surgery. The buffer capacity of the supernatants from ascending colon fluids from healthy human volunteers (aged 20-32 years) was ~18.9 mmol/L/ΔpH (Diakidou et al., 2009). However, a much higher buffer capacity (almost double) was estimated (37 mmol/L/ΔpH) when whole ascending colon fluids from humans were tested in place of the supernatants. The authors suggest that this is due to the consumption of titrated acid by the bacteria-mediated reactions prevalent in the whole colonic fluids (Diakidou et al., 2009).

2.1.4 Bile salts

Bile acids (bile salts) are the major organic components of the bile secretion, accounting for 50% of its solid content. Bile acids are released from the gall bladder into the duodenum after a meal. The average values of the total bile acid concentration are similar in the duodenum and the jenunum but decrease rapidly in the ileum due to reabsorption into the enterohepatic circulation (Dressman et al., 1998).

The mean values for the total duodenal bile acids concentration in the fasted state lie between 1.6-5.9 mM (Armand et al., 1996; Clarysse et al., 2009; de la Cruz Moreno et al., 2006; Deferme et al., 2003; Dressman et al., 1998; Lindahl et al., 1997; Persson et al., 2005). Postprandial luminal concentration of bile acids reaches a maximum within 30 minutes and gradually declines afterwards (Armand et al., 1996; Dressman et al., 1998; Fausa, 1974). The reported average bile acid concentrations were around 10 mM within 60 minutes after meal ingestion (Armand et al., 1996; Clarysse et al., 2009; Dressman et al., 1998; Fausa, 1974; Kalantzi et al., 2006; Persson et al., 2005). However, high intra- and inter-individual variability in total bile acid concentrations were reported in the literature, in both fasted and fed states. Clarysse et al., (2009) reported a time-dependent bile acid concentration variation within individual subjects in the fasted state ranging from 0.3 to 9.6 mM. The total bile salt concentration varied from 0.5 to 5.5 mM inter-individually in the fasted state in a study conducted by Moreno et al. (2006). High variability was also reported on postprandial bile salt concentrations, ranging from 0.5 to 37 mM (Hernell et al., 1990; Ladas et al., 1984).

In the early life of foetuses, the primary bile acids, cholic and chenodeoxycholic acids, are secreted by the liver, concentrated in the gallbladder and released into the fetal intestine (Watkins and Perman, 1977). However, the pool size and the concentration of bile acids in the gallbladder of new born infants (both pre-mature and full-term) was much lower than in older children and adults (Bongiovanni, 1965; Watkins et al., 1975), which might contribute to lower neonate duodenal bile acid concentrations. Challacombe et al., (1975) measured duodenal bile acid concentrations in three age groups of infants 2 hours after a 5% dextrose feed. The duodenal bile acids was significantly lower in infants under 2 days of age (1.65 ± 1.1 mM) and aged 2-7 days (3.33 ± 3.0 mM), compared to infants aged 10 days to 7 months (8.47 ± mM) which is similar to the value reported in adults. Low birth weight infants showed reduced duodenal bile acid concentrations (2.07 ± 1.3 mM in 10-19 days old and 5.8 ± 2.7 mM in 20-34 days old), compared to normal term infants (6.8 ± 2.7 mM in three weeks to 8 months of age) (Lavy et al., 1971). Unlike adults, lower concentrations of bile acids

in the duodenal content were observed in newborn infants during or within two hours after feeding and this is likely due to the dilution of gastric content (Norman et al., 1972; Senger et al., 1986).

The synthesis of bile acids by the liver was reported to decrease due to aging in older life (Bertolotti et al., 1993; Einarsson et al., 1985). (Einarsson et al., 1985) reported that the rate of bile acid synthesis in adult healthy human volunteers (age 20 years) was ~1.74 mmol/day, while the corresponding value in healthy elderly subjects (age 60 years) was only 0.91 mmol/day. Interestingly, this reduction was compensated by a reciprocal increase in cholesterol secretion from ~53 umol/h to ~73 umol/h in the same age group. Khalil et al., (1985) reported that the sensitivity of the gallbladder to plasma concentration of cholecystokinin (CCK) decreases with aging. This might be compensated by an increased release of CCK, which, in turn, could increase the circulatory concentration of CCK in the elderly. No effect of aging on gallbladder contractility and emptying kinetic was found (Khalil et al., 1985). Very limited work has been done in determining the bile acid concentration in human intestinal fluids in the older population. Annaert et al., (2010) found that there was no statistically significant change in the concentrations of 11 individual bile salts in older age compared to young adults. However, large inter-individual variation was observed in the intraluminal bile salt concentrations which may partly contribute to the absence of significant difference.

It is well known that the presence of bile salts increases the dissolution rate of poorly water soluble drugs (Dressman et al., 1998; Jantratid et al., 2008; Persson et al., 2005). This could account for an increase in absorption and bioavailability of these compounds after a meal compared to the fasted state (Charman et al., 1997; Fleisher et al., 1999; Jones et al., 2006). There are limited data available on the luminal bile salt content in children and older adults. No conclusions, therefore, can be drawn on whether changes in bile salt concentrations and/or compositions in the young and aged populations could affect drug solubility, dissolution and in turn bioavailability. Annaert et al., (2010) reported that in general drug solubility was not significantly different in the aspirated intestinal

fluids of older adults (62-72 years old) compared to young volunteers (18-25 years old). Again, large inter-individual variability in drug solubility was observed.

2.1.5 Osmolality

The information on osmolality and surface tension of human gastrointestinal fluids from different age groups is also limited. Wakayama et al. (1988) found a higher fasting gastric osmolality (253 mOsm/L) in infants (mean age of approximately 8 months) undergoing inguinal hernia repair in a study by Lindhal et al., (1997) in adults (191 ± 36 mOsm/L). Billeaud et al. (1982) reported a significant positive linear correlation between the osmolality of the diet and the osmolality in the stomach and duodenum among 15 low birth weight neonates during the 3 hours after feeding. In one study, osmolality of small intestinal aspirates from 62-72 years old (215 ± 37 $mOsm.Kg^{-1}$) and 18-25 years old (226 ± 35 $mOsm.Kg^{1}$) human subjects (Annaert et al., 2010) were not significantly different to one other.

The solubility of drug substances in the gut lumen is dependent on the interplay of a range of physiological factors, the luminal fluid volume, pH, buffer capacity, ionic composition, bile salt, viscosity and surface tension; however, there is limited data available on age-mediated changes regarding drug solubility in the human gut. In a recent study, Maharaj et al., (2016) have developed paediatric biorelevant media (i.e. P-FaSSGF, P-FeSSGF, P-FaSSIF and P-FeSSIF) reflective of age-related changes in neonates and infants based on available literature values. The solubility of seven BCS class-II compounds were compared in paediatric and adult biorelevant media and significant differences were found in some cases. For example, the solubilities of fenofibrate and carbamazepine in paediatric fasted-state gastric media (P-FaSSGF) were significantly different and outside the 80-125% bioequivalent criterion compared to that in adult-based media. In one study (Annaert et al., 2010), the average solubility of danazol (another neutral steroid) was found to be ~2 fold higher in intestinal aspirates from elderly humans (62-72 y, n=7) than in young subjects (18-25 y, n=8), though this increase did not achieve statistical significance. It has also been reported that the

solubility of an ionisable drug such as mesalamine in human gastrointestinal fluids is strongly influenced by the fluid pH and its buffer capacity (Fadda et al., 2010), and as such the solubility of such compounds is likely to be influenced by age-mediated differences in pH and the buffer capacity of gastrointestinal fluids.

2.2 Changes in the gastrointestinal mucosa

The appearance of the small bowel mucosa, in terms of villous morphology, was reported to show slight difference between young (mean age 43 years, range 13 to 59 years) and older (mean age 80 years, range 67 to 90 years) adults (Webster and Leeming, 1975). The study reported that leaf-shaped villi were more commonly seen in the older group than in younger subjects. The mean value of villous height for the older group was significantly smaller (371 µm) than weighted mean values (471 µm) from pooled results of previous publications in young subjects. However, Corazza et al., (1986) reported that there is no significant difference in the surface to volume ratio of the jejunal mucosa and the enterocyte height between young (mean age 36.8 years, range 15 to 60 years) and older (mean age 71.5 years, range 65 to 82 years) subjects.

2.2.1 Gut associated lymphoid tissue (GALT)

In the human small intestine, the Peyer's patches increase in size and number from early foetal life to puberty, with a general tendency to decrease in number rapidly after puberty, plateauing somewhat thereafter (**Figure 2**). They are well developed by the end of the fourth and the beginning of the fifth month of gestation, and the relative distribution of Peyer's patches in the duodenum, jejunum, and ileum was similar to that in older children (Cornes, 1965a).

The reduction in the number of patches with older age (70 and 95 years of age) was also gradual and fairly equal throughout the length of the small intestine, though with age, a gradual loss of lymphoid follicles within the patches was observed, and in some cases resulted in the appearance of 'fragmented' patches. In the latter case, this was seen to lead to the replacement of large patches by

a ‘collection’ of small ones; well-developed by the end of the fourth and the beginning of the fifth month of gestation. These patches were not seen to dissipate entirely in the oldest patient (95 year old female) studied where 59 patches were still clearly visible in the small intestine of the oldest patient (Cornes, 1965a, b).

Cornes (1965a, b) reported an increase in the number of Peyer’s patches in babies during gestation to birth and a continual increase until puberty (15 years) (**Figure 2**, **3A**). When the data reported by Cornes (1965a, b) was dissected in different age groups and reanalyzed using Microsoft Excel, the number of Peyer’s patches in the small intestine was found to decrease on further ageing, i.e., from 15 years to 95 years (**Figure 4A**). Where data was normalized to the number of patches per centimetre length of the small intestine, there was no difference in the number of patches present up to 15 years of age (**Figure 3B**), though a downward trend was still visible in individuals ranging from 15 years to 95 years (**Figure 4B**). Interestingly, when data was normalized to the subject body weights, an exponential decrease was observed with age until puberty (**Figure 3C**), which became less significant on further ageing (adult to elderly, **Figure 4C**).

It was suggested that the average number of lymphoid follicles in the colon of the child is comparatively greater than in adults, but there is otherwise no evidence of a decrease in the number of lymphoid follicles in old age (Dukes and Bussey (1926). When the data was dissected in different age groups and analysed using Microsoft Excel, a negative correlation was seen in the number of lymphoid follicles per cm^2 of the colon with age (**Figure 5A**). The average number of lymphoid follicles per cm^2 of the colon in younger subjects aged 15 years or less was 8.0 ± 2.3 (n=7), which decreased to half in individuals aged between 16-40 years (4.0 ± 1.6 follicles/cm^2, n=27). This decreased further with age to 3.5 ± 1.6 (41-60 years, n=57) and 3.1 ± 1.6 (61-88 years, n=15). More interestingly, when the data was grouped according to gender, it was found that the decrease in average follicles did not appear in females, whereas a clear downward trend was apparent in male subjects (**Figure 5B**).

2.2.2 The mucus layer

It has been suggested that the protective mechanisms of the human gastrointestinal tract are impaired with age (Newton et al., 2004), implicated by the higher statistical incidence of gastrointestinal diseases in the elderly ranging from peptic ulceration to gastric cancer and inflammatory bowel disease (Newton, 2004; Pullan et al., 1994). The normal gastro-duodenal mucosal protection is the consequence of complex interplay between bicarbonate secretion and the behaviour of the gastric mucus layer (Allen et al., 1993), to which end a decrease in bicarbonate secretion by gastric (Feldman and Cryer, 1998) and duodenal (Kim et al., 1990) mucosa. An increased production of mucus in the glands is stated as part of the development of the newborn GI tract (Xu, 1996). During the perinatal period, the undernutrition results in decreased mucus levels in the small intestine (Neu, 2007). For older people, reduced mucus thickness in the upper gut has been reported, particularly in patients with *H. pylori* infection (Newton et al., 2000).

The age-mediated decrease in the number of mucus producing cells (Goblet cells) has also been demonstrated in humans, which in turn results in a lower amount of secreted mucus over the epithelium. Also, the total sialic acid concentration in human gastric aspirates was found to decrease with age; suggesting a structural change in gastric mucus (Corfield et al., 1993). Here viewed on a molecular level, it has been shown that mucosal concentrations of prostaglandins A and E – which stimulate gastric mucus and bicarbonate secretion – are decreased in the elderly (Cryer et al., 1992a; Cryer et al., 1992b), whilst bicarbonate secretion by gastric mucosa (Feldman and Cryer, 1998) and duodenum (Kim et al., 1990) is also impaired in elderly subjects.

2.2.3 Mucosal enzymes and transporters

The intestinal epithelium accommodates a range of drug transporters (both influx and efflux) and a family of membrane-bound metabolic enzymes. These enzymes and transporters contribute

substantially to the absorption and bioavailability of a large numbers of drug substances. Limited research has been conducted on the effect of age on the expression of solute carriers in the intestine. Buddington et al. has reviewed ontogenetic development of intestinal nutrient transporters in humans (Buddington and Diamond, 1989). However, the effect of this on drug absorption is unclear.

The function of the efflux transporters is to expel compounds that have penetrated the intestinal epithelium back into the gut lumen, with P-glycoprotein (P-gp) being the most studied amongst them. P-gp is the product of the multidrug resistance gene MDR1 and is recognized as a member of the APT-binding cassette super-family of membrane transport proteins (Hunter and Hirst, 1997). Other efflux transporters include multidrug resistance-associated protein 2 (MRP2) and breast cancer resistance protein (BCRP). The intestinal metabolic enzymes, known as cytochromes, are responsible for the metabolism of variety of drug compounds, potentially decreasing their bioavailability. The most important membrane-bound enzyme is the cytochrome P450 (CYP450), with the most abundant form being CYP3A4 accounting for 80% of CYP enzymes in the small intestine (Doherty and Charman, 2002; Suzuki and Sugiyama, 2000).

A clear expression of MDR1-mRNA can be observed in human fetal intestinal epithelium from 16 to 20 weeks fetal age (van Kalken et al., 1992). Fakoury et al. detected MDR1-mRNA in the intestine of children aged 1 month to 17 years, however, they did not find a significant relation between P-gp expression and age (Fakhoury et al., 2005). Miki et al. (2005) demonstrated that MDR1-mRNA expression was 4-5 fold higher in the small intestine of young adults (15-38 years old) than in fetus and neonates (0 years old). Again, they did not show a significant correlation between the development and age. Mooij et al., (2014) found that intestinal transporter expression was statistically significantly associated with age for MRP2, but not for MDR1.

Miki et al., (2005) also reported a 4-fold higher mRNA expression of the CYP3A4 enzyme in the small intestine of young adults than of fetus and neonates with no significant difference. However, in the large intestine, the CYP3A4 mRNA expression was significantly higher (about 8-fold) in fetus than in neonates and young adults. In contrast, the study by Fakoury et al., (2005) reported that the duodenal mRNA expression of CYP3A4 was significantly higher in the first year of life and decreased with age to lower values in older children (1-6 years and 6-17 years). In an earlier study Johnson et al., (2001) demonstrated a developmental pattern of increasing CYP3A4 mRNA expression and enzyme activity from fetus to children older than 12 years (**Figure 6**). However, a statistically significant difference was only observed between neonates and children > 12 years.

Literature has provided limited evidence on the developmental effect on the activity of P-gp and CYP3A4 in the intestine of children, let alone their impact on the absorption of drug molecules that are substrates of these transporters and enzymes. de Wildt et al., (2002) have observed a nearly 10-fold lower rate in midazolam clearance after oral administration in preterm infants compared to adults. The oral midazolam clearance is dependent on the intestinal and hepatic CYP3A4 activities (Thummel et al., 1996). The possible low level of CYP3A4 activity in the intestine of neonates could explain this low clearance rate and a resultant higher oral bioavailability in pre-term infants (median 49%) compared to adults (24-38%) (de Wildt et al., 2002). Johnson et al. (2008) have suggested a list of drugs used in children with variable oral bioavailability which is believed to be due in part to intestinal first-pass metabolism (Johnson and Thomson, 2008).

The effect of older age on the intestinal CYP3A4 activity was unclear. Miki et al. demonstrated a nearly one-third decrease in CYP3A4 mRN expression in the elderly (67-85 years) compared to young adults (15-38 years); however, no significant correlation was found between age and the mRN expression (Miki et al., 2005). Gorski et al. investigated relative susceptibility of intestinal and hepatic CYP3A activity to induction by rifampin (a CYP3A inducer), measured by the clearance of CYP3A substrate midazolam (Gorski et al., 2003). They reported a nearly one-third reduction in the

oral clearance of midazolam in the elderly (age 70 ± 4 years men and 72 ± 5 years women, clearance rate 19 ± 18) compared to young adults (age 27 ± 4 years men and 26 ± 4 years women, clearance rate 36 ± 26), and again, no significant difference was detected. Fromm et al. studied the effect of rifampicin induction to intestinal CYP3A4 activity on pre-hepatic clearance of S-verapamil after oral administration in older adults (67.1 ± 1.2 years) (Fromm et al., 1998). Bioavailability of verapamil decreased significantly from 14.2 ± 4.3% to 0.6 ± 0.5% as a consequence of the rifampicin induction. However, this was not compared to the effect in younger adults. The impact of aging on the activity and expression of P-gp has been investigated in a number of tissues in human, especially in lymphocytes (**Table 2**). However, the effect on intestinal P-gp activity in human and its consequence in drug absorption in older age remains largely unclear.

2.3 Changes in gastrointestinal transit and motility

The paediatric data for gastrointestinal transit and motility has been well reviewed focusing on its impact on oral drug delivery design (Bowles et al, 2010). There are developmental oropharyngeal anatomical differences, mainly from birth into early childhood. The oesophageal transit time is comparable to normal younger adult values. The gastric emptying time of liquids is reported to reach adult values by around 6-8 months of age (Bowles et al, 2010). The intestinal transit time is similar between younger (2 months-3 year old) and older segments of paediatric population (3-12 year old) (Bowles et al, 2010). The colonic transit time in children is also comparable to normal adult values (Bowles et al, 2010).

The motor function of the gastrointestinal tract is relatively well preserved in healthy aged people while diseases and concomitant medicine use may alter the gastrointestinal system function at advanced age (Sinclair et al., 2012). In order to focus on the effect of the normal ageing process on gastrointestinal transit and motility, the changes that may occur due to age-related diseases and concomitant drug use are not mentioned in this paper. The age-related changes are significant in oropharyngeal and oesophageal motility, in particular reduction in lower oesophageal sphincter

pressure resulting in impairments including dysphagia and reflux (Grande et al., 1999; Grassi et al., 2011). The oesophageal dysmotility is most common in the very old (Hollis and Castell, 1974). The gag reflex is absent in 43% of elderly subjects (Davies et al., 1995).The oesophageal sphincteric and peristaltic function deteriorates with ageing (Grande et al., 1999; Ren et al., 1993; Robbins et al., 2006); changes in peristalsis are seen from as early as 40 years of age (Gregersen et al., 2008). Nevertheless, the magnitude of amplitude of oesophageal pressure changes is modest (Orr and Chen, 2002).

Although a modest slowing of gastric emptying time (Brogna et al., 1999; Evans et al., 1981; Kao et al., 1994) is reported to be associated with advanced age, the effect of natural healthy ageing process is reported to be minor on the GI motility (Fich et al., 1989; Husebye and Engedal, 1992; Kagaya et al., 1997; O'Mahony et al., 2002; Russell, 1992). The fasting and fed gastric motility do not differ between younger (18-39 year old) and older (40-69 year old) adults (Fich et al., 1989). An early study (Haboubi et al., 1988) indicated that small intestinal transit was slowed in elderly subjects but later studies using gamma camera (Madsen and Graff 2004) and video capsule endoscopy (Fischer and Fadda, 2016) confirmed that transit remains unaffected by age (**Figure 7**). However the influence of age on small intestinal transit time is still inconclusive and requires further investigation on larger cohorts with well-defined age bands. Healthy ageing does not substantially affect the small intestine motility in contrast to the altered colonic transit in elderly (Sinclair et al., 2012). In the colon, there is evidence of a prolonged transit time due to a decline in propulsive activity in the colon related to a reduction in both neurotransmitters and receptors (Britton and McLaughlin, 2013; Gomes et al., 1997; Madsen and Graff, 2004; Salles, 2007). The age-related loss of enteric cholinergic neurons is reported to be associated with the delay in colonic transit, leading to inefficient peristalsis (Wiskur and Greenwood-Van Meerveld, 2010). Further studies are required to be conducted in order to understand the true age-related changes in gut structured function by measuring gut function against age-related normal values rather than against data from younger adults (O'Mahony et al., 2002).

2.4 Changes in the gastrointestinal microbiota

The human gut is intensively colonised by microbes. The numbers of microbes and the composition of the microbiota vary significantly at different sites in the gastrointestinal tract (Ouwehand and Vesterlund, 2003). The stomach contains relatively low numbers of bacteria (< 10^4 CFU/ml). In comparison, a relatively higher density of bacteria colonise the small intestine, with approximately 10^3-10^5 CFU/ml in the duodenum and jejunum and 10^7-10^8 CFU/ml in the ileum respectively. The colon is the main site of microbial colonisation, containing an estimated 1.5 kg of microbes equivalent to approximately 10^{14} microbes (10^{10}-10^{11} CFU/ml) (Moore and Holdeman, 1974; Ouwehand and Vesterlund, 2003).

The intestines of new-born infants are considered to be sterile. Intestinal bacteria start to colonise within hours and a rapid increase in bacterial count occurs within the first few days. At the end of the first week, infant faecal bacteria reach a level of 10^9-10^{10} CFU/g of wet faeces (Palmer et al., 2007). However, infant intestinal microbiota is very different from that found in adults with low diversity and low complexity (Adlerberth and Wold, 2009). The neonatal gut presents high level of oxygen which prevents the growth of anaerobes. Therefore, enterobacteria dominate the early life flora including *E. coli* and enterococci (Adlerberth and Wold, 2009). During the first week of life, luminal anaerobic bacterial populations expand. These bacteria species consume the oxygen and create an anaerobic environment in the gut, which further favours the growth of anaerobic bacteria such as *Bifidobacterium*.

In the first months of life, the microbiota of the infant gut varies remarkably between individuals. The composition of the intestinal microbiota in early infancy is affected by many factors such as the mode of delivery, type of infant feeding, hospitalisation after birth and use of antibiotic (Penders et al., 2006). This inter-individual diversity decreases in the early months and the gut flora progresses toward an adult-like profile by the end of the first year. A Danish study reported significant changes in intestinal microbiota in infants before 18 months of life (Bergstrom et al., 2014). The intestinal

microbiota does not completely reach the adult state until much later in childhood; differences in viable counts of predominant bacterial species could still be seen between children up to 7 years old and adults (Hopkins et al., 2002; Ringel-Kulka et al., 2013).

In the older population, mean total anaerobe counts remain similar to that of young adults; however, shifts in dominant bacteria species are frequently reported (Woodmansey, 2007). One of the most marked changes in older people is the decline in numbers of bifidobacteria (Hopkins et al., 2002; Mitsuoka, 1992; Mitsuoka et al., 1974; Woodmansey et al., 2004). It was reported that the species diversity of bifidobacteria is also reduced in the elderly, with dominant species being *Bifidobacterium adolescentis, Bifidobacterium angulatum* and *Bifidobacterium longum* (He et al., 2001; Mitsuoka, 1992; Mitsuoka et al., 1974; Woodmansey et al., 2004). Studies have shown that total numbers and species diversity of bacteroides also decrease in older age (Claesson et al., 2011; Guigoz et al., 2002; Hopkins et al., 2002; Woodmansey et al., 2004). Bacteroides species contribute to the digestion of majority of polysaccharides to short-chain fatty acids in the colon. Reductions in amylolytic activities and overall fecal excretion of short-chain fatty acids have been reported in the older population (Woodmansey et al., 2004). This could be the consequences of the decline in bacteroides species in the colon. In contrast, numbers of facultative bacteria species were found to increase in the aged gut (Bouhnik et al., 2007; Guigoz et al., 2002; Hopkins et al., 2001; Woodmansey et al., 2004). In particularly, higher numbers of enterobacteria, streptococci, staphylococci and yeasts were reported in the elderly compared to young adults (Woodmansey et al., 2004).

A cross-sectional study examined fecal microbiota in older populations in four European countries, France, Germany, Italy and Sweden (Mueller et al., 2006). It was found that age effects in intestinal microbiota composition were dependent on the geographical location of the subjects. For example, higher levels of eubacteria were detected in the elderly compared to young adults in Germany; whereas a decrease in levels of these species with age was found in Italy. The older subjects in Sweden had the highest levels of *Fecalibacterium prausnitzii* in all study countries. However, higher

proportions of bifidobacteria were found in Italian subjects than in subjects from any other countries and this effect was independent of age. Another study suggested that many external factors affect the intestinal microbiota composition in the elderly. Significant correlations were found between microbiota composition in the elderly and health factors including diet, frailty, co-morbidity, nutritional status and markers of inflammation (Claesson et al., 2012). It was reported that older individuals with high frailty scores had a significant reduction in the number of lactobacilli compared to healthy older adults; whereas significantly higher levels of *Enterobacteriaceae* was found in these very frail older subjects (van Tongeren et al., 2005). **Table 3** summarizes the faecal microbiota composition of children, adults and the elderly in selected studies.

Bacteria present in the gut lumen are capable of metabolizing a range of drug substances (Sousa et al., 2008), affecting their bioavailability in modified release formulations. Drug delivery systems targeting to the colon are designed to utilize bacteria activity for the release of active substances. These include the inclusion of polysaccharides that are substrates to colonic bacteria metabolism and pro-drugs, such as the prodrugs of 5-aminosalicylic acid (mesalazine), including sulfasalazine, balsalazide, and olsalazine, for the treatment of ulcerative colitis. These prodrugs are poorly absorbed in the stomach and small intestine and rely on colonic anaerobic bacteria to cleave the azo linkage and liberate the active drug (Sousa et al., 2014). Changes in colonic microbiota, be it an increase or reduction in number, species and/or activity, in children and older adults might affect the absorption of drug compounds that either benefit from or be destroyed by these microorganisms.

3. Conclusion and future perspectives

Age is an important determinant that impacts on the absorption and metabolism of drugs. It is apparent from this paper that many physiological and functional aspects of the human gut differ in children and older individuals from young adults. However, there are many knowledge gaps on age-related changes in the gut. The heterogeneity of both age groups further complicates the situation, as children do not develop at the same rate nor do the elderly age uniformly. Considering the

rareness of healthy older patients, individual studies should be conducted on the geriatric population with co-morbidities and multiple medications, to consider the potential influence of concomitant diseases on the in-vivo fate of the drug in the gastrointestinal tract. Equally, further understanding on the chronological age-related changes (only healthy young and healthy older age-related) in the gut is important. In the case of the elderly, frailty should be a better indicator for the aged-gut compared to chronological age as the ageing status should be ideally defined by connecting the decline in physiological capacity and increased risk of vulnerability to disease.

Children and older patients are often underrepresented in clinical trials resulting in a lack of evidence based information on the effect of ageing on oral drug bioavailability. Although it is known that the function of GI tract is altered during developmental stages or with advanced age, the effects on pharmacokinetics and/or pharmacodynamics of the orally administered drug are often unclear. This data obtained from children and older subjects is essential as the prediction of clinical outcomes based on the gut physiological changes and/or extrapolation from healthy young adults may not be appropriate. The impact of prediction tools (e.g. physiologically-based pharmacokinetic/pharmacodynamics modelling and simulation) should be further explored to inform clinical trials in younger and older populations. Age related changes in barriers to drug delivery should be available to formulation scientists and adequately reflected in the design of personalized formulations to ensure the development of high quality, safe and effective drug therapies for use in young and older patients.

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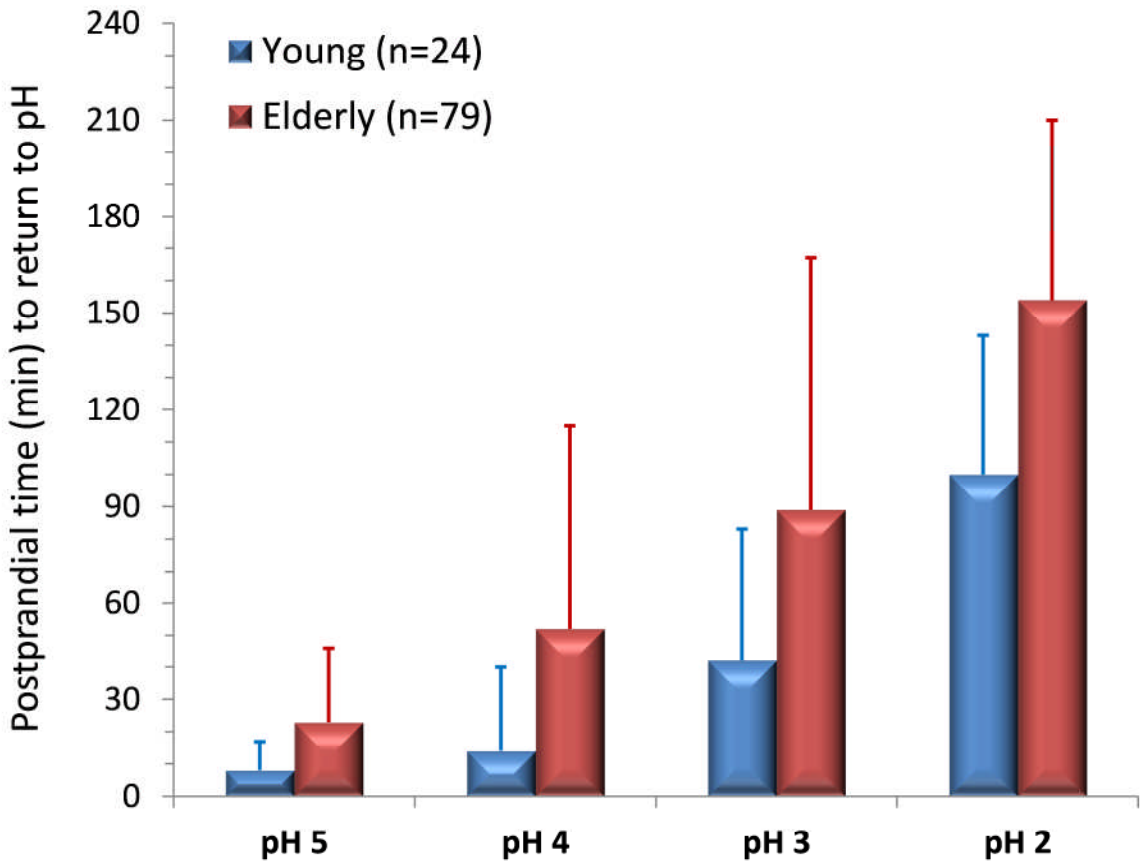

Figure 1. Postprandial time to return to pH 5, 4, 3 and 2 in elderly (65-83 years, n=79) and young (21-35 years, n=24) subjects. Figure plotted using data from (Russell et al., 1993).

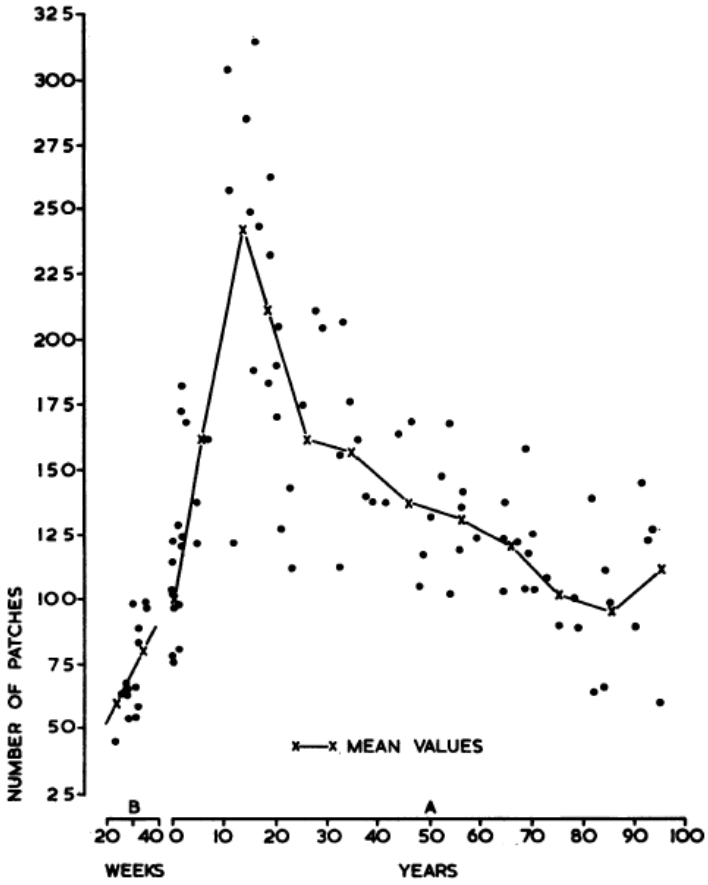

Figure 2. Effect of age on the number of Peyer's patches in human small intestine. B: before term (from 24 to 37 weeks gestation), A: after term (from birth to 95 years). Figure reproduced from (Cornes, 1965b).

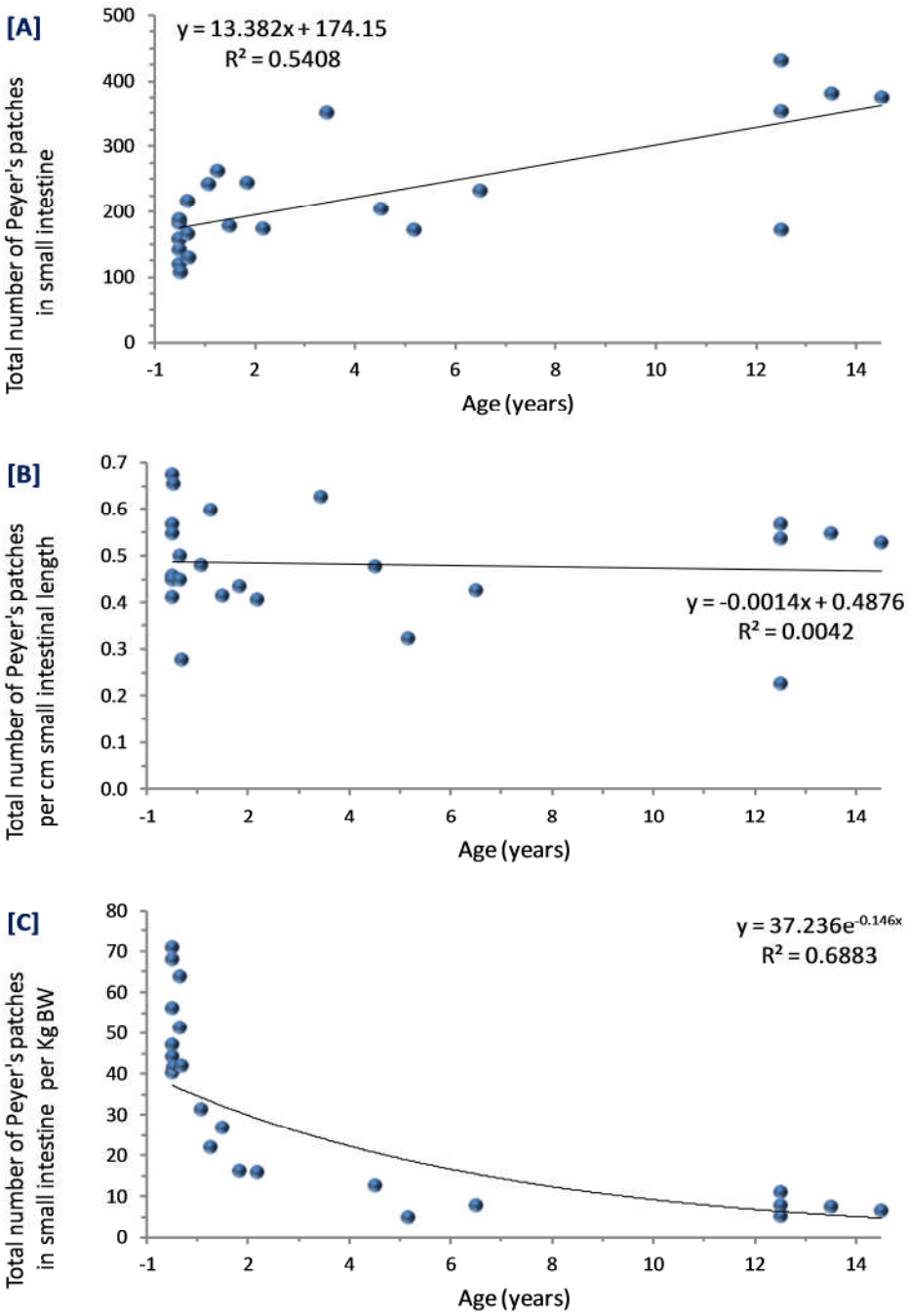

Figure 3. Effect of age on the number of Peyer's patches in human small intestine in subjects up to 14 years of age. Figure plotted using data from (Cornes, 1965b).

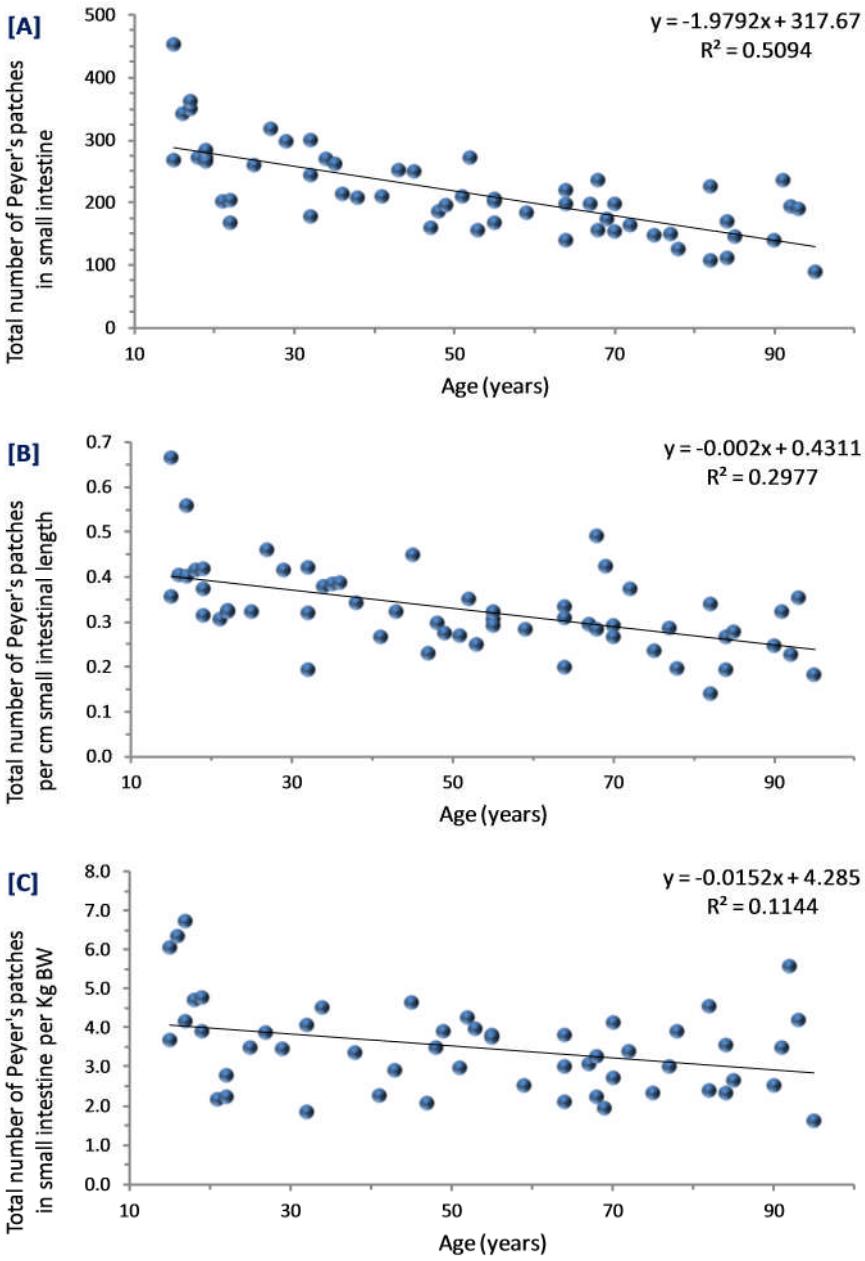

Figure 4. Effect of age on the number of Peyer's patches in human small intestine in subjects from 15 to 95 years of age. Figure plotted using data from (Cornes, 1965b).

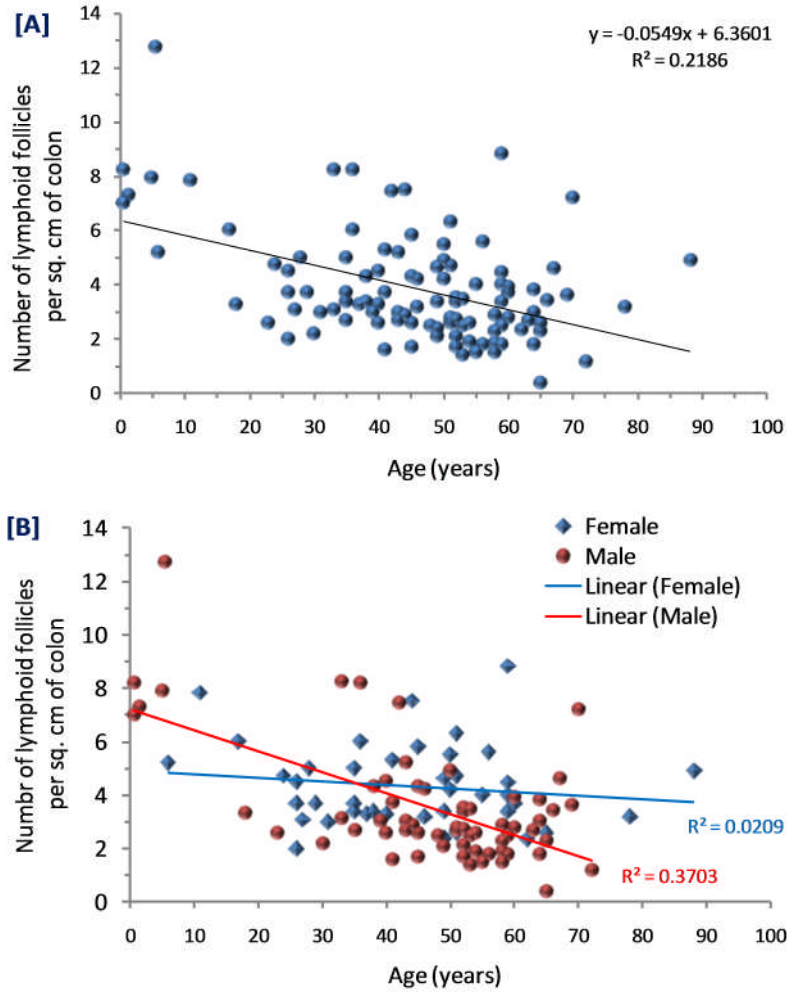

Figure 5. Effect of age on the number of lymphoid follicles per cm^2 of human colon. Figure drawn using data from (Dukes and Bussey, 1926).

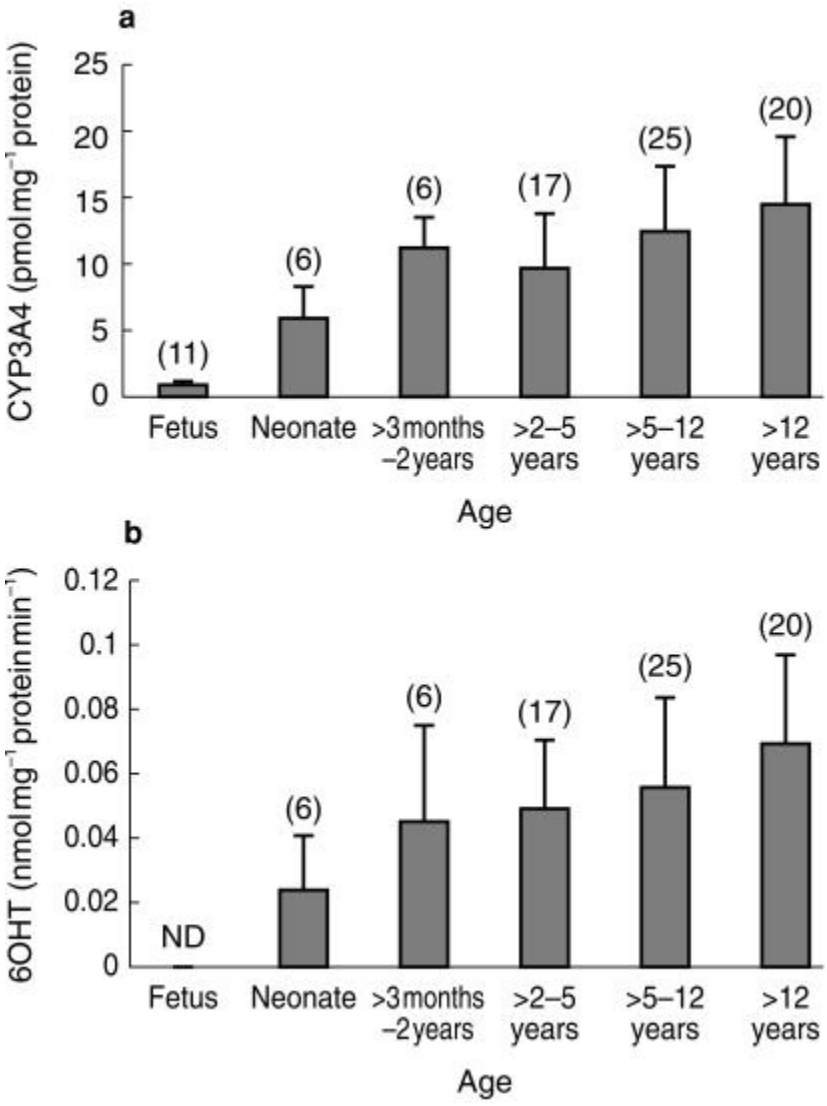

Figure 6. (a) Age related changes in the villi corrected expression of CYP3A4 in histologically normal duodenal sections. The numbers in each group are given in brackets and error bars are ± s.d. Statistical significance differences ($P < 0.05$) were achieved between foetus and all other groups and between neonate and children > 5 years. **(b)** Age related changes in villin corrected CYP3A4 activity measured by the rate of 6OHT formation in histologically normal duodenal sections. The numbers in each group are given in brackets and error bars are ± s.d. A statistically significant difference ($P < 0.05$) was observed only between neonates and children > 12 years. (Reproduced from (Johnson et al., 2001).

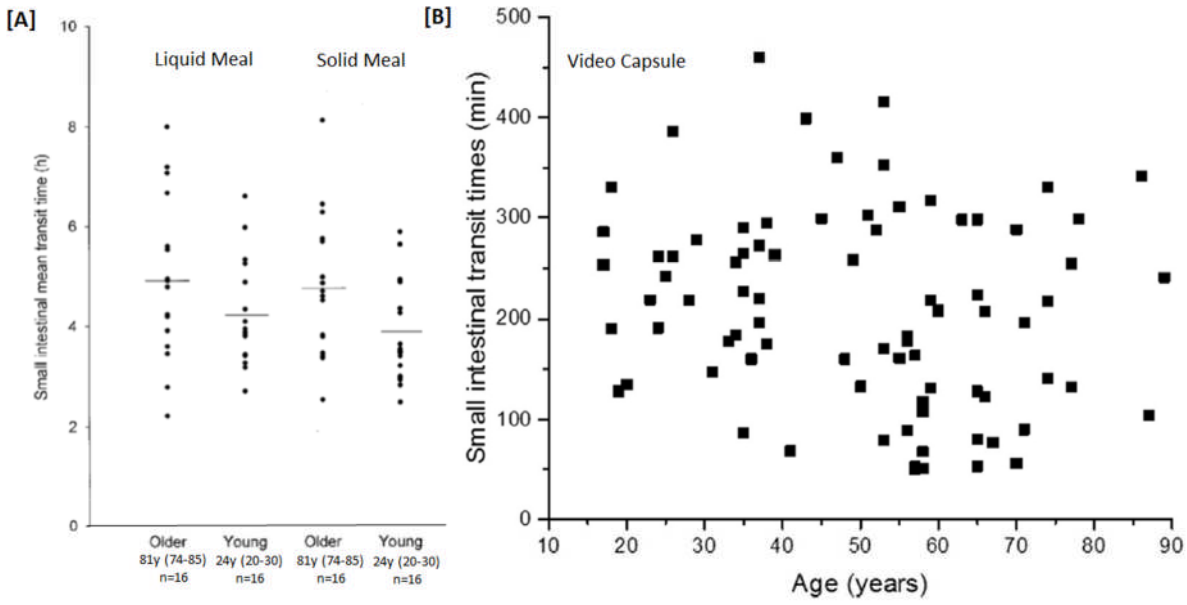

Figure 7. Effect of age on small intestinal transit time , figure adapted from [A] Madsen and Graff (2004) and [B] Fischer and Fadda (2016)

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Table 1. Effects of ageing on the human gastrointestinal environment.

GI characteristics	Mean ± SD		
	Young	**Adult**	**Elderly**
pH	8-14 y (n=12)[1]	18-65 y (n=39)[2]	65-83 y (n=79)[3]
Stomach	1.6	1.5	1.1-1.6
Small intestine (SI)			
Duodenum	6.5	6.4	6.5
Jejunum	6.6	6.6	
Mid SI	7.0	7.0	
Distal SI	7.4	7.3	
Caecum	5.9	5.7	
Colon			
Ascending	5.6	5.6	
Transverse	5.5	5.7	
Descending	6.0	6.6	
Rectosigmoid	6.5	6.6	
Faeces	6.4	6.5	6.57[4]
Buffer Capacity (mmol/L/ΔpH)			
Stomach		14 (20-32 y)[5]	
Small intestine (SI)			
Duodenum		18-30 (20-32 y)[5]	
Jejunum		3.2 ± 1.3 [6]	
Ileum		6.4 [6]	
Caecum		-	
Colon			
Ascending		18.9 (20-30 y) [7]	
Transverse			
Descending			
Rectosigmoid			
Faeces			
Bile salts (mM), Duodemum			
Fasted		1.6-5.9[10-16]	
Fed	1.7 (under 2 days)[19]	~ 10[10-13, 17,18]	
	3.3 (2-7 days)[19]		
	8.5 (10 days to 7 mo)[19]		
Osmolality (mOsm.Kg^{-1})		226 ± 35 (18-25 y)ref	215 ± 37 (62-72 y)ref
Gut associated lymphoid tissue (GALT)			
SI (Peyer's patches) [8]	222 ± 91 (0-14 y)	273 ± 67 (15-38 y)	181 ± 43 (41-95 y)
Colon (follicles/cm^2) [9]	8.0 ± 2.3 (≤15 y)	4.0 ± 1.6 (16-40 y)	3.5 ± 1.6 (41-60 y) 3.1 ± 1.6 (61-88 y)

[1] Fallingborg et al 1990, [2] Fallingborg et al 1989, [3] Russell et al 1993, [4] Bouhnik et al 2007, [5] Kalantzi et al., 2006, [6] Fadda et al 2010, [7] Diakidou et al., 2009, [8] Cornes 1965ab, [9] Dukes and Bussey 1926, [10] (Armand et al., 1996), [11] (Clarysse et al., 2009), [12](Persson et al., 2005), [13] (Dressman et al., 1998), [14] (Lindahl et al., 1997), [15] (de la Cruz Moreno et al., 2006), [16] (Deferme et al., 2003), [17] (Fausa, 1974), [18] (Kalantzi et al., 2006), [19] (Challacombe et al., 1975)

Table 2. Studies on the effect of aging on P-gp activity and expression in human.

Parameter tested	Cell type	Effects of aging	Reference
P-gp activity	B and T lymphocytes	↓	(Pilarski et al., 1995)
P-gp expression	T lymphocytes	↑	(Aggarwal et al., 1997)
P-gp activiety		↑	
ABCB1 expression		↑	
P-gp expression	Enterocytes	→	(Lown et al., 1997)
P-gp activity	B and T lymphocytes	→ or ↓	(Machado et al., 2003)
P-gp activity	Bone marrow stem cells	→ or ↑	(Calado et al., 2003)
P-gp activity	Natural killer cells	→	(Brenner and Klotz, 2004)
P-gp activity	Blood-brain barrier	↓	(Toornvliet et al., 2006)
ABCB1 expression	Liver	→	(Prasad et al., 2014)
P-gp activity	Blood-brain barrier	↓ in male, → in female	(van Assema et al., 2012)
P-gp activity	Intestine	→	(Larsen et al., 2007)
P-gp expression	Male lymphocytes	↑	(Vilas-Boas et al., 2011)
P-gp activity		→	

* Studies published prior to 2007 were adapted from (Mangoni, 2007). Increase (↑); decrease (↓); no change (→)

Table 3. Selected studies on the composition of the faecal microbiota in children, adults and the elderly*

Study population	Age	Total anaerobes	Bacteroides	Bifidobacterium	Enterobactria	Enterococci	Clostridia	Lactobacilli	Reference
Children	1 w		4.8 - 9.3	6.2 - 10.2	6.2 - 9.4	5.7 - 9.0	3.1 - 7.2	4.4 - 7.0	(Adlerberth and Wold, 2009)†
	5 w		6.0 - 10.1	4.3 - 11.3	6.1 - 9.6	4.5 - 9.6	3.0 - 8.1	5.0 - 9.1	(Adlerberth and Wold, 2009)†
	1 m		9.40 (5.74-10.36)	10.71 (6.84-11.56)			5.24 (2.70-9.57)	8.70 (7.92-10.73)	(Scheepers et al., 2015)
	16 m - 7 y	10.4 ± 0.2	9.9 ± 0.4	9.8 ± 0.3	8.0 ± 0.4	5.5 ± 0.5	7.2 ±0.8	6.6 ± 0.7	(Hopkins et al., 2002)
Adults	21 - 34 y	10.5 ± 0.1	10.0 ± 0.1	9.1 ± 0.2	5.9 ± 0.5	6.1 ± 0.7	6.6 ± 0.4	6.7 ± 0.6	(Hopkins et al., 2002)
	19 - 35 y		9.9 ± 0.1	9.5 ± 0.2	5.8 ± 0.6	6.5 ± 0.9	5.6 ± 1.0	6.3 ± 1.0	(Woodmansey et al., 2004)
	21 - 39 y	9.11	9.42	9.54					(Tiihonen et al., 2008)
Elderly	67 - 88 y	10.1 ± 0.2	9.6 ± 0.2	7.3 ± 1.0	6.7 ± 0.8	6.0 ± 0.8	6.9 ± 0.6	5.4 ± 1.0	(Hopkins et al., 2002)
	67 - 75 y		6.5 ± 2.1	8.1 ± 1.6	7.3 ± 0.4		5.3 ± 1,7	4.1 ± 1.8	(Woodmansey et al., 2004)
	> 62 y	10.3 ± 0.5		8.6 ± 1.0				6.0 ± 1.4	(Bartosch et al., 2005)
	69 ± 2 y	10.09 ± 0.07		8.5 ± 0.26	7.69 ± 0.21		3.25 ± 0.25		(Bouhnik et al., 2007)
	77 - 97 y		8.8	6.0	7.7	6.1	3.5	5.1	(Guigoz et al., 2002)
	68 - 84 y	9.29	9.59	9.59					(Tiihonen et al., 2008)

*Amounts are given as $\log_{10}$ number of bacteria/g fresh faecal weight, †Adapted from reference (Adlerberth and Wold, 2009), summarising studies on intestinal microbiota in children performed until 1990.

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