Critical review evaluating the pig as a model for human nutritional physiology

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Abstract

The present review examines the pig as a model for physiological studies in human subjects related to nutrient sensing, appetite regulation, gut barrier function, intestinal microbiota and nutritional neuroscience. The nutrient-sensing mechanisms regarding acids (sour), carbohydrates (sweet), glutamic acid (umami) and fatty acids are conserved between humans and pigs. In contrast, pigs show limited perception of high-intensity sweeteners and NaCl and sense a wider array of amino acids than humans. Differences on bitter taste may reflect the adaptation to ecosystems. In relation to appetite regulation, plasma concentrations of cholecystokinin and glucagon-like peptide-1 are similar in pigs and humans, while peptide YY in pigs is ten to twenty times higher and ghrelin two to five times lower than in humans. Pigs are an excellent model for human studies for vagal nerve function related to the hormonal regulation of food intake. Similarly, the study of gut barrier functions reveals conserved defence mechanisms between the two species particularly in functional permeability. However, human data are scant for some of the defence systems and nutritional programming. The pig model has been valuable for studying the changes in human microbiota following nutritional interventions. In particular, the use of human flora-associated pigs is a useful model for infants, but the long-term stability of the implanted human microbiota in pigs remains to be investigated. The similarity of the pig and human brain anatomy and development is paradigmatic. Brain explorations and therapies described in pig, when compared with available human data, highlight their value in nutritional neuroscience, particularly regarding functional neuroimaging techniques.

Key words: Pig model: Nutrition: Chemosensing: Food intake: Microbiota

Introduction

Rodents, mainly mice, have become the model for studies related to human physiology and genetics. However, the differences between rodents and humans in dietary habits, digestive strategies including coprophagy, nutrient requirements and nutrient–nutrient interactions suggest the pig may be a closer match than rodents for studying physiological functions relating to these research areas[1–4]. The suitability of the pig as a model for human research is becoming accepted, particularly related to physiological factors and biomedical applications[2,5–7]. Both humans and pigs are classified as omnivorous mammals and share similarities related to anatomical features of the gastrointestinal tract (GIT), such as the size of the compartments and the predominance of the colon rather than the caecum as the main fermentation site of plant/fibrous dietary components[2,8]. Pigs have been used to study several areas relevant to human nutritional sciences[1,2,9–11], including metabolic syndromes, obesity, bariatric surgery[2,5], neuroscience, brain imaging[3,12], food allergies[13], alcohol intake[14,15] and mother–offspring interactions[16]. In addition, the sequencing of the pig genome has widened support for using the pig as a model for humans by revealing the similarities and differences between pigs and humans at the gene and chromosomal levels[17].

The present review provides a critical evaluation of the pig as a model for studies related to humans in areas that encompass nutritional chemosensing and hormonal profiles relevant to feed/food intake, the gut barrier function, the host–microbiota interactions and the relationship between nutrition and brain development and metabolism. In addition, the present paper assesses, as quantitatively as possible, how appropriate it would be to use the pig as a model for humans for each of the areas discussed.

Nutritional chemosensing

The chemosensory system has evolved to allow animals to discriminate between foods in their environment, and is believed to

reflect species-specific dietary needs[18]. For example, humans have developed trichromatic vision, which was a key event relevant to food recognition and may have contributed to decrease the reliance on olfaction[19]. In contrast, pigs appear to have the largest olfactory gene repertoire of any animal studied, with 1,301 potential olfactory receptor genes[20]. The potential for pigs to become a model for human research in olfaction is relevant in the case of maternal transfer of dietary cues[21–24].

Nutritional chemosensing is the science studying the perception of nutrients in biological systems which relates molecular mechanisms to changes in genomic, metabolic, physiological and behavioural parameters. Dietary nutrients are perceived in the oral cavity through the taste system in both pigs and humans[25,26].

Taste perception and the nutrient chemosensory system

Five basic tastes are accepted in humans: sweet, umami, salty, sour and bitter. In addition, fat perception and other nutrient sensing have been related to taste[25]. Sapid compounds solubilised in saliva are presented to the taste buds in the tongue papillae[27]. Taste perception involves a large set of taste receptors (referred hereinafter as TR for the receptors and TASR for the corresponding genes), which are expressed in the sensory cells of the taste buds and account for recognition of the tastants triggering a depolarisation of the cell and the subsequent signalling to the gustatory cortex of the brain through dedicated neuronal fibres[28]. Salty and sour sensing has been related to ligand-gated, transmembrane-channels: the epithelial Na channels (involving three genes $ENaC\alpha$, β, γ) and the hydrogen-gated channels (involving two genes PKD1L3 and PKD2L1), respectively[25]. All other taste and nutrient receptors known to date are G-protein-coupled receptors (GPCR), including the family 1 (T1R), family 2 (T2R) and several other receptors related to amino acid (AA) and fatty acid (FA) sensing. In humans, the three genes of the taste receptor type 1 (TAS1R) subfamily have been related to umami and sweet tastes. They sense a limited range of L-AA (TAS1R1 and TAS1R3) and sugars (TAS1R2 and TAS1R3). The taste receptor type 2 (TAS2R) subfamily has evolved to identify potentially toxic compounds and elicits the bitter taste. In addition to the Tas1R family, AA and peptones are sensed in the oral cavity by five other GPCR receptors (mGluR1, mGluR4, GPRC6A, CaSR and GPR92). Medium- and long-chained FA are sensed by another set of five GPCR (GPR40, GPR41, GPR43, GPR84 and GPR120). Detailed reviews of the TR/TASR, including the standard nomenclature, are given for humans and rodents by Bachmanov & Beauchamp[25], Wellendorph et al.[26] and Foster et al.[29,30].

The pig nutrient-sensing repertoire is less known but several in silico comparisons of taste receptors have been published in the last 3 years, after the appearance of the second annotation of the pig genome[18,20,31–33]. Single gene sequencing in pigs has characterised the porcine TAS1R3[34]. The gene sequences for the porcine umami dimer, TAS1R1/TAS1R3, and the glutamate receptor, mGluR1, have also been published[4,35]. More recently a study including allelic variation and oral expression of the porcine TAS2R family indicated the potential role of the bitter receptors in environmental adaptation[31].

Evidence for expression of TR in the GIT outside the oral cavity has emerged from both pig and human genome studies[26,30]. For example, expression of the Tas1r3 gene has been identified in the tongue, heart, lung, stomach, intestine, liver, kidney and testis[34]. Other nutrient receptors have been found along the GIT including T1R in the small intestine and stomach[36–38]; AA and peptone receptors, GPRC6A, GPR92 and CaSR, in gastric mucosa[39]; and FA sensors, GPR40, 43 and 120, and seven TAS2R, in five different tissues of the GIT[40].

Comparisons between pigs and humans in nutritional chemosensing

Pigs have a high number of taste buds (n 19 904) relative to other animal species. The number recorded for humans (n 6074) is about 30 % that for pigs. Most of these taste buds are on the tongue surface as part of the fungiform papillae or on the circumvallate papillae in pigs and humans[41,42]. Pigs have a larger number of fungiform papillae than humans and only two circumvallate papillae compared with eight to twelve in humans[43]. However, the ratio of taste buds to adult body weight is similar for pigs and humans, as it is across many animal species[44].

Sensitivity of pigs compared with humans for the basic hedonic human tastes (sweet, umami, salty, sour and bitter) and homology between taste gene sequences are shown in Tables 1 and 2, respectively. Molecular mechanisms and taste sensitivity are generally similar between the two species for sweet, umami, sour and FA tastes. However, similarities are less for salty and low for bitter tastes.

In relation to sweet tastes, the first studies in pigs from the 1950s were on appetite for sucrose (SUC)[45,46]. Kennedy & Baldwin[47] also measured preferences by pigs for several sweet compounds and found similar recognition threshold concentrations in the diet for SUC and sodium saccharin (SAC) of about 5 to 10 mM. The taste recognition threshold was higher for glucose (GLU) at between 10 and 30 mM. Humans show a similar sensitivity to pigs for simple sugars and for two high-intensity sweeteners, sucralose and rebaudioside A[48,49]. However, pigs do not respond to aspartame, neohesperidin and thaumatin[48,49], which are recognised as sweet by humans. SAC solutions above 100 mM were rejected by pigs[45], suggesting an unpleasant sensation at high concentrations. This response is similar for humans, where high SAC concentrations elicit bitterness[50].

Pigs have a preference for free L-AA including glutamic, aspartic, alanine, glutamine and lysine which have been related to the porcine umami taste receptor[4]. In addition, preferences for serine, threonine, asparagine and hydroxyproline have also been reported in pigs[51]. However, pig preferences for AA or sugars seem to increase with dietary deficiencies[52,53]. In contrast to the AA listed above, pigs have been shown to avoid branched-chained AA and tryptophan, presumably related to the onset of bitter taste[51,54,55]. Compounds known to be bitter to humans such as pharmaceuticals, including antibiotics, caffeine, quinine HCl, among others, have also been shown to trigger avoidance in pigs[56,57].

Humans appear to be less sensitive to umami tastants than pigs. Umami taste in humans is stimulated by only two L-AA

Table 1. Efficacy of the pig model for humans in nutritional chemosensing; endocrine system; microbiota; and brain anatomy, development and imaging

Topic	Parameter	Pigs	Humans	Relevance of the pig as a human model	References
Nutritional chemosensing					
Anatomy	Taste bud number	19 904	6974	Low	Chamorro et al.[41]; Miller & Reedy[43]
	Taste bud number:body weight ratio*	103·7	102·6	High	Chamorro et al.[41]; Miller & Reedy[43]
	Circumvallate papillae number	2	8–12	Low	Kumar & Bate[313]; Miller & Reedy[43]
Molecular mechanisms	Carbohydrate sensing	TAS1R2 and TAS1R3	TAS1R2 and TAS1R3	High. Same genes. High gene homology ranging from 75 to 76 % in nucleotide sequence. Low SNP incidence	Roura[62]; Da Silva et al.[31]; Bachmanov & Beauchamp[25]
	Amino acid sensing	GPRC6A, mGluR1 and 4, CaSR and GPR92	GPRC6A, mGluR1 and 4, CaSR and GPR92	Very high. Same genes. Very high gene homology ranging from 77 to 99 % in nucleotide sequence. Low SNP incidence	Roura[62]; Da Silva et al.[31]; Wellendorph et al.[314]
	Fatty acid sensing	GPR40, 41, 43, 84 and 120	GPR40, 41, 43, 84 and 120	High. Same genes. High gene homology ranging from 75 to 90 % in nucleotide sequence. Low SNP frequency	Roura[62]; Da Silva et al.[31]; Wellendorph et al.[314]
	Bitter taste	12 functional genes and 3 pseudogenes	25 functional genes	Very low. Different genes. Eleven human genes without pig ortholog. Two pig genes without human ortholog. Medium to very low gene homology ranging from 54 to 73 % in nucleotide sequence. High SNP frequency	Da Silva et al.[31]; Kuhn et al.[315]
Taste sensitivity threshold	Sweet, sucrose (mM)†	6	8·8	Very high for simple sugars. Low or very low for artificial sweeteners	Roura et al.[4]; Newman & Keast[331]
	Umami, MSG (mM)†	3	1·6	High for glutamate. Very low for other L-amino acids	Roura et al.[4]; Newman & Keast[331]
	Sour, citric acid (mM)†	0·4	0·8	High for citric acid. Medium or low for other acids since pigs show preference for several organic and inorganic acids (unpublished results)	Roura et al.[4]; Newman & Keast[331]
	Salty, NaCl (mM)†	18	4·2	Medium	Roura et al.[4]; Newman & Keast[331]
	Bitter (mM)†	Unknown	Diverse, but often ranging in the μM range	Unknown	Brochoff et al.[316]
Endocrine system (blood hormone levels; pmol/l)					
Postprandial response	CCK	2- to 5-fold	2- to 5-fold	Very high. Quantitative and qualitative changes are similar	Corring & Chayvialle[71]; Clutter et al.[72]; Ripken et al.[73]; Feinle et al.[76]; Seimon et al.[77]; Blom et al.[78]; Mossner et al.[79]
	GLP-1	About 3-fold	About 3-fold	Very high. Quantitative and qualitative changes are similar	Souza da Silva et al.[95]; Hooda et al.[96]; Knapper et al.[97]; Blom et al.[78]; Lavin et al.[98]; Verdich et al.[99]
	PYY	1- to 2-fold	About 4-fold	Low. Levels are different and changes in response to nutritional status as well	Souza da Silva et al.[95]; Ito et al.[107]; Seimon et al.[77]; Degen et al.[106]
	Ghrelin	2- to 3-fold	2- to 3-fold	Medium. Changes are similar but plasma levels differ significantly	Barretero-Hernandez et al.[65]; Govoni et al.[108]; Zhang et al.[110]; Inoue et al.[111]; Scrimgeour et al.[112]; Blom et al.[78]; Cummings et al.[113] Pigs[65,108,110–112] Humans[78,113]
Effect on food intake	CCK	Reduction	Reduction	Very high[89,90]. Same effect on appetite	Anika et al.[80]; Houpt[81]; Baldwin et al.[82]; Ebenezer et al.[85]; Reidelberger & O'Rourke[86]; Woltman et al.[87]; Ebenezer et al.[88]; Gregory et al.[89]; Baldwin & Sukhchai[90]; Farmer et al.[91]; Baldwin et al.[92]; Zhang et al.[66]; Cummings & Overduin[67]; Perry & Wang[68]; Wolkowitz et al.[93]

Table 1 *Continued*

Topic	Parameter	Pigs	Humans	Relevance of the pig as a human model	References
	GLP-1	Circumstantial evidence for reduction	Reduction	Very high. Same effect on appetite	Ribel *et al.*[101]; Knudsen[103]; Zhang *et al.*[66]; Cummings & Overduin[67]; Perry & Wang[68]
	PYY	Reduction	Reduction	Very high. Same effect on appetite	Ito *et al.*[107]; Zhang *et al.*[66]; Cummings & Overduin[67]; Perry & Wang[68]; Degen *et al.*[106]
	Ghrelin	Increment	Increment	Very high. Same effect on appetite	Salfen *et al.*[114]; Zhang *et al.*[66]; Cummings & Overduin[67]; Perry & Wang[68]; Le Roux *et al.*[115]
Microbiota					
Bacterial enterotypes	Number of major enterotypes	Two	Three	Medium	Arumugam *et al.*[173]; Mach *et al.*[175]
	Enterotype-like clusters	*Prevotella* *Ruminococcus*	*Prevotella* *Ruminococcus* *Bacteroides*	Medium	Arumugam *et al.*[173]; Mach *et al.*[175]
	Over time variation of enterotype clusters	Yes	Yes	High	Arumugam *et al.*[173]; Mach *et al.*[175]
	Dominant phyla	Firmicutes Bacteroidetes	Firmicutes Bacteroidetes	High	Eckburg *et al.*[182]; Ley *et al.*[183]; Leser *et al.*[185]; Guo *et al.*[186]; Mach *et al.*[175]
	Dominant genera of Bacteroidetes	*Prevotella*	*Bacteroides*	Low	Dore & Corthier[199]; Kim *et al.*[211]
	Relative abundance of bifidobacteria (in Actinobacteria phyla)	Very low amount in 5-month-old pigs (<0·01 % of total sequences)	Predominant in infants (40 % of total bacteria)	Low	Adlerberth & Wold[317]; Dore & Corthier[199]; Kim *et al.*[211]; Mach *et al.*[175]
	Early colonisers of the intestine	*Bacteroides* *Escherichia/Shigella* *Lactobacillus* *Streptococcus*	*Bacteroides* *Escherichia/ Shigella* Bifidobacteria	Medium	Fallani *et al.*[212]; Wang *et al.*[318]
	Environmental factors that modify early microbial colonisation	Mode of delivery Nutrition Antibiotic treatment Biodiversity in the surrounding environment and in family members	Mode of delivery Nutrition Antibiotic treatment Biodiversity in the surrounding environment and in family members	High	Adlerberth & Wold[317]; Donovan *et al.*[177]; Fallani *et al.*[212]; Le Huërou-Luron *et al.*[206]; Mulder *et al.*[202]; Pinsk *et al.*[204]; Wang *et al.*[318]
	Evidence for long-term consequences of early modification of microbiota	Yes	Yes	High	Arnal *et al.*[137]; Boudry *et al.*[158]; Chatelais *et al.*[157]; Le Huërou-Luron *et al.*[206]
Firmicutes and Bacteroidetes abundance	In lean adults	Firmicutes 68–79 % Bacteroidetes 12–16 %	Firmicutes 74 % Bacteroidetes 26 %	High	Ley *et al.*[184]; Guo *et al.*[186]; Kim *et al.*[211]
	In obese adults	Firmicutes 71 % Bacteroidetes 5 %	Firmicutes 89 % Bacteroidetes 2 %	High	Ley *et al.*[184]; Guo *et al.*[186]
Brain anatomy, development and imaging					
Brain anatomy	Brain weight	180 g	1·3–1·4 kg	Fairly good. Pig brain comparable with the brain mass of several non-human primate species, which enables a good resolution for brain imaging	Sauleau *et al.*[12]

Table 1 *Continued*

Topic	Parameter	Pigs	Humans	Relevance of the pig as a human model	References
	Brain shape (isomorphic factor)	50	65	Good. In comparison, the isomorphy factor of the rat brain is 10	Lind et al.[319]; Sauleau et al.[12]
	Total number of neocortical neurons	325–430 million	19–23 billion	Fairly good. In comparison, the total number of neocortical neurons is 21 million in the rat, and 1·35 billion in the rhesus monkey	Pakkenberg & Gundersen[320]; Jelsing et al.[321]; Christensen et al.[322]
	General anatomy and vascularisation			Good. Pig brain similar to that of humans in terms of anatomy and vascularisation	Vodicka et al.[323]; Lind et al.[319]
	Presence of cortical circumvolutions	Yes	Yes	Good. Contrary to the rat brain, the pig brain is gyrencephalic such as the human brain	Sauleau et al.[12]
	Description of brain neurotransmitters involved in eating behaviour	Serotonin, dopamine, opioids, etc.	Serotonin, dopamine, opioids, etc.	Good	Niblock et al.[324]; Lind et al.[319]
Brain development	Brain growth spurt	From late prenatal to early postnatal	From late prenatal to early postnatal	Very good	Lind et al.[319]; Conrad et al.[325]
	Maturation of postnatal brain (% of adult brain volume)	50 % at 3·81–4·08 weeks	36 % at 2–4 weeks	Good. The large increase in brain volume in the postnatal period is similar to human neonates and indicates that pigs can be used to investigate brain development	Conrad et al.[325]
Brain imaging	Validated brain imaging techniques	CT, PET, SPECT, MRI, MEG, ECoG, NIRS	CT, PET, SPECT, MRI, MEG, ECoG, NIRS	Very good. All major brain imaging techniques in the human have been implemented in the pig model	Bowyer et al.[283]; Lind et al.[319]; Sauleau et al.[12]; Clouard et al.[3]; Uga et al.[284]
	Validated brain PET radioligands	39	178 (specifically for brain imaging over 569 PET radioligands in the MICAD database)	Good. PET radioligands were validated in pigs to study the cerebral blood flow, the metabolism of glucose, dopamine, serotonin, noradrenaline, NMDA, multitarget antidepressant, nicotine, oxygen, monoamines, phosphodiesterase, benzodiazepine, amino acids, neurokinin	Lind et al.[319]; Alstrup & Smith[280]; Zimmer & Luxen[326]
	Maximum number of labels/structures in stereotaxic brain atlases	219	1105	Fairly good. Even though the number of brain structures in pig atlases is much lower than that of the human atlas, major structures are identified and labelled. Moreover, a digital pig brain atlas is freely available, as in the human	Talairach & Tournoux[327]; Félix et al.[328]; Lancaster et al.[329]; Saikali et al.[330]

TAS1R, taste receptor type 1; TAS2R, taste receptor type 2; MSG, monosodium glutamate; CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; PYY, peptide YY; CT, computed tomography; PET, positron emission tomography; SPECT, single photon emission computed tomography; MEG, magnetoencephalography; ECoG, electrocorticography; NIRS, near-IR spectroscopy; NMDA, N-methyl-D-aspartate.

* The adult body weights for a pig and for a human used in the calculation were 192 and 68 kg, respectively.

† The preference tests in pigs consisted of double-choice tests including two solutions as described in Roura et al.[4]. The threshold values for pigs refer to the lowest concentration of the taste active compound tested which resulted in significant ($P < 0.05$) preference over water compared with 50 % (neutral value) (adapted from Roura et al.[4]). The threshold values for humans refer to the lowest concentration tested following the ascending forced choice triangle test method (Newman & Keast[331]), which resulted in significant ($P < 0.05$) detection (E Roura, unpublished results).

Table 2. Studies on taste receptor and nutrient sensor genes in *Sus scrofa* compared with *Homo sapiens**

Pig and human gene	Taste (human)	Pig to human sequence homologies: amino acid/nucleotide (%)	Ligand examples in mammals	Reported sites
TAS1R1	Umami	84/80	ʟ-Glutamate, ʟ-aspartate, ʟ-2-amino-4-phosphonobutyric acid and potentiated by IMP and GMP[44,332,333]	Pig: tongue[31,334,335]; stomach, jejunum[51] Human: spermatozoa, testis[336]; stomach[337]; liver, HuCCT1 cells[338]; pancreatic Min6 cells, H9C2 cells and HeLa cells[339]; tongue fungiform papillae[340]; adrenal, brain, breast, colon, heart, kidney, lung, ovary, skeletal muscle, testis, thyroid[341]; stomach antrum, duodenum, proximal jejunum, mid-jejunum, colon, rectum[342]; pancreas, liver[343]; intestinal endocrine cells[344]; GLP-1-producing cells[345] Other: evolutionary analysis (pig)[45]; sequencing, cloning, cell reporter system (pig)[4,48]
TAS1R2	Sweet	84/76	Carbohydrates[314], for example: sucrose, saccharin, dulcin and acesulfame-K[346]; aspartame, cyclamate[347]	Pig: tongue[31,334,335]; small intestine[348] Human: stomach[337]; duodenum, NCI-H716 cells and GLP-1-producing cells[345]; liver, HuCCT1 cells[338]; duodenum, jejunum[349]; HeLa cells, DU145 cells[350]; bladder urothelium[351]; skeletal muscle[341]; duodenum, jejunum[342]; tongue[352] Other: evolutionary analysis (pig)[45]
TAS1R3	Umami and sweet	82/75	Proteins: ʟ-amino acids (same as TAS1r1 and TAS1r3); Ca[353]; carbohydrates[314]	Pig: tongue[31,334,335]; tongue, heart, lung, stomach, intestine, liver, kidney, testis[47]; stomach, jejunum[51]; small intestine[348]; stomach[50]; duodenum, jejunum, ileum, colon[354] Human: duodenum, jejunum, ileum, colon[354]; enteroendcrine cells and caecum[355]; colon, gut Hu-Tu 80 cells, NCIH716 cells[356]; stomach[337]; duodenum, NCI-H716 cells and GLP-1-producing cells[345]; liver, HuCCT1 Cells[338]; duodenum, jejunum[349]; pancreatic Min6 cells, H9C2 cells and HeLa cells[339]; heart[357]; bladder urothelium[351]; circumvallate and fungiform papillae[358]; pancreas and liver[343]; adipose, adrenal, brain, breast, colon, heart, kidney, liver, lung, lymph node, ovary, prostate, skeletal muscle, testis, thyroid, leucocytes[341]; stomach antrum, duodenum, proximal jejunum, mid-jejunum, colon, rectum[342]; oral taste receptor cells[358] Other: evolutionary analysis (pig)[45]; sequencing, cloning, cell reporter system (pig)[48]
TAS2R1†	Bitter	71/54	Amarogentin, arborescin, cascarillin[61]	Pig: tongue[31,334]; stomach, jejunum, colon[53] Human: circumvallate papillae[359]; colon[360]; heart[357]; airways[361]; airway smooth muscle[362]; brain, kidney, ovary, testis[341]; T lymphocytes[363] Other: genome analysis (pig)[20]
TAS2R3	Bitter	82/72	Chloroquine[61]	Pig: tongue[31,334]; stomach, jejunum, colon[53] Human: colon, gut Hu-Tu 80 cells, NCIH716 cells[356]; heart[357]; airways[361]; airway smooth muscle[362]; stomach[364]; adrenal, brain, heart, kidney, ovary, testis[341]; T lymphocytes[363] Other: evolutionary analysis (pig)[365]; genome analysis (pig)[20]
TAS2R4	Bitter	80/70	Amarogentin, arborescin, artemorin[61]; epicatechin[366]	Pig: tongue[31,334] Human: colon, gut Hu-Tu 80 cells, NCIH716 cells[356]; colon[360]; heart[357]; airways[361]; airway smooth muscle[362]; adrenal, brain, breast, colon, heart, kidney, lung, lymph node, ovary, testis, thyroid[341]; mixed leucocytes, lymphocytes, monocytes, neutrophils[367]; T lymphocytes[363]; embryonic kidney cells[368] Other: evolutionary analysis (pig)[18]; genome analysis (pig)[20]
TAS2R7	Bitter	83/73	Caffeine, quinine[61]; malvidin-3-glucoside and procyanidin trimer[366]	Pig: tongue[31,334]; stomach, jejunum, colon[53] Human: caecum, NCIH716 cells[355]; heart[357]; airways[361]; brain, heart, ovary[341]; T lymphocytes[363] Other: evolutionary analysis (pig)[18]; genome analysis (pig)[20]
TAS2R9	Bitter	82/70	Ofloxacin, procainamide and pirenzapine[355]	Pig: tongue[31,334]; stomach, jejunum, colon[53] Human: enteroendcrine cells and caecum[355]; heart[357]; airways[361]; airway smooth muscle[362]; adrenal, brain[341]; T lymphocytes[363] Other: evolutionary analysis (pig)[18]; genome analysis (pig)[20]
TAS2R10	Bitter	84/69	Absinthin, arborescin, arglabin[61]	Pig: tongue[31,334]; stomach, jejunum, colon[53] Human: colon, gut Hu-Tu 80 cells, NCIH716 cells[356]; heart[357]; airways[361]; airway smooth muscle[362]; frontal cortex[369]; adipose, adrenal, brain, breast, heart, kidney, ovary[341]; mixed leucocytes, lymphocytes, monocytes, neutrophils[367]; T lymphocytes[363] Other: evolutionary analysis (pig)[18]; genome analysis (pig)[20]

Table 2 *Continued*

Pig and human gene	Taste (human)	Pig to human sequence homologies: amino acid/nucleotide (%)	Ligand examples in mammals	Reported sites
TAS2R16	Bitter	76/61	Sinigrin, sodium benzoate, diphenidol[61]	Pig: tongue[31,334]; stomach, jejunum, colon[53] Human: circumvallate papillae[359]; heart[357]; T lymphocytes[363] Other: evolutionary analysis (pig)[18]; genome analysis (pig)[20]
TASR20	Bitter	73/59	Unknown	Pig: tongue[31,334] Human: heart[357]; airway smooth muscle[362]; colon, cut Hu-Tu 80 cells, NCIH716 cells (the alias TAS2R49 is used instead of TAS2R20)[356]; adipose, adrenal, brain, breast, colon, heart, kidney, liver, lung, lymph node, ovary, prostate, skeletal muscle, testis, thyroid, leucocytes[341]; mixed leucocytes, lymphocytes, monocytes, neutrophils[341,367] Other: genome analysis (pig)[20]
TAS2R38	Bitter	78/68	Allylisothiocyanyte, phenylethyl isothiocyanate, sinigrin[61]	Pig: tongue[31,334]; stomach, jejunum[53] Human: circumvallate papillae[370]; colon, gut Hu-Tu 80 cells, NCIH716 cells[356]; colon[360]; heart[357]; 16HBE cells[371]; upper respiratory epithelium[372]; intestinal endocrine cells[344]; GLP-1-producing cells[356]; T lymphocytes[363] Other: evolutionary analysis (pig)[18]; genome analysis (pig)[20]
TAS2R39†	Bitter	82/70	Amarogentin, chloramphenicol, colchicine[61]; epicatechin, pentagalloylglucose[366]	Pig: tongue[31,334] Human: brain[341]; T lymphocytes[363] Other: evolutionary analysis (pig)[18]; genome analysis (pig)[20]
TAS2R40†	Bitter	78/62	Quinine, chlorpheniramine, dapsone[61]	Pig: none reported Human: colon, gut Hu-Tu cells, NCI-H716 cells[356]; T lymphocytes[341,363] Other: whole genome sequence analysis (pig)[20,46]
TAS2R41	Bitter	79/70	Chloramphenicol[373]	Pig: tongue[31,334] Human: heart[357]; T lymphocytes[363] Other: evolutionary analysis (pig)[18]; genome analysis (pig)[20]
TAS2R42	Bitter	76/59	Unknown	Pig: tongue[31,334] Human: colon, gut Hu-Tu 80 cells, NCIH716 cells[356]; heart[357]; airway smooth muscle, trachea[362]; adipose, adrenal, brain, kidney, ovary[341]; T lymphocytes[363] Other: evolutionary analysis (pig)[18]; genome analysis (pig)[20]
TAS2R60	Bitter	77/67	Unknown	Pig: tongue[31,334] Human: colon, gut Hu-Tu 80 cells, NCIH716 cells[356]; heart[357]; ovary, leucocytes[341]; T lymphocytes[363] Other: genome analysis (pig)[20]
TAS2R134‡	Bitter	N/A	Unknown	Pig: tongue[31,334] Human: not applicable Other: genome analysis (pig)[20]
GPR120, also known as FFAR4, O3FAR1	Fatty acid sensing	88/88	Upper range of medium- and long-chain SFA and unsaturated fatty acids[314,374]	Pig: tongue[31,334]; stomach, jejunum, colon[53]; duodenum, jejunum, ileum, colon[354] Human: brain, thymus, lung, ileum, colon, rectum, spleen, kidney, adrenal gland[374]; duodenum, jejunum, ileum, colon[354]; adipose tissue, stomach, small intestine, colon and lung[375]; stomach antrum, duodenum, proximal jejunum, mid-jejunum, colon, rectum[342] Other: next-generation sequence analysis (pig)[376]
GPR40, also known as FFAR1	Fatty acid sensing	84/85	Shorter medium- and long-chain SFA and unsaturated fatty acids[314,377,378]	Pig: tongue[31,53,334] Human: pancreatic islets[379]; stomach antrum, duodenum, proximal jejunum, mid-jejunum, colon, rectum[342]

Table 2 *Continued*

Pig and human gene	Taste (human)	Pig to human sequence homologies: amino acid/nucleotide (%)	Ligand examples in mammals	Reported sites
GPR43, also known as *FFAR2*	Fatty acid sensing	86/84	SCFA[314,380,381] (for example, acetate, propionate and butyrate)	Pig: tongue[31,334]; stomach, jejunum, colon[53]; small intestine, caecum, colon[382] Human: stomach antrum, duodenum, proximal jejunum, mid-jejunum, colon, rectum[342]; peripheral blood mononuclear cells, B/T lymphocytes, T cells, B cells, monocytes, neutrophils, immature dendritic cells, W529 B cells, Raji B cells, Jurkat T cells, THP1 monocytes[380]; pancreatic islets[383]; spleen, bone marrow, peripheral blood mononuclear cells, monocytes, polymorphonuclear cells, adipose, breast[381], ascending colon (mucosa) enteroendocrine L cells[384]
GPR41 aka *FFAR3*	Fatty acid sensing	75/79	SCFA[314,380,381] (for example, propionate, butyrate, acetate)	Pig: tongue[31,334]; small intestine, caecum, colon[382] Human: stomach antrum, duodenum, proximal jejunum, mid-jejunum, colon, rectum[342]; bone marrow, placenta, prostate, pancreas, adipose, peripheral blood mononuclear cells, spleen, intestine, stomach, muscle, kidney, fetal liver, liver, lung, heart, pituitary, brain[380]; pancreatic islets[383]; thymus, spleen, lymph node, bone marrow, peripheral blood mononuclear cells, dendritic cells, polymorphonuclear cells, small intestine, adipose, breast[381]; colonic mucosa[385]
GPR84	Fatty acid sensing	88/90	Medium-chain fatty acids[314]; capric acid[386]	Pig: tongue[31,334] Human: brain, fetal brain, trachea, lung, stomach, small intestine, colon, pancreas, liver, fetal liver, kidney, fetal kidney, fetal heart, thyroid, thymus, fetal thymus, spleen, fetal spleen, bone marrow, peripheral leucocytes, placenta, bladder, prostate, uterus, adrenal gland, mammary gland, salivary gland, adipose[386]; stomach antrum, duodenum, proximal jejunum, mid-jejunum, colon, rectum[342]
CD36	No taste	86/83	Long-chain fatty acids[387]	Pig: tongue[388,389]; monocytes[390] Human: tongue[388]; monocytes[390]
GPRC6A	No taste	90/88	L-Amino acids (subclass: basic, polar, aliphatic)[314,332,391]	Pig: tongue[31,334]; stomach antrum[52] Human: embryonic kidney cells[332]; stomach antrum[52]; brain, lung, liver, heart, kidney, pancreas, skeletal muscle, placenta, spleen, ovary, testis, leucocytes[392]
mGluR1, also known as *GRM1*	Umami candidate	92/99	L-Glutamate[393]	Pig: tongue[31,334]; stomach, jejunum[51] Human: brain cortex and cerebellum[394]; melanoma cells[395]; melanocytes[396]
mGluR4 aka *GRM4*	Umami candidate	93/93	Tricyclic thiazolopyrazole derivatives[397]	Pig: tongue[31,334]; stomach, jejunum[51] Human: stomach antrum, duodenum, proximal jejunum, mid-jejunum, colon, rectum[342]
CASR	No taste	91/94	Ca^{2+}; proteins: L-amino acids[398,399], subclass: basic, acidic, aliphatic, aromatic, branch chain[332]	Pig: tongue[31]; stomach antrum[52] Human: stomach antrum[52]; stomach antrum, duodenum, proximal jejunum, mid-jejunum, colon, rectum[295]; thyroid[400], parathyroid[400,401]; lens epithelial cells[402]; breast cancer cells[403]; antral gastrin cells[404]; pancreatic ducts, exocrine acinar cells in the islets of Langerhans, intrapancreatic nerves, blood vessels[405]; distal convoluted tubule[406]; kidney[407]; kidney, parathyroid gland[408]; antral gastrin cells[409]; oesophagus[410]; bone[411]
GPR92, also known as *GPR93, LPAR5*	No taste	84/77	Proteins (peptones)[314]	Pig: tongue[31,334]; stomach antrum[52] Human: stomach antrum[52]; mast cells[412]; stomach antrum, duodenum, proximal jejunum, mid-jejunum, colon, rectum[342]
Human bitter genes without pig orthologs: *TAS2R5*[356,357,362,367,369]; *TAS2R8*[357,361,362]; *TAS2R13*[350,356,357,361,362,367,369]; *TAS2R14*[341,357,361,362,367]; *TAS2R19*[357,362,367]; *TAS2R30*[357,362]; *TAS2R31*[357,362,367]; *TAS2R43*[356,357,361]; *TAS2R45*[356,357,362,367]; *TAS2R46*[356,357,361,362,367,371,413]; *TAS2R50*[356,357,362,367,369]			Various[61,355,366,373]	Airway smooth muscle, trachea[362]; frontal cortex[369]; colon, gut Hu-Tu cells, NCI-H716 cells; airways[356]; HeLa cells, DU145 cells[361]; heart[357]; 16HBE cells[371]; bone marrow stromal-derived cells, vascular smooth muscle cells[413]; mixed leucocytes, lymphocytes, monocytes, neutrophils; leucocytes[367]

TAS1R, taste receptor type 1; TAS2R, taste receptor type 2; IMP, inosine monophosphate; GMP, guanosine monophosphate; GLP-1, glucagon-like peptide-1.

* The homology percentage for pigs compared with humans was calculated between the amino acid sequences and between the nucleotide sequences for each gene.

† Porcine genes that have been identified as pseudogenes according to Da Silva *et al.*[31].

‡ The porcine gene *TAS2R134* has no known human homolog (Da Silva *et al.*[31]).

(glutamic and aspartic), whereas pigs recognise a wider array of L-AA[51,54]. The strong preference for non-glutamic AA in pigs is an important differential feature compared with humans. However, the umami receptor in laboratory rodents responds to an even wider array of L-AA compared with pigs[4].

Umami tastants in pigs, for example, monosodium glutamate, either alone or in combination with nucleotides, and some sweet tastants, stimulate nerve fibres identified with umami sensing[56]. Umami and sweet compounds are both perceived by dimeric taste receptors (TR) sharing one common receptor T1R3[58].

Characterisation of the umami dimer, TAS1R1/TAS1R3, and the glutamate receptor, mGluR1, revealed higher homologies of these porcine genes with the human orthologs compared with laboratory rodents[4,35]. Studies of divergences and similarities in gene structure and expression between pigs and humans for the whole TASR repertoire showed high homology for the TAS1R subfamily coding for carbohydrates, AA and FA, but low homology for the TAS2R subfamily coding for bitter tastes[31]. Recent publications indicate the pig has sixteen and the human twenty-five TAS2R genes[25,31]. It may be speculated that the similarity in *TAS1R* gene structure between pigs and humans is consistent with the theory of parallel evolution, where both species are omnivorous and consumed diets with similar nutrient profiles. The lower number of *TAS2R* genes in pigs may indicate a higher resilience of pigs to bitter dietary compounds[20]. It has been suggested that the TAS2R receptors unique to humans tend to have a narrow specificity and may not have food-related significance particularly in light of their extra-oral expression[59]. Da Silva *et al.*[31] also reported a high incidence of non-synonymous polymorphisms in the porcine TAS2R repertoire when comparing fourteen different pig breeds across the globe. These findings are consistent with the view that bitter taste gene diversity is an adaptation within animal species[18,60], including humans[61], to their specific ecosystems. The porcine TAS2R is not a good model for the study of bitter agents in humans. However, the global widespread nature of pig breeds following human expansion may represent a unique model to study the role of taste in the adaptation to specific geo-ecosystems.

One limitation for using the pig as a model for humans to study taste sensitivity is a difference in methodology used between the two species. Threshold nutrient concentrations are determined in pigs using preference tests, often compared with water, whereas recognition thresholds are used in human studies. Compared with a recognition threshold, a preference threshold may require higher doses. Results from these two types of comparisons suggest that pigs are more sensitive to citric acid and SUC, but less sensitive to NaCl than humans[4,62]. In addition, pigs and humans show strong discrepancies in the responses to most high-intensity sweeteners tested to date[48,49].

Conclusion on using the pig as a model for human nutritional chemosensing studies

The chemosensing anatomy appears to be similar for pigs and humans with a similar ratio of taste buds in the mouth to mature body weight and the location of sensors throughout the GIT

and other organs. The two species also show similarity in studies related to tasting simple sugars (sweet), glutamate (umami) and citric acid (sour). In contrast to humans, pigs do not have the same ability to taste some high-intensity sweeteners or non-glutamate AA and appear less sensitive to NaCl (salt). Overall, pigs are an excellent model for humans, when based on carbohydrate-, AA- and FA-sensing mechanisms. In contrast, the bitter sensory system of pigs and humans is diverse and characterised by species-specific features evolving from the adaptation to different ecosystems. Studying gene expression in human tissues can be difficult, particularly when using well-controlled nutrition intervention studies. The similarity in nutrient receptors and taste sensitivities between pigs and humans creates the opportunity to use the pig as a model in place of rodents for behavioural studies into chemosensing.

Endocrine regulation of food intake: gut–nutrient sensing and gut–brain communication

Sensory cells expressing TR in the GIT are part of the entero-endocrine system. Signalling of appetite or satiety in the gut can be both GIT hormone and nutrient specific[63]. The release of GIT hormones is activated by fasting or food intake via intestinal receptors that respond to mechanical and/or chemical stimulation[64] including TR[65]. Over forty GIT hormones have been identified with specific bioactivity[66] of which eight GIT hormones (cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), oxyntomodulin, peptide YY (PYY), apoA-IV, gastrin-releasing peptide and neuromedin B, gastric leptin and ghrelin) have been implicated in the regulation of food intake in mammals[67]. This section focuses on the following four hormones specifically released by the stomach and/or small and large intestine with published results in humans and pigs: CCK, GLP-1, PYY and ghrelin. The importance of these four GIT hormones in appetite regulation has been reviewed by Perry & Wang[68]. In the context of nutrient-induced release of satiety hormones it has been reported by Geraedts *et al.*[69] that the release of CCK and GLP-1 in response to dietary proteins differs substantially between humans and rats, underlining the need for alternative, more human-like, animal models to study food intake regulation.

Once GIT hormones are released, they may exert their action via a neural or an endocrine route. The neural route involves binding of GIT hormones to local GIT receptors and subsequent signal transduction via afferent fibres of the abdominal vagal nerve to the brain. The endocrine route involves systemic transport of GIT hormones to the brain and by binding to brain receptors present in the area postrema, a brain structure serving as an interface between blood and brain, or by crossing the blood–brain barrier (BBB) and subsequent binding to receptors in specific brain regions. For proper translational research from pig to human, it is important that the kinetics of GIT hormones in blood follow a similar profile. For instance, the kinetics of circulating GLP-1 are similar in pigs and humans, but different in rats[70]. This difference between the species is probably caused by the rapid inactivation of GLP-1 by circulating protease dipeptidyl peptidase-4 (DPP-4), which is more active in rats than in pigs and humans.

Similarities and differences of nutrient-induced release of satiety hormones and endocrine gut–brain communication in pigs and humans

Cholecystokinin. Depending on the amount and composition of the diet, CCK is secreted by the I cells in the duodenal and jejunal mucosa and also by neurons in the enteric nervous system. CCK contributes to satiation and satiety effects. The major effects of CCK are stimulation of gall bladder contraction, delay of gastric emptying and stimulation of pancreatic secretion[63]. In pigs, CCK release is stimulated by a mixed meal from fasting to postprandial (30–120 min) concentrations going from about 10 pmol/l to about 30 pmol/l, respectively, with a faster response when diets are enriched with starch than with fat[71]. Clutter et al.[72] measured plasma CCK-8 in pigs, at fasting about 2 pmol/l increasing to 10 pmol/l at mixed meal feeding whereas Ripken et al.[73] reported concentrations of 0·5 pmol/l and 2 pmol/l for fasting and postprandial, respectively. All experiments showed a 2- to 5-fold increase in plasma CCK-8 concentrations after mixed meal feeding. Another study in pigs[74], using intraduodenal fat infusion, showed plasma CCK concentrations of approximately 5 pmol/l both prior and following fat infusion. CCK release was higher to long-chain FA than to medium-chain TAG. In humans, CCK release is mostly stimulated by the presence of dietary protein, whereas carbohydrate provides a weaker stimulus[75]. To our knowledge, the effect of dietary protein on plasma CCK secretion has not been investigated to date in pigs. Plasma CCK concentrations are similar in pigs and humans, on average for humans at fasting 2 pmol/l and at feeding 10 pmol/l[76–79]. In both species the magnitude of the CCK response is dependent on the composition of the meal. The effects of single macronutrients or combinations thereof on CCK release have not been investigated systematically in humans or pigs. The pig is a valid model to investigate these relationships.

Anika et al.[80] administered CCK intravenously at a rate of 0·2 nmol/kg per min for total doses of 0·4, 0·8, 1·7 and 3·4 nmol/kg to pigs. The lowest dose of CCK reduced food intake by 13 % and the highest dose by 94 %. Houpt[81] showed that intravenous (iv) CCK-8 (the synthetic and bioactive part of the CCK molecule) infusion to pigs of 14, 28 and 56 pmol/kg per min decreased meal size in a dose-dependent manner. A dose of 28 pmol/kg per min reduced food intake by 30–40 %. Similar results were found by Baldwin et al.[82] who studied hungry pigs working for food, thirsty pigs responding for water, and non-deprived pigs working for SUC. In this study CCK produced significant dose-related decreases in response rates for all three conditions.

Nutrients exert much of their effects through the CCK-1 receptor[83]. There are two regions where CCK acts to produce satiety: the nucleus *tractus solitarius* in the hindbrain and the medial-basal hypothalamus[84]. CCK acts both at CCK-1 receptors beyond the BBB and by a CCK-1 receptor-mediated mechanism involving abdominal vagal nerves to inhibit food intake[84]. CCK-1 antagonists, such as devazepide (DVZ), are commonly used in pig appetite and satiety studies[85]. DVZ easily passes the BBB following iv administration inducing a dose-related increase in food intake at doses ranging from 17·5 to 140 µg/kg with maximum increases occurring at about 70 µg/kg[86–88]. Arterial injection of DVZ (0·1 mg/kg) in pigs[89] abolished the inhibition of food intake to duodenal infusion of emulsified fat and monoacylglycerols. However, it did not alter the inhibition of intake in response to oleic acid, to glycerol or to GLU, suggesting that monoacylglycerol-induced CCK secretion is mainly responsible for the satiety resulting from duodenal fat infusion in the pig. Baldwin & Sukhchai[90] reported that intracerebroventricular (icv) injection of DVZ (100 µg) before the administration of 1 µg CCK abolished the inhibition of GLU intake produced by CCK in pigs. However, DVZ itself had no effect on GLU intake. In a study by Farmer et al.[91], pigs were fasted for 24 h, injected intravenously with DVZ at 70 mg/kg and subsequently subjected to a feed motivation test (operant conditioning). The number of pushes, duration of eating and amount of feed eaten during the feed motivation test were all increased by fasting, and were further increased by DVZ injection, indicating that CCK induces satiation in pigs. An alternative CCK receptor antagonist used in pigs is 2-NAP (2-naphthalenesulfanyl-l-aspartyl-2-(phenethyl) amide), which does not cross the BBB. Baldwin et al.[92] revealed that iv administration (20 or 40 mg/kg) of NAP injected before iv administration of CCK-8 (1 µg/kg) abolished the inhibitory effects of CCK-8 on food intake in hungry pigs. Also, NAP abolished the inhibitory effects of CCK-8 on food intake in hungry pigs after icv injection of both compounds. Overall, these results indicate that endogenous CCK is involved in the regulation of satiety in pigs, in a way which is similar to what is known in humans[93], albeit the pig offers the possibility to conduct invasive mechanistic studies on CCK action.

Glucagon-like peptide-1. GLP-1 is mostly produced by the L cells in the distal small intestine and colon and contributes to satiety. It performs a variety of functions in the body such as inhibiting acid secretion and gastric emptying, while increasing insulin secretion from the pancreas in a GLU-dependent manner[94]. The secretion of GLP-1 is mediated by indirect, duodenal activated neurohumoral mechanisms, as well as by direct contact of nutrients with the distal small intestine[82]. Souza da Silva et al.[95] showed that fasting plasma GLP-1 concentrations of 15 pmol/l rose to 35 pmol/l 1 h after the beginning of a complete mash feed. Hooda et al.[96] showed that fasting plasma GLP-1 concentrations in pigs were approximately 8 pmol/l and rose postprandial to 20 pmol/l, 1–2 h after initiation of the mixed-nutrient meal. The rise of plasma GLP-1 in response to intraduodenal infusion of GLU, fat and GLU–fat in pigs was modest (+5 pmol/l) for GLU and intermediate (+10 pmol/l) for fat, while the effect of combining GLU and fat was additive (+15 pmol/l)[97]. To our knowledge, the effect of dietary protein on plasma GLP-1 secretion has not been investigated to date in pigs. In general, the GLP-1 responses to fasting/feeding in pigs are comparable with those in humans, with fasting concentrations of about 13 pmol/l rising to 30–40 pmol/l postprandial[78,98,99].

Although GLP-1 can cross the BBB, several studies suggest that peripheral GLP acts to reduce food intake primarily via activation of the vagal afferent nerve[100]. Little information is

available on the effect of GLP-1 on food intake in pigs. Ribel et al.[101] showed that administration of a synthetic GLP-1 analogue reduced gastric emptying and therefore may induce satiation in pigs. In general, the pig is recognised as being a good model for studying human conditions[102], and in the case of GLP-1 analogues, it has found to be predictive of clinical findings in both reduction of hyperglycaemia (through improved insulin secretion and decreased glucagon secretion) and body-weight loss (through reduced gastric emptying and increased satiety)[103]. The icv administration of GLP-1 inhibited feeding in fasted rats and was counteracted by the icv injection of exendin 9–39, a GLP-1 receptor antagonist[104]. No data are available in pigs.

Peptide YY. PYY is an anorexigenic hormone. It is a member of the pancreatic polypeptide (PP)-fold family of peptides. It is secreted by the L cells of the distal small-intestinal mucosa. The PYY-containing endocrine cells are found in highest numbers in the lower small intestine and colon. The gastrointestinal functions of PYY include inhibition of gastric acid and pepsin secretion, inhibition of pancreatic exocrine secretion, delay of gastric emptying and inhibition of jejunal and colonic motility[105]. The secretion of PYY is stimulated by the presence of nutrients in the intestine. In humans, fats provide the strongest stimulus followed by carbohydrates and protein[106]. Little is known on the effects of intestinal nutrient loads on PYY secretion in pigs. Plasma PYY concentrations were higher (500 pmol/l) in *ad libitum*-fed pigs compared with fasted pigs (200 pmol/l)[107]. On the other hand, Souza da Silva et al.[95] showed that fasting plasma PYY concentrations were approximately 700–800 pmol/l, showing no response to a mixed-nutrient meal. Plasma PYY concentrations are lower in humans, ranging from 30 pmol/l at fasting up to 116 pmol/l after an intestinal nutrient load[77,106]. In the fast–refed condition, both single bolus injection (30 mg/kg body weight) and iv infusion (0·25 mg/kg per min) of PYY3–36 (the synthetic and bioactive part of the PYY molecule) suppressed feed intake in pigs[107], suggesting that circulating PYY3–36 influences satiety and contributes to the termination of a meal such as in humans[68].

Ghrelin. Ghrelin is an orexigenic intestinal hormone. In pigs, it is mostly produced in the oxyntic and cardiac gland and less commonly in the pyloric glands of the stomach[108], with a similar pattern to that in humans[109]. Ghrelin functions as a neuropeptide signal that reduces satiety and increases hunger. Govoni et al.[108] and Zhang et al.[110] showed that fasting increases plasma ghrelin concentrations from 10–25 to 50 pmol/l in prepubertal gilts and weanling pigs, respectively. Ghrelin in pigs responded to changes in energy balance and the concentrations increased during fasting from approximately 25 to 75 pmol/l[108,111]. Barretero-Hernandez et al.[65] reported postprandial plasma ghrelin concentrations of 6 pmol/l, increasing to 10 pmol/l at fasting in pigs. Scrimgeour et al.[112] suggested that the dynamics in plasma ghrelin concentrations in the pig appear to be strongly influenced by prolonged fasting and change from 15 pmol/l (feeding) to 100 pmol/l (fasting). These ghrelin responses to fasting/feeding are comparable with those observed in humans[78,113]. However, the concentrations in humans are higher, being approximately 150 pmol/l postprandial and 250 pmol/l at fasting.

Salfen et al.[114] showed that there was an increase in body weight of weanling pigs given exogenous ghrelin chronically, suggesting that feeding behaviour was influenced by the treatment. Ghrelin stimulates food intake in humans too. However, in vagotomised patients, ghrelin does not increase food intake, suggesting that an intact vagus nerve is required for exogenous ghrelin to increase appetite in humans[115]. No pig studies have been conducted addressing the contribution of the vagus nerve to the orexogenic action of ghrelin.

Conclusions on nutrient-induced secretion of satiety hormones and on endocrine gut–brain communication in pigs and humans

Basal or fasting plasma concentrations of CCK and GLP-1 are similar in pigs and humans. The nutrient-induced increase of CCK and GLP-1 concentrations is also similar in both species. Plasma CCK increases postprandial 2- to 5-fold, whereas GLP-1 increases 3-fold in pigs and in humans. Therefore, pigs are a useful model for the investigation of the effects of single (macro) nutrients or combinations of nutrients on CCK- and GLP-1 release. Fasting and postprandial plasma concentrations of PYY in pigs are 10- to 20-fold higher compared with humans. The PYY response of pigs to feeding or intestinal infusion of nutrients (in terms of percentage increase of PYY concentration) seems to be smaller than in humans. For ghrelin the reverse is true; humans show 2- to 5-fold higher plasma concentrations than pigs. The responses of ghrelin to fasting or feeding are comparable in pigs and humans: 2- to 3-fold increases of ghrelin at fasting as compared with fed conditions. The relevance of the pig as a model for human GIT hormone dynamics seems therefore CCK and GLP-1 > ghrelin > PYY.

Many pig studies support the human-like action of CCK on food intake regulation, but far fewer studies are available on the actions of GLP-1, PYY and ghrelin. Nevertheless, for GLP-1, PYY and ghrelin, human-like actions on food intake regulation have been reported in pigs[116]. Therefore, from an endocrine perspective, the pig is a suitable large animal model for the study of the humoral pathways of gut–brain communication as summarised in Table 1.

Gastrointestinal tract permeability and detoxification systems

Current outline of research in nutrition, gut-barrier and defence systems

An important aspect of the GIT function refers to metabolic disorders and obesity, which in humans are partially driven by excessive intake of high-energy diets and may be programmed early in life[117,118]. Unbalanced, high-energy/fat and low-fibre diets may alter GIT permeability, allowing translocation of gut pro-inflammatory microbial-associated molecular patterns (MAMP) such as lipopolysaccharide (LPS) into the body and the development of metabolic inflammation, as demonstrated

in mice[119]. These MAMP may then promote adipose tissue expansion, insulin resistance, metabolic disturbances and fat deposition.

The GIT is complex, comprising the mucus, the epithelial monolayer and the enteric immune system that includes intestinal epithelial cells[120]. The intestinal epithelium participates in digestion and absorption, while tightly restricting body access of deleterious components. Passage of compounds across the epithelium is mainly regulated by tight paracellular junctions and by macromolecular uptake transcellular mechanisms[121]. LPS entry can be transcellular via the chylomicron pathway, following FA absorption, but also paracellular when the transcellular route is altered[122]. These mechanisms appear relevant to stress-related diseases (for example, gut chronic inflammation), the metabolic syndrome and obesity in humans[123–125].

Besides permeability, the intestinal mucosa is equipped with defence systems including epithelial intestinal alkaline phosphatase (IAP) and inducible heat shock proteins (HSP). IAP is produced by the enterocyte and acts as a major anti-inflammatory enzyme through two mechanisms: detoxification, by dephosphorylating pro-inflammatory MAMP (for example, LPS), and control of local (and systemic) inflammation through a down-regulation of the Toll-like receptor 4-triggered NF-κB activation and of inflammatory cytokine production[126]. Intestinal epithelial cells are chronically exposed to a harsh environment and toxic substances and they have developed inducible HSP (HSP27, HSP70) as anti-inflammatory and antioxidant cytoprotection mechanisms[127]. Inducible HSP are involved in intracellular protein trafficking, with many functional implications, including protection against potentially invasive compounds and organisms[127]. Inducible HSP are produced in response to diverse microbial components and related metabolites (for example, LPS, butyrate) *in vitro*[120]. However, *in vivo* data are scarce.

Alterations in the function of the intestinal barrier and defence systems may lead to chronic inflammatory diseases[128]. Conversely, dietary approaches aimed at reducing intestinal permeability and/or stimulating IAP and inducible HSP may contribute to prevent or treat such diseases.

This section summarises comparisons between the pig and the human for intestinal physiology of permeability and its neuroimmune regulation, detoxification and defence systems, their dietary modulation and their early programming.

Similarities between pigs and humans

The pathophysiology, molecular basis and neuroimmune regulation of the intestinal barrier when under stress have been described for rodent models[121]. In summary, the mechanism involves hypothalamic corticotropin-releasing factor (CRF), central and peripheral CRF receptors, degranulation of mucosal mast cells and release of various bioactive mediators. Large quantitative inter-species differences exist for intestinal permeability of small, medium-size and large molecules. Notably, pigs are closer to humans than rodents for both trans- and para-cellular permeability values[129,130]. Intestinal permeability regulation in pigs also involves enteric nerve activation, CRF,

mast cells and released tryptase and TNF-α[120,131,132]. Data are limited in humans, but they indicate essentially the same regulations[130,133].

MAMP detoxification function by IAP is conserved across species[134]. IAP is highly expressed along the villous epithelium of the small intestine in pigs[135] and humans[136]. IAP activity is 10- to 15-fold higher in the distal ileum compared with proximal colon in both species[136,137]. In pigs, IAP activity is drastically reduced after weaning and may cause post-weaning intestinal alterations[135]. Depressed IAP is also suspected in various inflammatory diseases in humans[126]. Administration of exogenous IAP has strong anti-inflammatory effects in both species[126,136,138,139]. Plasma LPS is a marker of metabolic inflammation in humans[140]. Intake of saturated fat is consistently reported to increase plasma LPS in humans[141] and pigs[142], intestinal transport of LPS in pigs[142] and plasma IAP in humans[143].

Differences between pigs and humans, or pig studies with no equivalent in humans

Differences exist between humans, pigs and rodents for IAP gene copies (*n* 1, 2 and 2, respectively) and their chromosome location[134]. IAP distribution along the small intestine is opposite between pigs and rodents, the former having higher IAP activity in the ileum and lower in the duodenum compared with rodents[126,144]. The distribution of IAP along the human intestine is yet to be elucidated. Human cell line Caco2 and porcine IPEC-I display inducible HSP (for example, HSP70)[145,146]. However, data on intestinal HSP25 or HSP70 in human tissues are lacking. As a prototypic example of dietary modulation of intestinal defence systems, L-glutamine supplementation has been shown to improve the morphological integrity and barrier function of the intestines in humans[147] and pigs[148,149]. However, these studies are difficult to compare due to many differences in experimental conditions. Zn is a key element for intestinal and body homeostasis. One *in vitro* study with human (Caco2) and porcine (IPEC-J2) intestinal epithelial cells revealed cell line differences in permeability and Hsp70 responses to Zn, suggesting inter-species differences[150]. However, *in vivo* data in humans are lacking.

The concept of 'developmental origin of health and disease', linking early-life malnutrition (deficiency or excess) to metabolic diseases was formulated two decades ago[151]. The effects of nutrition in early life on gene expression and potential long-term effects have become a discipline of high interest in human[152,153] and animal[21,154] models. However, data on how early-life gene programming may affect intestinal function and defence systems are limited in humans[155,156]. Various dietary components, including protein, fat, methyl donors and fibre, influence gene expression in the intestines[155,156]. The pig as a model for humans has a high potential value in this area of research, but comparative studies are limited[137,157–159]. For example, high-protein milk formula transiently altered *ex vivo* ileal permeability in piglets and increased responses to LPS challenge in young adults[157]. Neonatal dietary protein excess also led to long-term alterations in colonic barrier function under oxidant stress in female pigs[158]. Finally, alterations in

mother-to-offspring transmission of GIT microbiota (for example, using antibiotics) had long-term consequences on IAP and iHSP along the GIT in pigs[137,159].

Intra-uterine growth retardation (IUGR) is a risk factor for the metabolic syndrome and obesity, possibly through low-grade inflammation[160,161]. For example, the prevalence of the metabolic syndrome was found to be 10-fold higher in human subjects weighing less than 3·0 kg at birth compared with those with more than 4·3 kg of birth weight[161]. IUGR in the pig occurs naturally for some pigs in litters[162]. IUGR piglets display immature gut, higher HSP70 both *in utero* and after birth, and altered pro-inflammatory NF-κB signalling pathway[163–165]. The IUGR piglets fed high-protein milk formula had higher ileal permeability and altered neuronal regulation of the gut barrier function later in life[166]. Finally, the GIT microbiota is an important modulator of gut development[156,167].

Conclusion

The available literature suggests that basic mechanisms of intestinal permeability and defence systems are conserved across species, including pigs and humans. Functional permeability studies *ex vivo* suggest the pig to be close to the human. However, data are scant for intestinal IAP and inducible HSP defence systems in humans, and only indirect evidence suggests some similarities and differences between pigs and humans. Data on pig nutrition and gut health are numerous[168–170], making this species valuable to human nutrition research, due to anthropometric, dietary and GIT anatomical and physiological similarities. Moreover, nutritional programming of intestinal gene expression and long-term effects on growth and health can be assessed in this out-bred species and should be relevant to humans.

Host–microbiota interactions

One of the main aspects in the study of GIT function is the microbial population. Animals are associated with a diverse microbial community, primarily consisting of symbiotic and commensal bacteria. Mammalian bacterial diversity has been related to phylogeny and claimed to be influenced by the host diet, increasing in meat eaters compared with non-meat eaters[171]. The GIT microbiota of modern humans is that of omnivorous primates. Original studies of the GIT microbiota focused on their role in inflammatory diseases, with the view that bacteria were pathogens only. However, the importance of the microbiota has been revisited in the past decade. It is now widely accepted that the GIT microbiota play a crucial role in maintaining homeostasis. The complex and intimate relationship between GIT microbial communities and its host is becoming clearer, due, in part, to large-scale microbial genome-sequencing programs[172]. Metagenomic sequencing of total community DNA provides information about both the phylogenetic representation as well as functional genes. Interrogation of metagenomic information has revealed three distinct 'enterotypes' in the human microbiota that are identifiable by changes in the population of at least one of the three genera: *Bacteroides*, *Prevotella* and *Ruminococcus*[173].

Enterotypes are not limited to humans, but also occur in mice[174] and pigs[175]. Advancement in sequencing total RNA (metatranscriptomics), identifying total proteins (meta-proteomics) and total metabolites (metametabolomics) has added further knowledge on the GIT ecosystem complexity both in humans and in pigs[176,177]. The use of an ecosystems biology approach, in association with 'omics approaches, will lead to a more complete understanding of the complex interactions between the thousands of bacterial species in the GIT and the host[178].

The physiological similarity between humans and pigs in GIT development, digestive function, and gastrointestinal fermentation profiles (the colon being the main site of bacterial fermentation in pigs and humans) suggests that the pig is preferred over other non-primate models for digestive and metabolic disease studies relating to humans[16,179,180]. Moreover, the pig is a human-sized omnivorous species. The pig has been extensively used as a model for nutritional studies as its protein and lipid metabolism is comparable with humans[102]. Increased knowledge of the pig microbiota composition and structure along the intestinal compartments being similar to humans further supports the pig as an ideal biomedical model for humans[175,181]. This section outlines the similarities and differences of the pig and human in host–microbiota interactions.

Similarities between pigs and humans in gut microbiota

Dominant phyla. The largest microbiota of the body is located in the GIT and the set of gene products provides a diverse range of biochemical and metabolic activities to complement host physiology. Hundreds of species are present in the GIT lumen, only belonging to a few microbial phyla. Firmicutes and Bacteroidetes are the two dominant bacterial phyla in the human and mouse gut, with the Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia phyla as subdominant phyla[171,182–184]. Similarly, the GIT microbiota in pigs, as well as in wild *suidae*, mainly consists of the Firmicutes and Bacteroidetes phyla[185,186](Table 3). Recently two different enterotype-like clusters, primarily distinguished by unclassified *Ruminococcus* and *Prevotella*, have been identified in pig faeces[175]. Their phylogenetic composition was highly similar to two of the enteroptypes described in humans[173]. Interestingly, in pigs, as in humans, enterotype-like clustering distribution can vary within an individual over time[187].

Postnatal and early life microbial colonisation. During the first few hours after birth (postnatal), contact with environmental and colonising bacteria is essential for healthy intestinal and immune maturation. The major role of microbiota in the development of the neonatal GIT was confirmed in conditions where colonisation was modified early in life through exposure to micro-organisms of maternal and environmental origins, through nutrients consumed and through antibiotic treatments. During infancy, the composition of the intestinal microbiota is unstable and more variable than in older children and adults. Diet-induced adaptation of the microbiota may vary from the proximal to the distal parts of the intestine. The composition

Table 3. Mean values of the amount of total SCFA throughout life in pig and human faeces

	Age in months													
	0	1	1·1	1·6	2	3	6	6·4	7·5	9	12	18	24	Adults
Humans														
SCFA (mmol/kg faeces)	6*	63	–	–	–	66	84	–	–	89	101	110	120	77
Pigs														
SCFA (mmol/kg DM faeces)	–	61†	284†‡	383†	225†	–	–	51§	72§	–	–	–	–	50§

* SCFA content in meconium; values adapted from Midtvedt & Midtvedt[414].
† Commercial pigs (Large White × Landrace × Pietrain breed) weaned 28 d of age, data expressed per kg of faecal DM (Montagne et al.[415]).
‡ 5 d post-weaning.
§ Post-pubescent (6·4 and 7·5 months-old Large White × Landrace × Pietrain breed) and gestating sows (1·5–2 years-old Large White × Landrace breed), data expressed per kg of faecal fresh matter (I Le Huerou-Luron, unpublished results).

that is typically measured from faecal samples does not reflect the large bacterial diversity along the intestinal tract. Animal models, specifically cannulated pigs, are useful for obtaining a better understanding of the interactions between microbiota present in different niches of the intestine and physiology relevant to humans[188]. Studies with germ-free piglets clearly show that bacteria are essential for the growth and development of the digestive tract[189]. The comparison of gene expression profiles in enterocytes of germ-free compared with conventional piglets has brought insight on the impact of microbiota on the GIT function[189]. Bacterial colonisation induces the maturation and function of several components of the mucosal immune system and defence in order to prevent inflammatory responses that would compromise the barrier function[189–191]. In humans, neonates compared with older individuals have a decreased innate defence, a low production of IgA and a defective interaction between dendritic cells, T lymphocytes and regulatory T cells[192]. Similarly, the mucosal immune system is essentially absent in the neonatal piglet, even though the systemic immune tissue is well developed[193], and piglets begin to synthesise secretory IgA from the second week of age[194]. The balance between T lymphocytes helper 1 and helper 2 responses in human and pig neonates is skewed toward the helper 2 profile, resulting in a high susceptibility to intracellular pathogen infection[195,196]. The impaired protection of the neonate against infections may be partly attributed to a deficient secretion of interferon[195,197].

It only takes a few hours for bacteria to appear in the faeces of mammalian neonates[198,199]. Facultative anaerobic bacteria, such as Proteobacteria, are the first colonisers. These bacteria reduce oxygen concentration in the GIT and allow strict anaerobes, such as members from the genus *Bacteroides* and the phyla Actinobacteria and Firmicutes, to colonise the intestine. During the first year of life in humans and the first 6 months in pigs, the intestinal microbiota composition fluctuates widely between individuals and over time, before resembling the adult status (Table 4). The potential use of piglets as a model for human studies is reinforced by the early colonisers, *Bacteroides* and *Escherichia/Shigella* being similar in humans. However, the substantial presence of *Lactobacillus* and *Streptococcus* in pigs is unparalleled in humans.

Early disturbances of the microbial colonisation process, such as induced by high-hygiene environments or by antibiotic treatment, have major consequences for the developmental

sequence of the GIT microbiota and for host metabolism[200]. An advantage of the porcine model is the flexibility to compare different early environmental-rearing conditions, using, for example, outdoor and indoor sow-reared piglets or isolator-reared neonates. Mulder et al.[201] showed large differences in composition of ileal-adherent microbiota between outdoor and indoor sow-reared animals, which corresponded to major differences in intestinal immune activation. Excessive hygiene appears to interfere with the normal processes of bacterial stabilisation and alters immune development. Mulder et al.[202] showed that the succession of events that lead to a stable adult microbiota depends on colonisation during the first 2 d of life, and also on continuous exposure to highly diverse microbiota during the early development at least up to weaning at 4 weeks of age. The use of antibiotics, in combination with stressors in early life, was shown to affect adult pig microbiota and intestinal gene expression, including genes involved in immune-related processes[203]. This observation in pigs corroborates human studies indicating that changing the environmental conditions, and in particular microbial exposure, throughout early life affects the development of immune diseases[204]. Whether inducing early change in immune homeostasis by modifying microbiota would lead to different sensitivity of pigs to infectious or inflammatory challenge, such as recently reported with early spray-dried supplementation[205], warrants further investigation.

Many beneficial strategies have been suggested to strengthen the postnatal development of presumably beneficial microbiota and GIT functions, including: changing the composition of maternal food during gestation and lactation; changing the composition of infant formulas; and favouring breast-feeding over formula-feeding during the suckling period[180,206]. For example, feeding neonatal piglets with formula supplemented with prebiotics increased the bacterial numbers by 5-fold, the content of folic acid by 53 % and growth of the colon[207]. Similarly, supplementation of the sow diet with prebiotics during gestation and lactation was associated with 50 % greater fermentative activity of the caecal microbiota, accelerated development of the intestinal immunity, and improved intestinal protection by increasing ileal Peyer's patch production of secretory IgA in the offspring by 46 %[195]. Faecal secretory IgA also increased by 170 % in healthy infants who receive a prebiotic-supplemented formula[208]. These results in pigs and humans underline the key role of maternal nutrition during

Table 4. Comprehensive summary of the existing literature on the relationship between nutrition and brain composition/development in pig models

Nutritional treatments	Pig models	Exposure periods	Impacts on brain composition and development	References
Studies on maternal diets and FA				
Maternal diet with vegetable v. fish oil	Yorkshire sows and 15 d-old piglets (n 7 and 6)	Lactation period	Higher DHA levels (fish oil increased DHA and EPA milk levels)	Arbuckle & Innis[416]
Diet with supplements (30 g/kg) of either soyabean oil or tuna oil	Multiparous sows (n 6 per diet) and their piglets	Last 21 d of pregnancy	Tuna oil increased the proportionate amount of total n-3 FA (especially DHA) but proportion of ARA was decreased; umbilical plasma cannot be used to predict the FA status of piglet brain	Rooke et al.[417]
Gestation and lactation diet with lard or linseed oil	Sows (Landrace × Large White) and their piglets (Landrace × Large White × Pietrain) (n 20)	Last 3 months of gestation and lactation	No difference for n-6 PUFA; proportions of DPA and DHA were greater in the brain of linseed oil-fed piglets than in that of lard-fed piglets during the suckling period	de Quelen et al.[418]
Conventional low-fat diet (3 %) v. three high-fat diets (6 %) i.e. high-fat saturated, high-fat oats (rich in LA), and high-fat linseed (rich in ALA)	Multiparous Swedish Yorkshire sows and their litters (n 63)	From weaning of the previous litter until weaning of the experimental litter	Increased proportion of ALA in the diet increased long-chain PUFA, EPA, DPA and DHA in piglet brain	Sampels et al.[419]
Studies on maternal milk, formulas and FA				
Mother's milk compared with formula containing SFA as MCT oil or formula with MCT and fish oil	Male Yorkshire piglets (six per diet)	From birth to 18 d	Piglets fed formula with fish oil had similar brain ARA and EPA but higher DHA than did piglets fed sows' milk; data from plasma and erythrocytes were not reliable predictors of differences in brain long-chain PUFA	Wall et al.[420]
Maternal milk v. infant formula	14- to 21-d-old piglets	Suckling period	Higher deposition of DHA with formula – no difference for 20 : 4n-6 and precursors – maternal milk and formula are not equivalent with respect to precursor and bioavailability and processing	Alessandri et al.[421]
Artificial feeding with sows' milk, control formula, or formula enriched with n-3 FA with low-EPA fish oil at high or low concentration, or formula enriched with n-3 and n-6 FA from either egg yolk- or pig brain-phospholipids	Newborn piglets	2 weeks	DHA levels in the brain correlated with plasma; 4·5 % fish oil decreased ARA in the cortex and cerebellum whereas 1·5 % limited this decline in the cerebellum; ARA level was 10–20 % higher in brain temporal lobe than in parietal, frontal and occipital lobes; egg phospholipids increase both DHA in the brain and ARA in the temporal lobe	Goustard-Langelier et al.[422]
Pig milk formula devoid of DHA for 2 weeks, formula with DHA TAG (1·8 % of total fat) for 2 weeks, control formula for 4 weeks, or control formula for 2 weeks followed by supplemented formula for 2 weeks	36-h-old male large white piglets (n 16)	2 to 4 weeks	DHA incorporated efficiently during both early and late supplementation periods; the piglet brain cortex is responsive to dietary DHA; higher rate of DHA synthesis in the piglet compared with the human infant	Morris et al.[423]
Four milk diets differing in their FA composition: deficient, contemporary, evolutionary, supplemented	Male Yorkshire piglets (six per diet)	From birth to 30 d	Contemporary diet high in LA compromises DHA accretion and leads to increased n-6 adrenic acid and DPA in the brain; evolutionary low in LA supports high brain DHA; DHA increased brain levels of DHA but not n-3 EPA and DPA; n-6 DPA is efficiently acylated and preferentially taken up over DHA in primary cultures of cortical neurons; DHA but not n-6 DPA supports growth of secondary neurites	Novak et al.[424]
Supplementation with 0·2 % cholesterol, 0·2 % DHA, or cholesterol plus DHA to the basal milk formula	Newborn piglets (n 16)	0–49 d	Cholesterol reduced the brain levels of glutamate, serine, glutamine, threonin, β-alanine, alanine, methionine, isoleucine, leucine and γ-aminobutyrate but increased glycine and lysine; DHA similarly affected these amino acids and taurine; DHA reduced ammonia in plasma and brain	Li et al.[425]
Three diets containing ARA (0·64 %) and DHA (0·34 %), ARA being provided by three different sources	Piglets (eight per diet) selected from five sows (Yorkshire Landrace bred to Hampshire boars)	3–22 d	ARA bioequivalent across treatments in the brain; DHA brain levels unaffected by diet	Tyburczy et al.[426]
Liquid diets with varying concentrations of Fe: control, mildly deficient, severely deficient (i.e. 100, 25, or 10 mg/kg milk solids)	Female (n 12) and intact male (n 12) Yorkshire piglets	4 weeks	Decreased Fe concentration in the hippocampus (but not in the prefrontal cortex) in deficient piglets compared with control; level of the transferrin receptor mRNA greater in the prefrontal cortex of control piglets (but not in the hippocampus); cognitive impairment in a hippocampal-dependent task in deficient piglets	Rytych et al.[427]

Table 4 *Continued*

Nutritional treatments	Pig models	Exposure periods	Impacts on brain composition and development	References
Unsuckled control piglets (*n* 7), suckled piglets with sows for 24 or 72 h (*n* 14), or experimentally fed piglets given milk colostrum (*n* 14), an elemental diet (*n* 14), or the elemental diet supplemented with purified plasma Ig (*n* 14)	Crossbred (Yorkshire × Swedish Landrace) × Hampshire piglets (*n* 63)	From birth to 24 h or 72 h	Positive correlation between growth, level of total plasma protein and IgG, and hippocampus development in sow-reared piglets; decreased level of astrocyte-specific protein	Pierzynowski et al.[428]
Studies on solid diets and FA				
Diet containing partially hydrogenated fish oil, partially hydrogenated soyabean oils, or lard	Norwegian Landrace 3-week-old female pigs	From 3 weeks to 2 years	No *trans*-FA detected in brain phosphatidylethanolamine; increased level of *n*-6 DPA with dietary *trans*-FA	Pettersen & Opstvedt[429]
High-fat high-cholesterol diet *v.* low-fat low-cholesterol diet	Female pigs selected for three generations for high-serum and low-serum cholesterol (*n* 36)	92 d	No difference in the brain or other tissues, apart from the liver	Harris et al.[430]
High-PUFA diet *v.* low-PUFA diet	Male Yorkshire piglets (six per diet)	From birth to 30 d	Frontal cortex phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine were higher in piglets fed the low-PUFA diet; fewer arm entries on the maze but no difference between groups when given L-DOPA	Ng & Innis[249]
Diet containing 4 % fish oil or 4 % high-oleic acid sunflower-seed oil	7-week-old piglets	8 weeks	Higher proportion of DHA in the frontal, parietal and occipital lobes but not in the temporal lobe with fish oil – less DHA in temporal than in other lobes	Dullemeijer et al.[431]
Diet with 0 *v.* 0·5 % cholesterol	Male (*n* 18) and female (*n* 18) pigs genetically selected for high (HC) or low (LC) plasma total cholesterol	From weaning (24–36 h) to 42 d	Brain weight greater in HC than LC pigs but not affected by sex or diet; dietary cholesterol tended to increase brain cholesterol	Pond et al.[432]
Four different diets with low (3 %) or high (25 %) fat, with (1 %) or without conjugated LA	Newborn piglets (*n* 24; six per group)	16 d	Significant decrease in *n*-6 long-chain PUFA biosynthesis by inhibition of LA elongation and desaturation; inhibitory effect more pronounced in pigs fed a low-fat diet (3 %) than a high-fat diet (25 %)	Lin et al.[433]
Control *v.* high-fat diet	19-week-old Ossabaw minipigs	19- to 37-week-old with two phases	The high-fat diet influenced the lipid metabolome in the brain cortex; not necessarily correlated with plasma and urine	Hanhineva et al.[434]
Dietary mono-conjugated ALA isomers compared with conjugated ALA, non-conjugated *n*-3 PUFA and *n*-6 PUFA	3-week-old piglets (*n* 32)	15 d	*n*-3 PUFA composition decreased in the conjugated ALA compared with *n*-3 PUFA group	Castellano et al.[435]
Other studies on solid diets or drinking water				
Water with sulfate in excess at 2000 ppm *v.* 1000 ppm	Sample of two sows, six live piglets, six dead piglets from a herd of 125 gilts and sows		Myelin deficiency in spinal cord and brain of sows and piglets	Jericho et al.[436]
Chloroquine intoxication via the diet in doses of 2·0–3·5 g/kg food *v.* control diet	Göttingen minipig	From 100 to 240 d and then from 177 to 219 d	12 % increase of ganglioside concentration in the cerebrum, with allocortex much more affected than isocortex, but no alteration of phospholipids or cholesterol in the brain	Klinghardt et al.[437]
Control, swainsonine*-fed and locoweed-fed animals	Pigs		Alterations in the structure of brain glycoproteins	Tulsiani et al.[438]
Diet containing 1 mg Cd per kg feed, as well as 0, 50, 100 or 200 mg Cu per kg feed	Male castrated weanling crossbred pigs (Deutsche Landrace × Pietrain)	3 months	Cd retention increased in correlation with Cu content of the feed	Rambeck et al.[439]
Experiment 1 (*n* 16 obese pigs): adequate diet (21 % protein, 3 % fat) *v.* protein-deficient diet (5 % protein, 23 % fat) for 7 or 8 weeks; experiment 2 (*n* 16 genetically lean or obese pigs): adequate or protein-deficient diet for 10 weeks	3-week-old genetically lean or obese pigs	7, 8 or 10 weeks	Experiment 1: reduced brain weight and cellularity after 7 weeks but not 8 weeks or protein restriction; experiment 2: lean and obese pigs responded similarly, with no interaction between diet and genotype for brain weight	Pond et al.[440]
Diet containing 500 mg quercetin†/kg body weight	Cross-bred castrated male pigs (*n* 2; 122–138 kg)	3 d	Low concentration in the brain compared with liver and kidney	De Boer et al.[441]

FA, fatty acids; ARA, arachidonic acid; DPA, docosapentaenoic acid; LA, linoleic acid; ALA, α-linolenic acid; MCT, medium-chain TAG; L-DOPA, L-3,4-dihydroxyphenylalanine; HC, high cholesterol; LC, low cholesterol; ppm, parts per million.
* Swainsonine is a plant toxin.
† Quercetin is a dietary polyphenol with potential health benefits.

The pig as a model in human nutrition

pregnancy in supporting neonatal development of the GIT immune system via modulation of microbiota.

The health benefits of breast-feeding have been recognised for a long time. Breast-feeding is associated with earlier colonisation with bifidobacteria, partly in relation to the presence of oligo-saccharides in human maternal milk[177]. As in humans, breast-fed piglets showed lower intestinal growth and permeability compared with high protein formula-fed ones[166,177]. One major issue in human studies on the effect of breast- *v.* formula-feeding on gut function is the great number of confounding factors which are difficult to circumvent, including quantification of food intake in breast-fed infants, variable length of exclusive breast-feeding, and variability of the composition of milk formulas. Animal models are used to help control these confounding factors, in particular, use of an automatic milk feeder that provides neonatal piglets with artificial milk as similar as possible to maternal milk[209]. These studies using a formula-fed piglet model provide strong support for the idea that short dietary changes before weaning associated with a modification of the early intestinal bacterial colonisation can have a long-term impact on the severity of inflammatory responses without changing the basal physiology of the intestinal barrier and cytokine profile in the intestine[157,158]. No similar data are available from human studies due to the invasive procedure required for intestine functionality research. However, breast-feeding is clearly associated with lower incidence of necrotising enterocolitis and diarrhoea in both human and pig neonates[206,210].

Differences between pigs and humans in gut microbiota

Dominant phyla. Differences in the most abundant genera exist between the human and the pig intestinal microbiota[180]. Belonging to the Bacteroidetes phylum, the most abundant genus is *Bacteroides* in humans, averaging 9 to 42 % of total bacteria[199], while the most abundant genus is *Prevotella* in weaned pigs, accounting for more than 20 % of total bacteria[175,211]. In adult humans, the phylum Actinobacteria may represent up to 15 % of total bacteria. It comprises bifidobacteria, the most predominant group detected in infants (40 % in average in faecal samples of 6-week-old European infants)[212]. The population of bifidobacteria present in the intestine of pigs is considerably lower, with less than 0·1 % of total sequences in the faecal samples of 22-week-old commercial pigs[211]. Dietary, environmental and behavioural (such as feeding habits) factors contribute to the species-specificity of the composition of microbiota.

Epigenetic mechanisms. The concept that early developmental dietary insults (poor or inadequate pre- or postnatal nutrition, for example) can have long-term consequences on health later in life has been termed developmental programming, or 'developmental origins of health and disease'. The GIT microbiota appears to have an important role in the GIT programming as its initial composition creates distinct individuality during the lifespan[167,213,214]. A mechanism leading to these long-term effects may be due to epigenetically active fermentation metabolites such as in histone acetylation[215,216]. However, no studies on epigenetic modifications underlying

long-term effects on early microbial colonisation and gut function are available in pigs. Despite limited studies, use of the pig as a model for humans to assess the effects of early nutrition on the development of microbiota is gaining acceptance amongst the scientific community.

Conclusion

Although similarities exist between humans and pigs in terms of dynamics of postnatal maturation of microbiota diversity and structure, responses to environmental factors, including dietary factors, and phyla composition, differences in the most abundant genera exist. As reviewed by Heinritz *et al.*[180], understanding the crucial role and complexity of human microbiota could be improved by the use of human flora associations in pigs. This model has been successfully established taking advantage of the higher similarity between pigs and humans compared with the widely studied rodent models. In addition to the similarities in anatomy, physiology and metabolism between the pig and humans, the pig is more similar to humans than rodents with increased *Bacteroides* spp. and bifidi-bacteria[217,218]. The pig has already successfully been used as a model for humans to study nutritional interventions[219,220]. In addition, the human flora-associated pig can be considered as a useful model for human infants. However, stability of the implanted human microbiota in the gut of pigs during the life-span remains to be investigated in studies regarding developmental programming.

The relationship between nutrition and the brain in the pig

Current outline on research in nutrition and neurosciences in pigs

Nutrient intake is driven by homeostatic and hedonic signals of peripheral, gastrointestinal, endocrinological and metabolic origin that convey in the central nervous system where they are integrated in a cognitive process referred to as the hunger–satiety cycle. Dietary nutrients also have an impact on brain development and function. Neuroscientific studies in pigs have progressed in recent years, partially to address scientific matters that cannot be studied in humans for ethical reasons.

This section summarises the pig studies, mostly *in vivo* and the minimally invasive neurocognitive explorations that are paralleled in human studies. Second, we will summarise the pig studies, mostly post-mortem explorations on brain tissues, which present differences or no equivalent in humans.

Similarities between pigs and humans in terms of brain functions

Brain responses to food signals. Describing brain responses to food signals is important to investigate food pleasure and motivation, or to decipher the brain networks underlying sensory and nutrient perception. An extensive literature is available on this topic in the human, describing mostly via functional MRI the brain responses to various food signals, according to different internal states (for example, hungry *v.* sated) or conditions (for example, lean *v.* obese), and many review papers

are available on this topic (for example, Rolls[221]; Stice et al.[222]; Carnell et al.[223]). Studies using large animal models have not yet completely made use of the opportunities provided by in vivo brain imaging. The very first studies using functional imaging to describe food-induced brain responses in pigs used ^{99m}Tc-HMPAO (technetium hexamethylpropylenea-mine oxime) SPECT (single photon emission computed tomography) and [^{18}F]fluorodeoxyglucose positron emission tomography to map cerebral blood flow[224–226] and brain GLU metabolism[227], respectively. These studies addressed the use of the pig to study food conditioning, looking at specific modulations of the response of the brain reward circuit after exposure to flavours with positive or negative hedonic values[226,227]. These studies provide two major outcomes: (1) functional imaging in anaesthetised pigs can be imple-mented to explore brain responses to different food signals, as in humans; and (2) the brain circuits activated by the perception of food signals are similar to those described in the human (for example, frontostriatal areas, amygdala and insular cortex). Boubaker et al.[224] showed in pigs that duodenal and portal GLU infusions led to different systemic and brain responses in areas regulating food intake and pleasure. Clouard et al.[225] compared congruent v. dissociated oral and duodenal SUC perception, and found different brain responses in the limbic and reward circuits. Studies in human subjects showed that brain responses to energy-providing sugars and sweeteners are not the same in the reward circuit (for reviews, see Low et al.[228] and Ochoa et al.[229]), which resembles the results obtained by Clouard et al.[225] in pigs. These studies are important to understand how the human brain correlates sugar cravings, as well as the neurobehavioural changes that could emerge due to the chronic consumption of, for example; sugars or non-energy sweeteners.

Impact of diet on brain activity, neurotransmission and cognition. Minipigs have become a widely accepted model for studying obesity and the metabolic syndrome[230–234]. They can be used to investigate the obesity-induced central modi-fications in humans, including decreased activity of the pre-frontal cortex[235–237] and altered dopaminergic function[237,238]. Val-Laillet et al.[239] demonstrated in the Göttingen minipig that brain alterations similar to those described in obese humans exist in this model and that they are an acquired feature of obesity correlated to weight gain. In Pitman–Moore minipigs, Val-Laillet et al.[240] also described the effects of three high-lipid diets differing in their lipid sources and found that the basal GLU metabolism of the anterior prefrontal cortex and nucleus accumbens was highest with a diet enriched with sunflower-seed oil, intermediary with a diet enriched with lard, and lowest with a diet enriched with fish oil. These results demonstrate that specific dietary nutrients can modify brain metabolism independently from body weight, and that specific nutrients in excess might favour the onset of brain metabolism anomalies.

In humans, cognitive test scores were positively related to breast milk DHA and negatively related to linoleic acid, suggesting that high levels of dietary linoleic acid may impair cognition[241].

Individual consumption of dietary FA had an impact on cogni-tive measures in children[242], with n-3 FA being positively related to cognitive test scores in male and female children, while n-6 showed the reverse relationship. Higher scores in tests of neurodevelopment were found in infants fed formula with DHA than in infants fed formulas without DHA[243]. DHA supplementation in young boys increased the prefrontal cortex activation during sustained attention[244]. These results are consistent with the hypothesis that dietary DHA is assimilated by the brain and has a positive influence on cognition. Autopsy data show lower DHA in brain of human infants fed formula rather than those who were breast-fed[245,246], which resembles the results obtained in pigs (Table 4).

In pigs, dietary FA significantly made an impact on the frontal cortex and striatum concentrations of neurotransmitters (for example, dopamine, serotonin)[247,248]. Another study showed fewer arm-entries in a maze in pigs receiving a low-PUFA diet compared with a high-PUFA diet, with the effect being probably dependent on central dopamine metabolism[249]. Epidemio-logical and clinical studies also suggest a relationship between dietary FA and altered functions of the nervous system, including neurocognitive disorders[250]. Dietary n-3 FA could also protect against brain disorders on neurotransmission, neuroprotection and neurogenesis[251]. However, data are lacking for pigs providing an opportunity to investigate the relationship between diet, brain activity and cognitive functions relevant to humans via in vivo imaging.

Peripheral neuromodulation to regulate eating behaviour in pigs. Vagal nerve stimulation (VNS) is a therapy for refractory epilepsy and psychiatric disorders[252,253], but it has also received attention as a way to modulate food intake. Animal models, including pigs, were used to investigate this question[254]. Diaz-Guemes et al.[255] found that VNS increased central nervous activity, but no effect was observed on feeding behaviour. In contrast, other authors demonstrated a decreased weight gain, decreased fat gain and plasma insulin-like growth factor I[256], or decreased food intake and specific activations in several brain areas associated with altered gastric myoelectric activity[257] in growing pigs[258]. Another on-going study[259] showed VNS-induced metabolism differences in the brain reward circuit only 7 d after VNS onset, meaning that quick central neuroplasticity phenomena can be induced by VNS, possibly modulating homeostatic and cognitive processes. In Göttingen minipigs fed a Western diet, VNS prevented further weight gain, decreased food intake and sweet cravings[254], proving the therapeutic potential of this strategy. Similarly, some studies assessing the impact of VNS on eating behaviours and weight in individuals with other psychiatric and neuro-logical disorders showed significant modulation of food cravings and body weight[260,261], but there are significant dis-crepancies between studies. Overall, functional imaging in pigs has the potential to help validate and optimise therapies before their application to human patients.

Central neuromodulation to regulate eating behaviour in pigs. Recent development suggests that the deep-brain stimu-lation (DBS), a procedure for the treatment of Parkinson's or

depression, might also be used to combat obesity[262–265]. The minipig has emerged as an ideal model for basic and preclinical studies on DBS[266]. Hypothalamic DBS was validated in the Göttingen minipig[267,268] and resulted in reduced weight gain[269], as well as in behavioural and physiological changes that could be related to the activation of limbic and autonomic brain networks[270]. These results with pigs are similar to those described in human studies (for reviewes, see McClelland *et al.*[260] and Val-Laillet *et al.*[261]). Shon *et al.*[271] showed that DBS of the subthalamic nuclei in pigs can stimulate striatal dopamine release, which is related to food motivation and obesity[272,273], whereas Sauleau *et al.*[12] managed to modify food motivation and learning. Knight *et al.*[274] demonstrated that DBS of the nucleus accumbens, a putative target to combat obesity[262], modulated the activity of the prefrontal, cingulate and insular cortices, which are brain regions involved in eating behaviour. A similarly low metabolic activity of the prefrontal cortex was observed in obese humans[235–237] and minipigs[239], an anomaly that was normalised via DBS of the cortex. The combination of DBS and MRI has been explored in pigs, in terms of image-guided brain navigation[275], network activation[274,276] and safety[277–279]. These studies show the (mini) pig is a convenient model to study the impact of DBS on eating behaviour and nutritional diseases, and to test medical innovations in preclinical trials before being safely applied to humans.

New imaging approaches in pigs. In addition to the aforementioned neuromodulation studies, there are many innovative methods related to nutrition and brain activity used in humans that could potentially be investigated in pigs. Alstrup & Smith[280] reviewed 10 years of positron emission tomography findings on neuromolecular processes in the living porcine brain and listed all the validated brain radio ligands including several molecules of interest for nutrition studies. Other methods can be used for molecular imaging in pigs, such as the wireless instantaneous neurotransmitter concentration system which allows the measure of neurotransmitter release in specific brain areas[281,282]. The non-invasive magnetoencephalography and electrocorticography[283], as well as the functional near-IR spectroscopy and cortical imaging have been successfully implemented in the pig model[284] and could be applied in the future to map brain responses to food and nutrient stimulations.

Differences between pigs and humans, or pig studies with no equivalent in humans

The most important corpus of literature investigating the impact of nutrition on the pig brain has been focused on brain composition and development, and especially on the role of dietary FA. The health consequences of dietary deficits or supplements of *n*-3 and *n*-6 FA are still controversial areas of human nutrition due to conflicting results. However, animal models such as the pig have the potential to bring new insight through better-controlled experimental designs[285,286]. Table 4 provides a comprehensive summary of this literature. Most of these results have no equivalent in human studies (other than exceptional

post-mortem case studies), because it is not possible to assess, non-invasively, human brain composition or the administration of specific toxicants.

The BBB is important for the regulation of food intake and the blood-to-brain transport of dietary compounds[287]. The passage of xenobiotics[288] or bismuth[289] into the brain has been investigated in pigs via *post-mortem* tissue analyses. However, the emergence of *in vitro* models of pig BBB[290–293] have significant advantages for investigating the transport mechanisms of compounds into the brain. BBB transport of glutamate[294], alkaloids[295], mycotoxins[296] and central nervous system-active drugs[297] was assessed with this model, which might help to understand the neural toxicology of dietary compounds and the effectiveness of medicines. An *in vivo* imaging study in obese minipigs showed increased BBB permeability with a diet enriched with fish oil characterised by an excessive amount of *n*-3 FA[240]. Transport of nutrients and their impact on the BBB integrity should receive more attention in the future to understand how the gut–brain axis is altered by nutritional diseases[298], and to provide nutritional recommendations in humans. If the increased BBB permeability induced by high doses of *n*-3 FA is confirmed in humans, this could also have unexpected beneficial outcomes, for example to improve drug delivery to the brain in Alzheimer's disease[299] or other neuropsychiatric disorders.

In pigs, central concentration of serotonin can modulate operant food intake behaviour[85]. In addition, brain DHA, which depends on dietary DHA, promotes central dopamine metabolism[249]. Dietary AA can also affect brain neurotransmitters in the hypothalamus[300–303], suggesting that dietary manipulation of AA precursors of neurotransmitters may offer a practical means of reducing stress responses. Further studies are needed to verify whether these results may be of benefit in human nutrition, for example to help patients with stress disorders. Elmquist *et al.*[304] suggested that central CCK increases with time in piglets in parallel to the ability to assimilate nutrients from a solid diet. Kenk *et al.*[305] demonstrated via positron emission tomography imaging of cAMP, a strong region-specific signal in the brain, as well as an impaired cAMP-mediated signalling in obese pigs, giving some insight into pathological progression with potential for directing therapy in humans. Mycotoxin[306,307] and feed additives[308] were also found to alter behaviour, neurotransmitter activity and metabolism in pigs. The decreased feed intake and increased aggressive behaviour observed in subjects contaminated with mycotoxins might be related to anomalies in brain monoamines, including dopamine and serotonin. Gbore[309] demonstrated that the acetylcholinesterase activity in the hypophyses, hypothalamus and amygdala decreased with increased mycotoxin concentrations in the diet. These results are important to identify the risk associated with mycotoxin contamination and possible therapeutic interventions in humans.

Pig studies exploring the brain responses to specific diets relying on *post-mortem* methods have no equivalent human data. Kanitz *et al.*[310] showed that low protein:carbohydrate dietary ratio during gestation may alter the brain expression of genes encoding key determinants of glucocorticoid activity in the fetus, with potential long-lasting consequences for stress

adaptation and health. Also, increased c-Fos (a transcription factor) immunoreactivity in several brain structures was described after oral administration of fungi extracts in pigs[311]. Madsen et al.[312] found that expression of the fat mass and obesity associated gene (FTO) transcript was detected at high levels in brain tissues and that these levels varied through the development and between specific brain areas. These results demonstrate a relationship between the genetic propensity to develop obesity and dietary habits at the cerebral level. Since the FTO gene has recently been associated with increased BMI in several human populations, the pig model might be used to investigate the epigenetic mechanisms that could lead to obesity-related brain anomalies in humans.

Conclusions on the relationship between nutrition and the brain

The general comparison between the brain of pigs and humans (Table 1) shows that, even if there are some differences in terms of size and structure, the overall brain anatomy and development in pigs are similar to those of humans. In addition, similar functional neuroimaging approaches have been transferred from humans to pigs. Understandably, many pig studies do not have any equivalent in humans (especially the invasive and terminal experiments). However, most of the *in vivo* functional brain explorations and therapies described in pig models are echoed in human studies, which highlight the fantastic potential of pig models for translational research in nutrition and neurosciences. In addition, the pig model is of high value to perform mechanistic, toxicological and epigenetics studies that could not be performed in humans for practical and ethical reasons.

Acknowledgements

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