MINIREVIEW

The Pig as an Experimental Model for Elucidating the Mechanisms Governing Dietary Influence on Mineral Absorption

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This review highlights the similarities between pigs and humans and thereby the value of the porcine human nutritional model, and reviews some of the more recent applications of this model for nutritional research. Exp Biol Med 233:651–664, 2008

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Introduction

Nutrition plays an intrinsic role in human health with nutritional deficiencies and poor dietary habits being a leading cause of many intestinal and extra-intestinal diseases and disorders, including diarrhea, irritable bowel syndrome, celiac disease, inflammatory bowel disease, obesity, insulin resistance syndrome, type 2 diabetes mellitus, cardiovascular disease, hypertension, gall bladder disease and anemia (1–12). In many instances the onset of these conditions can be partly, or wholly, attributed to the diet; be it from malnutrition, mineral and/or vitamin deficiencies, malabsorption of nutrients, inappropriate reaction by the body to dietary antigens, or the failure to maintain what is considered to be a healthy balanced diet (2–3, 13–15).

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Currently, much attention is being paid to elucidating the exact mechanisms by which the diet can initiate the consequences outlined above, as well as investigating means of preventing or alleviating these conditions using various diet regimes and dietary supplements. This could be achieved through fortifying commonly consumed food products with the necessary vitamins, minerals, and other nutrients, or through the consumption of dietary supplements such as probiotics, prebiotics, and organic acids (16–20). To elucidate the mechanisms involved in dietary effects on health, particularly with regard to mineral absorption across the intestinal epithelium, it is sometimes necessary to access the different compartments of the gastrointestinal tract (GIT). While access to the human GIT can sometimes be obtained in a hospital environment using patients with ileostomies and colonoscopies, or patients that have been intubated, such procedures are costly and laborious, and may be hampered by the inability to locate enough willing participants and ensure their full compliance (21). Often, the more suitable approach is the use of animal models, with the option of using cannulas and catheters to access the GIT of live animals, or euthanasia to allow for excision of different GIT compartments.

Traditionally, rats have been the animal model of choice when performing nutritional studies. However, the rat model has a number of limitations which makes extrapolation back to a human situation questionable, including a significantly different food intake and energy expenditure for body size, a different lifespan and body proportion, differences in intestinal morphology and enteric microbiota, as well as other distinct physiological differences (21, 24). Another major problem with using rat models for mineral studies is their propensity for practicing coprophagy. While this is an effective way for the animals

Table 1. Comparison of Some Biochemical and Physical Characteristics of Pigs and Humans

	Humans	Pigs
Average birth weight (kg)	3.4	1.4
Average mature weight (kg)	60–100	200–300
Initial growth rate at birth (g/day)	19	150–200
Composition at birth		
Water (%)	82	82
(Fat-free basis)		
Protein (%)	14	13
Ash (%)	3	3
Composition at maturity[a]		
Water (%)	69	72
(Fat-free basis)		
Protein (%)	21	23
Ash (%)	9	4
Average lifespan (years)	80	20
Average age at chemical maturity (days)[b]	1285	270–420
Percentage of total life at chemical maturity [b]	4.4	4.6
Hemoglobin concentration at birth (g/L)	180–226	80–90
Serum electrolyte balance at birth		
Calcium (mg/dL)	8–11	9
Potassium (mg/dL)	14–26	18
Sodium (mg/dL)	310–340	353
Phosphorus (mg/dL)	4–8	7
Chlorine (mg/dL)	350–400	330–400
Circulating serum mineral levels at birth		
Magnesium (mg/dL)	1.0–1.4	2.4
Copper (mg/dL)	0.080–0.163	0.058
Zinc (mg/dL)	0.088–0.112	0.102
Iron stores at birth (mg)	250	50
Blood glucose levels at birth (mg/dL)	40–150	59–171
Intestinal length at maturity [a]		
Small intestine (m)	5.5–7	15–22
Large intestine (m)	1.5	4–6
Intestinal weight at maturity [a]		
Small intestine (g)	1040	2310
Large intestine (g)	590	1970

[a] Maturity is considered to be 33 years in humans and 3 years in pigs.
[b] Chemical maturity refers to the point at which the concentration of water, proteins and salts reaches a relative constant in all bodily organs (49).

to recycle nutrients and maximize nutrient absorption, it may have a dramatic impact on the results of a nutritional study.

Although no animal model will ever perfectly mimic the human condition, the pig has emerged as a superior non-primate experimental animal model because of its much closer resemblance to humans. This review highlights the similarities between pigs and humans and thereby the value of the porcine human nutrition model, and reviews some of the more recent applications of this model for nutritional research.

Comparative Gastrointestinal Tract Anatomy of Pigs and Humans

While externally there are many obvious morphological differences between humans and pigs, their internal anatomy and physiology is very similar (1, 14, 25–36). Table 1 (27, 41–49) provides a comparison of some biochemical and physical aspects of pigs and humans. For nutritional studies, the digestive system and its accompanying metabolic processes are the focus of concern when selecting an appropriate experimental research model. To this end, the porcine model is superior over other non-primate animal models because, despite some anatomic differences, the physiology of digestion and associated metabolic processes are very similar between humans and pigs (1, 26, 32, 37–38). Pigs are also the only widely utilized animal model that is truly omnivorous, and they have strikingly similar nutritional requirements to that of humans (37, 39–40).

The most apparent macroscopic difference between the human and porcine intestine is their lengths. The small intestine of adult pigs is around 15–22 meters, while the large intestine has an average length of 4–6 meters (Table 1) (27, 41–48). In contrast, the small intestine of a human adult averages around 5.5–7 meters, while the large intestine is around 1.5 meters (26, 44, 50–51). However, although the pig intestine is considerably longer than the human intestine, when one accounts for the much higher adult bodyweight of pigs (around 200–300 kg), and considers these values in terms of length per kilogram bodyweight, the end result is a value of around 0.1 meters of intestine per kilogram bodyweight for humans and pigs alike (44).

Anatomically, the gastrointestinal tract of pigs is similar to that of humans, although the division between the duodenum, jejunum, and ileum are not as distinct in the porcine small intestine as they are in humans (29, 50). In addition, the stomach of pigs has a muscular outpouching of uncertain function termed the torus pyloricus, in the pyloric region near the gastro-duodenal junction (29, 52–53). This outpouching is not present in the human stomach. However, by far the most distinct difference between humans and pigs is the spatial arrangement of the intestine within the abdominal cavity (Fig. 1), particularly that of the large intestine. The small intestine in humans is, for the most part, situated behind the large intestine in the abdominal cavity; whilst the small intestine of pigs is arranged in the right side of the abdomen. In humans, the large intestine is arranged in a square-like configuration. The ascending colon extends upwards from the ileo-cecal junction, where it turns left and becomes the transverse colon which stretches laterally from

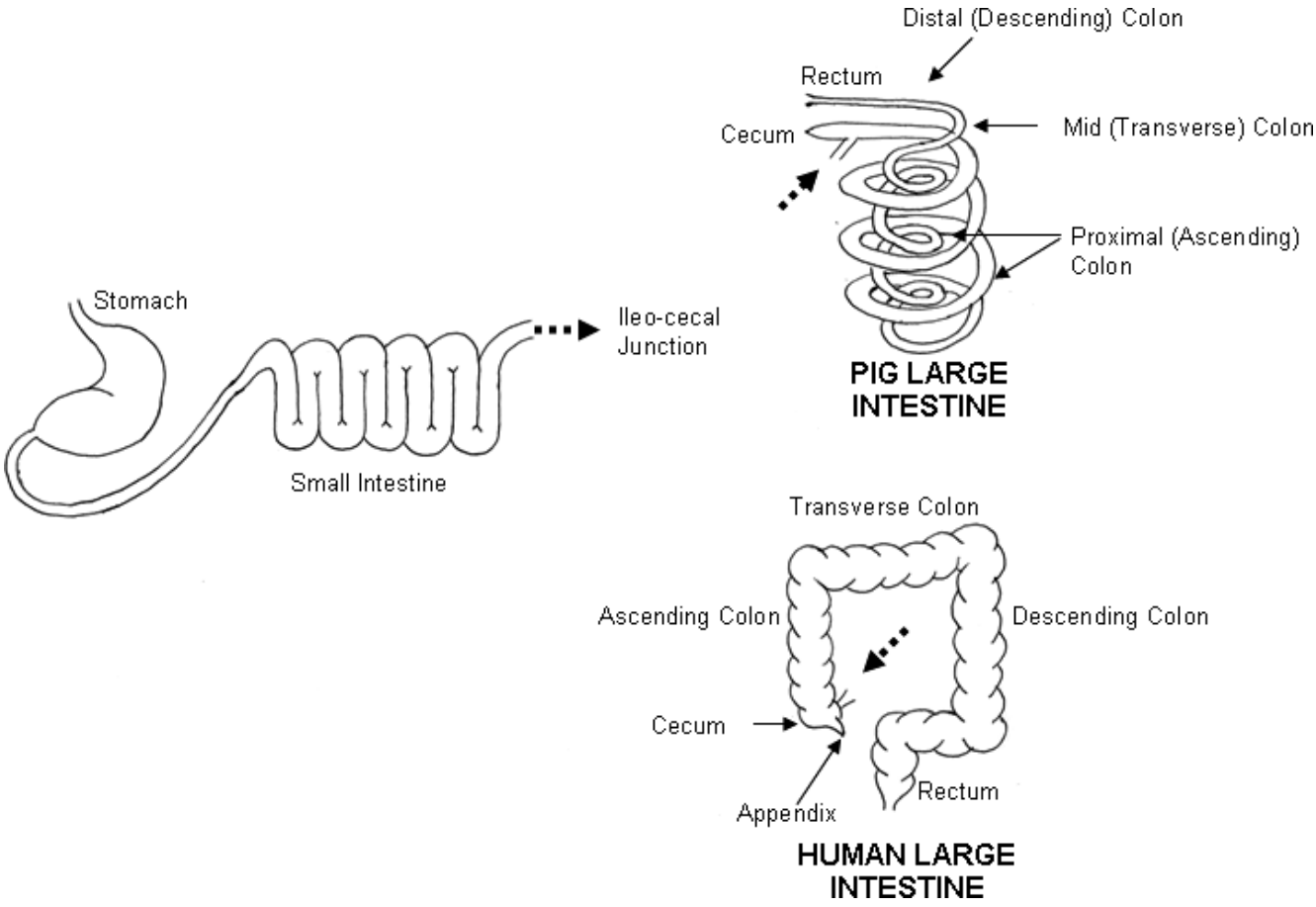

Figure 1. Comparative structure of the intestine of humans and pigs. The stomach and small intestine are very similar between humans and pigs; the large intestine is strikingly different in its conformation but not functionality.

right to left in the abdomen. Once there, it makes a downward turn to become the descending colon, which continues downwards and becomes S-shaped to form the sigmoid colon that lies posterior to the urinary bladder and empties into the rectum (26, 50). In contrast, the greater proportion of the pig large intestine, consisting of the cecum, proximal (ascending), mid (transverse), and the majority of the distal (descending) colon, is found in a spiral conformation beginning mid-abdomen and spiralling toward the left upper quadrant of the abdomen in a series of clockwise and anticlockwise coils (26, 29, 54). The remainder of the descending colon passes posteriorly along the left abdominal wall to the rectum. Incidentally, pigs also do not possess an appendix at the terminal end of the cecum (54).

In spite of these differences in absolute intestinal length and the internal spatial layout of the alimentary canal, the porcine digestive and metabolic processes function in much the same way as those of a human, and digesta transit times are also similar between the two species (26, 55). Microscopically, the intestinal villus structure and component epithelial cell types are also very alike (26, 55). This makes pigs an ideal model for human nutritional studies investigating the bioavailability and digestibility of various dietary factors in different gastrointestinal compartments.

Application of the Porcine Model in Nutritional Research

Pigs share many similarities with humans making them a valuable experimental model for a multitude of research applications, including dentistry, ophthalmology, the integumentary and renal systems, the cardiovascular system, and digestive anatomy and physiology. For nutritional research, the digestive system is of primary interest, and examination of this region in the pig model can provide insight into the mechanisms involved in human digestion and absorption processes. The pig model is particularly ideal for nutrient bioavailability and absorption studies because of the remarkable similarities between pigs and humans with respect to their nutritional requirements, as well as their digestive and metabolic processes (1, 37–38). Some pertinent research questions relating to mineral bioavailability that are currently being addressed using the porcine experimental model include: an examination of the absorptive capacity of the intestine with respect to different micronutrients, and the homeostatic controls that play a role

in their uptake; the effect of different dietary components on micronutrient uptake; and the bioavailability of iron and other minerals from foods. The subsequent section will review some of these studies.

A. Mineral Bioavailability Studies. Mineral absorption has been a primary concern of much nutritional research over the last few decades, with a lot of attention being paid to understanding the "normal" absorptive capacity of the intestine for different trace minerals, and the homeostatic controls that regulate their uptake. A variety of techniques have been developed for assessing nutrient retention and absorption, each with their own advantages and disadvantages. One of the simplest methods for indirectly measuring absorption of an ingested nutrient is to assess fecal and urinary excretion levels, and then differentially calculating the absorption/retention based on the dose ingested. Another option involves the use of radioisotopes. Whole body counting can then be performed to determine retention levels, while individual tissues can also be assayed to determine distribution patterns throughout the body. To avoid the hazards associated with the use of radioactive materials, the radioisotope could be replaced with a stable isotope. Isotope absorption can then be determined by measuring the changing isotopic ratios in tissue, blood, and/or urine against the more abundant, natural isotopic form (8). The availability of cannulation procedures also provides a means of assessing nutrient absorption on a compartmental basis. Other nutrient specific techniques may also be available, such as the hemoglobin repletion assay reported by Perks & Miller (40) which can be used to obtain a relative measure of iron absorption. Blood is sampled at the start of the feeding period for measurement of initial hemoglobin concentrations. The animals are then fed the experimental diets for a period of 2 to 5 weeks, after which a second blood sample is drawn for determination of final hemoglobin concentrations. Feed intakes are measured throughout this entire period to allow calculation of iron intake. Blood volume is estimated from body weight and the following formulas are used to calculate "Hemoglobin Repletion Efficiency" (HRE):

$$Hb\ Fe\,(mg) = [BW\ (kg)\ x\ \ 0.067\,L\,blood/kg\,BW]\ x \times [Hb\ (g/L\,blood)]\ x\ [3.35\ mg\,Fe/g\ Hb]$$

$$\mathrm{HRE} = \frac{Hb\ Fe,\ mg\ (final) - Hb\ Fe,\ mg\ (initial)}{Total\ Fe\ Intake,\ mg} \times 100$$

Where, Hb Fe = total body hemoglobin iron, and Hb = hemoglobin concentration in blood.

This technique is particularly useful in anemic pigs who would respond rapidly to iron increases in the diet (19, 40). Table 2 summarizes some of the more recent studies which investigated mineral absorption and retention using the porcine model.

Mineral Absorption/Retention. One of the most extensively studied minerals is calcium, owing to its vital role in bone health and cell function (21). Using flame atomic absorption spectrophotometry (AAS) to measure urinary and fecal output of calcium from a corn-soybean meal diet, Mroz *et al.* (56) demonstrated that apparent calcium absorption in the intestine of swine averages 40–45%, although the exact value can be influenced by diet composition, including phytate content, organic acid content, and buffering capacity. In a similar study, also utilizing flame AAS to measure urinary and fecal calcium output, Armstrong & Spears (57) found the apparent absorption of calcium in swine to be 51.8%. Similar calcium retention values in swine have also been reported by Veum *et al.* (58–59) and Pointillart *et al.* (60). The results of these studies fall within the calcium absorption range for women of 17–58% reported by Wolf and colleagues (61).

Along with calcium, zinc is another whose deficiency is emerging as a widespread problem (62–63). Owing to the important consequences of zinc deficiency on growth, immunity, and everyday metabolic and physiological processes, a lot of research is being directed toward alleviating this problem, and the porcine model is increasingly being utilized for this research. Poulson & Larsen (64) studied zinc absorption and retention in swine by measuring fecal and urinary output following consumption of increasing levels of dietary zinc. They found that increasing the zinc content of the diet from the natural level of 42 mg zinc per kg diet up to 162 mg per kg could increase zinc absorption from 19% to 22–26%. However, homeostatic mechanisms ensured excessive zinc absorption did not occur, as further increases in dietary zinc concentration beyond 162 mg per kg did not yield further increases in percentage absorption. A similar result was also noted by Carlson and colleagues (65) who fed increasing dietary levels (0, 125, 250, 375 or 500 ppm Zn) of a proprietary zinc polysaccharide (Sea-Questra Min Zinc, Quali Tech, MN) to swine, and compared the absorption rates to that of control pigs administered a corn-soybean meal diet supplemented with 165 ppm Zn as $ZnSO_4$. They discovered that zinc absorption in pigs administered the control diet (165 ppm) averaged 20.9%, while that of the zinc polysaccharide averaged 25.0% and 25.9%, at concentrations of 125 ppm and 250 ppm in the diet, respectively. No further increases in percentage absorption of this zinc polysaccharide were attained with further increases in concentration beyond 250 ppm in the diet; in fact, percentage absorption was seen to decrease at higher Zn-polysaccharide concentrations. The zinc absorption values reported in the literature using the pig model are remarkably similar to those reported in human studies, which generally average between 20–40%, depending on other diet components and the zinc status of the individual (66–67).

The use of the pig as an experimental model for measuring iron absorption and retention has only recently become common. Previously, rodent models have been utilized for such determinations, in spite of their limitations. Nevertheless, in a recent study, Zinn and colleagues (68)

utilized the piglet as a model for human infants to study the absorption of radioactive iron (^{59}Fe), administered as either elemental iron or ferrous sulfate, from a rice-meal cereal diet. The apparent absorption of elemental iron in the piglets averaged 13%; as expected, the absorption of ferrous sulfate was significantly higher than this, averaging 26%. In contrast to this result, Apgar & Kornegay (69) saw much lower iron absorption values in their pig study. Absorption values averaged between 5 and 9%, based on an analysis of fecal and urinary iron excretion, in pigs administered a corn-soybean meal diet supplemented with an excess level (350 ppm) of iron (iron source not given). It is worth noting, however, that in the study of Zinn iron injections at birth were withheld, which would have affected the iron status of the piglets during the study period. It would be expected that anemic and/or iron deficient animals would absorb more dietary iron, in an attempt to rectify their mineral deficiency, than would pigs with ample body stores of iron. Similar variations in iron absorption levels have also been observed in humans, and can be the result of a number of interactions, including iron status and diet composition.

Chemical Form/Fortificant. An understanding of the "normal" absorptive capacity of the intestine with respect to different minerals is vital to human nutrition, as is an appreciation of the changes in these absorptive functions in deficient states. The studies discussed in the previous section have provided a valuable contribution to this knowledge of human nutritional requirements. Many of these studies, in addition to others, have also highlighted the critical importance of the chemical form of fortificant utilized in the treatment of mineral deficiencies, as the bioavailability of these different chemical forms can be significantly different. For instance, Pointillart *et al.* (31) used a pig model to analyze bone calcium content so as to compare calcium bioavailability from milk and supplemental calcium salts. They detected significantly higher bone calcium content in pigs administered milk, as compared to the groups administered supplemental calcium salts, given as either calcium carbonate or calcium sulfate. These results suggest a significantly higher bioavailability of calcium from milk over calcium salt supplements, a result of importance for human nutrition.

In a different study of zinc supplementation, Cheng *et al.* (70) examined zinc levels in the liver, kidney, and ribs of pigs following administration of differing dietary zinc levels from different zinc sources, in conjunction with increasing dietary lysine concentrations. As expected, tissue zinc levels increased with the dietary zinc concentrations tested in their experiment. In addition, zinc concentrations were lower in the kidneys and ribs of pigs administered the higher dietary lysine levels. However, no significant impact of zinc source or lysine concentration on zinc absorption across the intestine was noted in this particular experiment.

In a study of iron bioavailability, Maekawa *et al.* (71) utilized the porcine model to compare the bioavailability of hydrogen-reduced (HR) elemental iron powder, added to bread either before or after baking. The change in hemoglobin levels over the 16-day treatment period was used to calculate the hemoglobin repletion efficiency (HRE) and relative biological value (RBV) of the two breads, as compared to $FeSO_4$ fortified bread whose RBV was set at 100%. The HRE was found to be 8.7 ± 3.0 and 7.5 ± 1.3 for the HR Fe added to bread before and after baking, respectively; the HRE of $FeSO_4$ fortified bread was found to be 18.7 ± 2.8. As mentioned in a previous section, the HRE gives an approximation of iron absorption. The RBV of the bread diets were 53.5% and 40.1% for bread with Fe added before and after baking, respectively ($P > 0.05$). These results suggested that baking does not improve the bioavailability of hydrogen-reduced elemental iron powders in unenriched, refined wheat flour; and that hydrogen-reduced elemental iron has a significantly lower bioavailability than $FeSO_4$, which is the current "gold standard" for human iron fortification.

Impact of Dietary Mineral Content on the Bioavailability of Other Minerals. Another factor that can significantly affect the bioavailability of specific minerals is the presence (or absence) of other minerals within the diet, and their abundance relative to the mineral of interest. In a recent study, Atkinson and colleagues (72) used a pig model to study the effect of a combination of calcium and phosphorus on zinc, copper, and iron absorption across the intestine. Piglets were fed a complete liquid diet supplemented with calcium and phosphorus, as well as one of the following: zinc alone; zinc plus copper; or a combination of zinc, copper, and iron. After a 5-day adaptation period, piglets were orally and intravenously dosed with a radioactive isotope mix containing zinc, manganese, iron, selenium, and calcium. Isotope levels in the body were measured after a 15-day period, during which time fecal excretion of any unabsorbed isotope was monitored. Dietary supplementation with calcium, phosphorus, and zinc reduced isotopic zinc uptake compared to control pigs. Increased intakes of a combination of calcium, phosphorus, zinc, and copper tended to reduce iron absorption as well. Unfortunately, due to the study design it was not possible to determine whether only one, or a combination of these elements, was causing these alterations in zinc and iron absorption. Nevertheless, this study demonstrated that the presence of certain minerals in the diet can impact upon the absorption of other minerals, although the exact mechanisms behind these interactions still remain to be clearly elucidated. Such a result has important implications for animal and human nutrition.

In contrast to this, Zinn and colleagues (68) studied the effect of dietary iron and zinc on the retention of radioisotopes of iron, zinc, copper, and calcium in young pigs. They found no observable effect of dietary iron or zinc on the retention of orally administered zinc, copper, or calcium. A similar result was observed by Apgar & Kornegay (69), who studied the impact of increasing copper levels, administered as either copper sulfate or a copper-

Table 2. Summary of Recent Mineral Absorption and Retention Studies that Utilized the Porcine Model [a]

Authors	Study design	Results
Apgar & Kornegay (69)	Examined total copper, zinc and iron retention in pigs administered increasing copper dosages as either copper sulfate or a copper-lysine complex by measuring fecal and urinary excretion by flame AAS.	Absolute copper absorption increased with the elevated dose; no significant difference between copper sources; no significant effect on zinc or iron retention.
Morais *et al.* (122)	Studied calcium, phosphorus, iron and zinc retention in the small intestine of pigs administered a diet supplemented with resistant starch. Iron and zinc radioisotope tracers from the digesta were detected using liquid scintillation. Calcium and phosphorus were detected by a colorimetric method.	43% increase in calcium absorption; 91% increase in iron absorption in resistant starch groups.
Perks & Miller (40)	Examined the impact of ascorbic acid on iron absorption from iron-fortified milk in anemic pigs measured through hemoglobin regeneration efficiencies.	No significant impact of ascorbic acid on iron absorption.
Pointillart *et al.* (76)	Studied the impact of ascorbic acid on calcium and phosphorus absorption and retention by measuring fecal and urinary output of the two minerals.	No significant changes.
Cheng *et al.* (70)	Examined zinc retention and utilization in pigs administered either zinc sulfate or a zinc lysine complex in combination with various lysine levels. Zinc levels in serum, liver, kidney and intestinal contents were analysed by AAS.	Zinc absorption increased with zinc dosage but not zinc source; absorption not significantly influenced by lysine level.
De Schrijver *et al.* (118)	Studied calcium, magnesium, zinc, and phosphorus retention following diet supplementation with resistant starch. Small intestinal contents, feces and urine were assayed by AAS.	No significant changes observed.
Houdijk *et al.* (121)	Studied the impact of non-digestible oligosaccharides on calcium, phosphorus, magnesium, iron, copper and zinc absorption in the intestine measured by AAS on small intestinal contents, feces and urine.	No significant changes.
Stahl *et al.* (82)	Examined the impact of increasing phytase concentrations on free phosphorus and iron concentrations of intestinal contents and hemoglobin concentration in anemic pigs.	Free phosphorus concentration increased in a dose-dependent manner; free iron only increased at the highest phytase level; 19–27% increase in hemoglobin concentration.
Zinn *et al.* (68)	Studied the effect of dietary iron (as either elemental iron or ferrous sulfate) and zinc on *in vivo* retention of radioisotopes of iron, zinc, copper and calcium measured using a germanium detector.	Ferrous sulfate absorption two-fold higher than that of elemental iron; no significant effect of elemental iron on calcium, zinc or copper retention.
South *et al.* (19)	Examined the impact of meat on nonheme iron absorption and compared heme and nonheme iron absorption in anemic pigs measured by hemoglobin regeneration efficiency.	Meat increased nonheme iron bioavailability by 95%; heme Fe no more bioavailable than nonheme Fe.
Rideout & Fan (123)	Studied the impact of inulin extract on crude protein, calcium and phosphorus absorption and retention. Fecal and urinary protein levels were measured by a combustion method. Calcium and phosphorus in the feces and urine were measured using AAS.	No significant effect on calcium or crude protein absorption or utilization; post absorptive phosphorus utilization enhanced.
Kammlott *et al.* (86)	Examined the effect of pancreatic duct ligation on sodium, potassium, chloride, copper, zinc, iron, manganese, calcium, magnesium and phosphorus digestibility measured in small intestinal contents and feces by AAS (Cu, Zn, Fe, Mn); spectrometry (Ca, Mg); photometry (P); spectroscopy (Na, K) and titration (Cl).	Decrease in sodium and potassium absorption (24 and 68%, respectively); reduced calcium and magnesium absorption (59 and 76%, respectively); increased phosphorus absorption (51%).

Table 2. Continued

Authors	Study design	Results
Maekawa *et al.* (71)	Examined the bioavailability of hydrogen-reduced (HR) elemental iron powder added to bread before or after baking compared to $FeSO_4$. The rate of increase in hemoglobin levels over the study period were used to calculate the relative biological value (RBV).	The RBV of the HR Fe bread diet was 53.5% and 40.1% when Fe was added before and after baking, respectively, compared to the FeSO4 diet (100% RBV).

[a] AAS, atomic absorption spectrophotometry.

lysine complex, on copper, iron, and zinc absorption. While copper absorption was seen to increase with increasing copper dose, irrespective of the copper source, no significant impact of copper on zinc or iron absorption was observed.

Impact of Other Dietary Constituents on Mineral Bioavailability. The porcine model has also been utilized to determine the impact of other dietary nutrients/factors on mineral absorption. In a recent study, South *et al.* (19) demonstrated that in iron deficient swine, meat consumption increased non-heme iron absorption, a result that concurred with previously obtained human bioavailability data (73–75). In a study of ascorbic acid supplementation, Pointillart *et al.* (76) concluded that it had no detectable impact upon calcium or phosphorus absorption measured by fecal and urinary output and bone mineral content. In a 10-day feeding trial of ascorbic acid supplementation, Perks & Miller (40) demonstrated that ascorbic acid had no effect on the bioavailability of iron from an iron-fortified milk product. This result was in agreement with a number of human studies of similar duration, but was in direct contrast to numerous single meal studies which concluded that ascorbic acid can promote iron bioavailability (77–81). This suggests that single meal studies may overestimate the impact of some dietary factors on mineral absorption, and therefore may not reflect true long-term bioavailabilities. Hence, there exists a need to confirm any bioavailability data obtained from short-term studies over a longer period of time, before extrapolation of data back to humans. The use of a porcine model would permit studies of longer duration and would overcome the difficulties in ascertaining compliance of human subjects over longer time periods.

B. Absorption Site. The aforementioned studies utilizing pig models have provided valuable insight into the retention of minerals, and the interaction between different dietary minerals and their subsequent absorption. In most cases, however, the methods adopted do not provide more specific information about the absorptive capacity of the different GIT compartments, particularly the different regions of the small intestine. One of the major advantages of using animal models is that they can provide access to the different GIT compartments, regions which are not as readily accessible in humans. Although nutrient absorption primarily occurs in the small intestine, absorption can also occur to a limited extent in the colon. Therefore, measurement of colonic absorption rates can also be of importance, particularly when diets are geared toward altering the large intestinal environment.

Table 3 summarizes some recent studies that have investigated nutrient absorption in the large intestine. To date, most studies investigating colonic absorption of nutrients have been conducted using *ex vivo* approaches, such as the Ussing chamber technique. However, the availability of cannulation procedures provides a useful means of monitoring gastrointestinal uptake of nutrients on a compartmental basis, and allows for greater control over nutrient levels reaching the different intestinal regions (30, 84–86).

The use of the pig model to address large intestinal absorption of minerals is yet to receive a lot of attention. It is generally assumed that absorption of certain nutrients (e.g. iron) occurs primarily in the duodenum, and this has certainly been shown to be the case for many nutrients. However, it may be likely that during times of nutrient limitation the large intestine makes a more significant contribution to nutrient absorption, as the body attempts to maximize recovery of limiting nutrients from the diet. By up-regulating colonic absorption of nutrients, the body can scavenge any nutrients that may have evaded absorption in the small intestine, and can further take advantage of any nutrients that may be liberated or generated by the microbiota of the large intestine. Studies are currently underway in our laboratory to determine the contribution of the colon to total iron absorption during anemia. The porcine model is particularly useful for these kinds of investigations as the iron status of pigs can be readily manipulated from birth, owing to the fact that they are born with very limited iron stores and are highly dependent on supplemental iron. Another advantage of this model, which is also being exploited, is the ability to surgically implant cannulas into the large intestine. This enables specific delivery of iron isotopes into the large intestine and thus a differential determination of small and large intestinal iron absorption in anemic individuals.

C. Regulation of Nutrient Absorption. While adverse consequences can arise when certain nutrients are limiting in the body, there can also be significant consequences associated with nutrient overload. For instance, iron deficiency has been associated with impaired

Table 3. Summary of Recent Absorption and Retention Studies that Examined Large Intestinal Uptake of Nutrients in the Porcine Model[a]

Authors	Study design	Results
Murray *et al.* (124)	Examined lactose absorption in different aged pigs using the *ex vivo* Ussing chamber method and varying lactose concentrations. The correlation between lactose absorption and sodium and chloride uptake was also examined.	5 day old animals absorbed 67% more lactose than 18 day old animals. Absorption of intact lactose increased with lactose concentration up to 240 mM. Lactose absorption was correlated with both sodium and chloride uptake.
Darragh *et al.* (125)	Studied lysine and methionine absorption across the proximal colon. Deficient pigs were administered the amino acid/s via a colonic catheter and urinary excretion measured.	No significant absorption across the proximal colon.
Murray *et al.* (126)	Examined colonic absorption of 3 glucose-containing (glucose, maltose and a glucose polymer) and 3 galactose-containing (lactose, lactulose and galactose) carbohydrates using the Ussing chamber method.	A 3–4 fold higher mucosal to serosal flux of galactose-containing carbohydrates than those containing glucose up to a concentration of 40 mM.
Kien *et al.* (30)	Studied colonic uptake of intact lactose using both unlabeled and radio-labeled lactose.	Demonstrated that *in vivo* colonic absorption of intact lactose does occur.
Cheng *et al.* (70)	Examined apparent zinc absorption in pigs administered either zinc sulfate or a zinc lysine complex at 100 ppm in combination with various lysine levels. Zinc levels in stomach, small and large intestinal digesta were determined by AAS and used to calculate apparent absorption coefficients using the indicator method.	Zinc absorption in the large intestine averaged 26% with the different zinc supplemented diets, but was significantly higher ($P < 0.001$) at 35% in pigs fed the control diet with no added zinc.
Liu *et al.* (127)	Studied apparent calcium and phosphorus absorption in the small and large intestine by measuring digesta mineral concentrations and the impact of the Ca:P ratio in the diet. Control animals were administered a calcium and phosphorus adequate diet (0.6% and 0.5%, respectively) while treated animals were administered deficient diets containing 0.3% phosphorus, and calcium at a ratio to phosphorus of 1.5, 1.3 or 1:1. All deficient diets were supplemented with 500 units of phytase.	Lowering the Ca:P ratio caused an increase in apparent P absorption in the small intestine but not the cecum or colon, with no significant effect on apparent Ca absorption. Averaged across all diets, the apparent absorption of P was highest when measured at the cecum, and the apparent absorption of Ca was highest when measured at the colon.
Asrar & O'Connor (14)	Examined absorption of radio-labeled folic acid and para-aminobenzoic acid across the large intestine by measuring fecal and urinary excretion of radioisotope following surgical injection of tracer doses into the cecum of anesthetized piglets.	Large intestinal absorption was estimated to be around 18%. The authors also suggested that 18% of the daily requirement for folate in piglets could be met through colonic absorption.

[a] AAS, atomic absorption spectrophotometry.

physical work performance, cognitive impairment, adverse pregnancy outcomes, and irreversible developmental delays in infants and toddlers (87). In contrast, excess iron can be lethal because it can catalyze the formation of free radicals through the fenton reaction, leading to a cascade of deleterious outcomes for the host (88–90). To prevent the adverse effects associated with nutrient limitation or overload, the body must tightly regulate absorption and excretion processes. One way of controlling nutrient status, so as to prevent deficiency or overload, is to tightly regulate absorption rather than rely on excretion processes to eliminate excess nutrients. Certainly in the case of iron, as well as some other minerals, excretion is very limited, and therefore iron status must be closely regulated at the site of absorption (6). The most economical way to modulate absorption is through substrate dependent regulation of nutrient transporter protein levels on the cell membrane and within the cytoplasm. A reduction in nutrient transporter levels therefore leads to a reduced absorption, and has the added benefit of improving cell efficiency, as any surplus protein would monopolize valuable energy and space (91). During times of nutrient limitation, the appropriate transport proteins are up-regulated, so as to enable effective nutrient scavenging from the depleted environment.

While the regulation of nutrient absorption in this manner sounds quite simple, in reality this is a very complex

process, and there are many different pathways and intermediate products involved in this regulation. Recent investigations using murine and rat models have begun to shed light on the complex processes involved in these regulatory pathways for various minerals, including calcium, iron, and zinc; as well as the various interactions these pathways have with other bodily processes, including the immune system. The ease of inducing gene knockouts and nutrient disorders in these particular animal models, as compared to the porcine model, is a significant factor to consider when performing pioneer studies of this type. However, in light of the limitations of murine and rodent animal models it is important to confirm any findings with a more relevant model such as pigs to ensure those regulatory pathways, and their agonists and/or antagonists, are maintained between models. This can be of particular importance when examining expression of genes coding for nutrient transporters present on intestinal enterocytes, such as the divalent metal transporter, DMT-1, that is involved in transport of iron and other divalent metals. To compare the expression of the DMT-1 gene in different GIT compartments, or to measure functional protein levels, it may be necessary to isolate intestinal enterocytes from these regions. This can be a complicated process if using human subjects, and therefore the use of a porcine model in place of a human model for these analyses can supplement the evidence obtained using rat and mouse models.

Until now, the pig model has not been utilized to a great extent for studying nutrient regulatory pathways, as many of these have only recently been elucidated using rodent and/or murine models. Current studies are under way in our laboratories using a porcine anemia model to compare iron regulatory pathways that have recently been identified in mice and rats, and to assess their corresponding function during iron limitation in pigs.

D. Prebiotics, Probiotics and the Enteric Microbiota. An important and often overlooked effector of nutrient bioavailability is the enteric microbiota. Intestinal bacteria possess specific nutrient requirements for growth and largely meet these needs through metabolism of host diet components and exploitation of host resources. Whilst the maintenance of what is considered a beneficial balance of enteric microbes provides crucial protection against pathogen incursions, and thereby maintenance of intestinal health, the balance of enteric bacteria can also potentially affect nutrient bioavailability. For instance, most pathogenic bacteria are highly dependent on iron for survival, and the host immune system takes advantage of this as a means of combating infection. By sequestering iron away from the pathogens their growth can be impeded, allowing other components of the immune system to overwhelm them (92–93). Unfortunately, this critical immune response can also have adverse implications for the host in times of chronic infection and lead to what is termed the ''anemia of inflammation'', as iron availability to the host is also limited (94–95). While a large number of GIT pathogens are exogenous to the intestine, it is important to recognize that some are actually endemic to the GIT in limited numbers. These opportunistic pathogens are generally kept in check by the host immune system and by other resident commensal bacterial populations; that is until a situation arises (e.g. antibiotic administration; considerable change in diet) which provides them the opportunity to overwhelm this protection and proliferate to greater numbers, thereby causing infection. These pathogenic populations have an intrinsic iron requirement which is obtained either from the host diet or from host cells. In contrast, non-pathogenic microbes, such as lactobacilli and bifidobacteria, tend to have low iron requirements (96–97). For that reason, modulation of the enteric microbiota can potentially have a significant impact on mineral bioavailability to the host. For instance, a microflora with high proportions of lactobacilli and bifidobacteria should have a lower iron requirement, and this may lead to more available iron for the host.

Up to this point, the enteric microflora has not received significant attention relating to its impact on nutrient bioavailability, particularly with respect to minerals such as iron, which are believed to be primarily absorbed in the upper small intestine. However, the small intestine also possesses a commensal microflora, albeit in much smaller numbers than that residing in the large intestine. Nevertheless, it may be likely that this microflora plays an important role in nutrient bioavailability, and the promotion of a beneficial microflora may provide a valuable treatment option for many micronutrient deficiencies, either alone or in conjunction with a supplementation regime. Prebiotics and probiotics represent such a way of modulating the enteric microbiota.

Probiotics are defined as: ''Live microorganisms, which when administered in adequate amounts confer a health benefit on the host''; while a prebiotic is: ''A nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria that can improve host health'' (98–99). These dietary supplements are currently being advocated as a therapeutic/preventative measure for many intestinal and extra intestinal diseases and disorders including inflammatory bowel disease, diarrhea and metabolic syndrome, but may also have applications in mineral nutrition (20, 100–102). Prebiotics in particular perform a dual role in the gastrointestinal tract. These carbohydrates resist digestion by pancreatic and salivary amylases in the small intestine, thereby minimizing postprandial glucose availability and providing relief from metabolic syndrome (or insulin resistance) and other related extra-intestinal disorders, including type II diabetes mellitus and cardiovascular disease (5, 7, 103). They also fulfil a secondary function in the large intestine, as the undigested material accumulates in this region and promotes a favorable enteric microbiota through the promotion of ''friendly'' bacterial populations such as bifidobacteria and lactobacilli, at the

expense of pathogenic or opportunistic populations such as clostridia, enterobacteria and proteolytic bacteroides species (104–107). Another benefit derived from the beneficial modulation of the colonic flora is increased production of short chain fatty acids (SCFA), particularly butyrate, which is an important energy source for colonocytes (22, 108–109). Some better known prebiotics include inulin, fructose oligosaccharides (FOS), and galactose oligosaccharides (GOS).

As a consequence of their multi-faceted role in the gastrointestinal tract, prebiotics can potentially affect mineral bioavailability by a variety of mechanisms. This can include a reduction in intestinal pH through promoting the production of SCFA, which can in turn increase mineral solubility; the promotion of a reductive environment in the intestine, which may prevent mineral loss due to precipitate formation; promotion of epithelial cell proliferation in response to SCFA, thereby increasing the available surface area for mineral absorption; or by affecting the expression of mineral transport proteins or regulatory genes involved in the absorptive process (110–112). A prebiotic effect on mineral availability may also arise through modulation of the enteric microbiota. Each microbial population has differing requirements for specific minerals and the provision of these nutrients in the intestinal lumen can potentially be influenced by changing enteric microbial population dynamics. Currently, there is much research being directed toward understanding the mechanisms by which prebiotics can influence mineral availability (113–115).

Several studies utilizing rat models have demonstrated that prebiotic supplementation can influence mineral bioavailability (113, 116–119). At present, however, there is limited data on mineral availability in pigs arising from prebiotic supplementation, in spite of the abundance of literature pertaining to the impact of prebiotics on the porcine enteric microflora and intestinal environment (SCFA production etc). In one recent study, Yasuda *et al*. (120) showed that supplementation of a corn-soybean meal diet with 4% dietary inulin increased iron bioavailability to pigs measured by the hemoglobin repletion efficiency assay. In another study by Houdijk and colleagues (121), no effect of FOS or TOS on absorption of calcium, magnesium, iron, phosphorus, copper, or zinc in weaner and grower pigs was found. In contrast, the infant pig model adopted by Morais *et al*. (122) showed a promotion of intestinal calcium and iron absorption in response to dietary supplementation with resistant starch.

Evidently, more research utilizing the pig model is required to elucidate the impact of dietary supplements such as prebiotics on mineral bioavailability. The use of the porcine model for these studies is of particular importance because of the similarity between their enteric microbiota and that of humans, in addition to the other various similarities presented earlier. While it is possible to utilize a rodent model with a human associated enteric flora, the many variances between human and rodent digestive physiology and function still significantly limit the conclusions which can be made from such studies and their applicability to humans.

Limitations of the Porcine Model

Inasmuch as the porcine model bears some remarkable similarities with humans, it is important to recognize that there are some differences between the two species which may lead to a differing response to certain experimental regimes. Although the physiology of digestion and associated metabolic processes are alike between pigs and humans, it should be recognized that the absolute length and weight of the intestine does differ between the two (Table 1) (1, 26, 32, 37, 40). At maturity, the length of the pig intestine is fourfold greater than that of humans. This comparison holds true when the individual lengths of the small and large intestine are also contrasted between pigs and humans. A consequence of this difference in intestinal length is a corresponding difference in intestinal weight. The overall weight of the pig intestine is around 2.5 times greater than that of humans. On a compartmental basis, the small intestine of pigs is twofold heavier, while the large intestine is three to fourfold heavier than that of humans. Although the intestinal transit time of pigs is similar to that of humans, it cannot be completely ruled out that the described differences in intestinal length and weight do not impart some effect on experimental determinations, leading to a divergence in the response of pigs to that of humans.

As is the case in humans, body fat content and distribution in pigs varies markedly depending on age, energy balance, and genotype (128–129). Newborn humans have much higher levels of body fat (16%) compared to pigs (1%) (130). Body fat peaks at about 26% at 4 months in humans, and then gradually declines to 18% at 36 months (131). Pigs become severely obese when given *ad libitum* access to feed, and pigs fed high fat diets have higher body fat content than pigs fed low fat diets (132). There is little evidence that body fat content affects nutrient absorption in the intestine. However, Bekri and colleagues (133) reported a high prevalence of anemia in severely obese human patients. They showed elevated expression of hepcidin (both mRNA and protein) in the liver and adipose tissue of these patients, and suggested that this may be due to the chronic inflammation that is common in obese subjects. Hepcidin is known to inhibit iron absorption by blocking the basolateral export of iron from enterocytes (134). Therefore, it is possible that differences in body fat content between pigs and humans could translate into differences in nutrient absorption but this seems unlikely except in cases of severe obesity.

Incidentally, pigs have also been known to practice coprophagy which can be another confounding experimental factor. Albeit this practice is quite rare in pigs, as compared to rats that frequently practice coprophagy.

Summary

Clearly, there are many similarities between pigs and humans that make swine a valuable experimental model system for investigating a variety of scientific parameters. Of particular importance to the field of nutrition, is that despite some anatomic differences, the physiology of digestion and associated metabolic processes are analogous between humans and pigs. With the current surge in availability and consumption of dietary supplements designed to prevent, or provide relief from, many intestinal and extra-intestinal disorders it is vital to understand the impact of these products on the gastrointestinal environment and its related functionalities. The porcine model can be utilized for this purpose, not only to provide access to gastrointestinal compartments that are not as readily accessible in humans, but to also by-pass problems associated with obtaining willing human participants and ensuring their compliance over long-term studies.

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