Gastric emptying and plasma glucose response in men following ingestion of milk from different species

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Nutritional Science at Massey University, Palmerston North, New Zealand

Nicholas John Schnell

2005

Abstract

The ^{13}C Octanoic acid breath test (OABT) was used to measure the rate of gastric emptying of whole goat's milk (WG), whole cow's milk (WC), goat's milk infant formula (GIF) and cow's milk infant formula (CIF) in healthy, adult men.

Prior to the gastric emptying study, the integrity of the vacuum in two commonly used gas collection tubes was tested. The experiment showed that the Exetainer® brand of tube was more suitable for collecting expired air compared to the Vacutainer® brand based on the fact that it had less residual dead-space which could dilute expired air samples.

Fifteen healthy men were given one of the four test milks containing 100µg ^{13}C octanoic acid after an overnight fast. Breath samples were collected at regular intervals for four hours. Following analysis by ratio isotope mass spectrometry, gastric emptying parameters were calculated.

The gastric emptying half time ($t_{1/2}$) of CIF was significantly shorter ($P<0.05$) than that of GIF (120 min vs. 159 min), but there were no differences in the rate of emptying between WC (141 min) and WG (150 min). There were no significant differences between either of the infant formulas and the whole milks.

Blood samples were taken concurrently with the expired air samples. The samples were analysed to determine plasma glucose concentration. The results of showed that the timing of the peaks of plasma glucose levels and subsequent drop to below baseline concentration may be associated with the rate of gastric emptying.

The manner in which the four test milks coagulated was also tested. Milks were incubated *in vitro* at 37.5°C after acidification with 1 molar HCl (to gastric pH 3) and addition of the enzyme pepsin. Vastly different coagulation properties were observed. The WC formed large curds with a clear separation between the whey-containing liquid and the curd whereas the WG and GIF were more homogenous with finer curds and considerably less clear fluid. The CIF exhibited very fine curds.

Differences in composition between whole goat's milk and whole cow's milk did not appear to be sufficient to elicit different rates of gastric emptying. Thus any nutritional differences between milk from the two species may not be related to the rate at which they are emptied from the stomach.

Acknowledgements

I am extremely appreciative of my two supervisors, Dr. Alison Darragh, and Dr. Roger Lentle. They were a constant source of guidance, inspiration, motivation, and not to mention, enthusiasm. Thank you for stretching my brain, and for making it an enjoyable and rewarding experience.

I am indebted to my wife, Paulina, who supported me financially and emotionally through this endeavour. I hope that what the thesis will bring me in the future will be worth the effort it took enduring weekends spent at home instead of in the mountains; I will be forever grateful.

Chris Booth played a large role in setting up, and helping me with the trial, thank you. Thank you to Mirian Hendriks who in addition to being an excellent nurse to the subjects throughout the trial, also made the trial fun.

This trial would not have been possible without the subjects who volunteered to take part, I am sincerely grateful to them.

This work was funded by the Foundation for Research in Science and Technology and Dairy Goat Co-operative (N.Z.) Ltd.

This trial was approved by the Massey University Human Ethics Committee (Protocol No. 04/19).

Table of Contents

List of Tables

List of Figures

Chapter 1

General Introduction

Eating is one of life's great pleasures, yet it seems that we do not have enough time to devote to it anymore. Convenience foods are becoming more and more popular and people are eating more meals in front of the computer or in the car; taking the opportunity to eat when it presents itself. In essence, not much has changed since the earlier days of our existence in that eating also occurred in an opportunistic fashion i.e., when food was available. We are therefore perfectly adapted to this opportunistic/occasional way of eating. We have evolved with a stomach that can store our meals, and then gradually progress them through our digestive system, without us even being aware of the process.

The stomach is more than a simple storage reservoir. In addition to physically grinding up ingested food into particles small enough to be broken down by chemical means, the stomach carefully regulates the flow of food entering the small intestine. The time that it takes for food to be digested and subsequently absorbed is much greater than the time that it takes to ingest the meal. The rate at which nutrients can be absorbed from the intestine is limited, and before absorption can take place, polymeric food components such as proteins have to be enzymatically broken down to their component monomers. The stomach thus accommodates the meal, and then gradually allows the ingested food to pass distally to the small intestine. In light of the physiological factors that are known to regulate gastric emptying, it is tempting to assume that this occurs at a rate that is optimal for digestion of food and absorption of nutrients.

A range of factors are known to influence the rate of gastric emptying, but essentially the rate that food is passed into the small intestine is in part governed both by physical factors relating to the meal and the nutrient content of the meal. The physical factors include volume, temperature and size of the food particles. Large food particles such as chewed fragments of meat must first be reduced in size before they can pass from the stomach into the duodenum (which takes time), whilst smaller food particles such as those found in fruit juice require little reduction in size and can pass directly through. Receptors in the small intestine respond to the chemical composition of food; information on the nutrient content of foods in the small intestine is fed back to

the stomach through neural or hormonal responses, which then increase or decrease the rate at which nutrients are emptied into the small intestine (Buchan, 1999; Furness *et al.* 1999).

Whilst much work has been done on the rate of gastric emptying of solid materials and the gastric emptying of liquids has also been described, little work has been conducted on the gastric emptying of emulsions such as milk. Cow's milk comprises about 14% solids suspended in an aqueous fluid (Jenness, 1988). Milk contains proteins, carbohydrates and fats, which are all known to inhibit gastric emptying through negative feedback mechanisms (Guyton & Hall, 2001; Westphal, 2004). Further, when in the acidic environment of the stomach, certain milk proteins are precipitated, thus altering the physical characteristics of the food and causing changes in gastric emptying rate (Fox *et al.,* 2004; Hall *et al.;* 2003; Miller *et al.*1990).

Milk is designed to support life and sustain rapid growth of neonate mammals in the early stages of life. The milk composition of each mammalian species is highly variable and is assumed to best suit the needs of the particular neonate. Thus, the composition of milk across different species contains different proportions of proteins, carbohydrates and fats as shown in Table 1A.

Table 1A. *Composition of the milk from different mammalian species (per 100g liquid milk).*

Mammal	Protein (g)	Fat (g)	Carbohydrate (g)	Energy (kcal)
Cow	3.2	3.7	4.6	66
Human	1.1	4.2	7	72
Water Buffalo	4.1	9	4.8	118
Goat	2.9	3.8	4.7	67
Donkey	1.9	0.6	6.1	38
Elephant	4	5	5.3	85
Monkey, rhesus	1.6	4	7	73
Mouse	9	13.1	3	171
Whale	10.9	42.3	1.3	443
Seal	10.2	49.4	0.1	502

(Taken from: Webb et al., 1974)

Whales and seals live in extremely cold environments, and thus require a substantial amount of insulation (in the form of blubber or fat). Accordingly, their milk has a very high fat content in order to provide the whale calf or seal pup with the necessary substrate for blubber formation (MarineBio.org, 2005). Fat is higher in energy

compared to protein and carbohydrate (Rolls, 2000; Southgate & Durinin, 1970) and a single feeding of milk with more fat would therefore contain a higher energy content. Therefore, a diet with a very high fat content, and thus, a very dense energy level, will also support the seal pups while they are left alone on beaches or rocks for extended periods of time while their mother go out hunting for fish.

In the wild, bovine calves suckle from the cow, and then lie in hiding while the cow grazes for extended periods. The digestion of casein in cow's milk by the calf is linked to the action of the enzyme rennet, which promotes the formation of an irreversible curd that is slow to breakdown. The whey portion of the cow's milk empties with the aqueous phase of the milk while the curd can more slowly be released into the small bowel where it is rapidly digested (Miller *et al.*, 1990). Effectively, the components of the each milk feeding are passed on to the lower regions of the digestive tract over an extended period of time, providing sustenance for the calf in the time spent away from its mother.

The composition of human and primate milk is similar (Table 1A). Neonates of these species are kept close to their mothers, and therefore feed more frequently than some of the other mammals. It is therefore reasonable to expect their milks to have a relatively faster rate of gastric emptying compared to the high fat milk consumed by the seal pup or whale.

Humans are the only species in the animal kingdom to willingly and constantly drink milk from other species. We have known for centuries that milk from other species such as the cow, buffalo, goat and sheep is a nutritious and practical food, and today dairy foods are advocated as an important part of a balanced diet. Whilst human breast milk is best for the human infant, milk from other species is also a convenient alternative to breast milk for mothers who cannot or choose not to breastfeed. Thus an advanced industry has developed around the production of commercial infant formulas that are based on milk from other species.

Cow's milk is one of the most widely consumed milks in the world (Park & Haenlein, 2006). Goat's milk is also used as an alternative to cow's milk in infant formula. There are differences between the composition of whole goats' milk and whole cow's milk that could elicit different rates of gastric emptying and therefore impact on an infant's health and wellbeing.

This thesis describes a research programme specifically designed to compare the rates of gastric emptying of whole goats' milk and whole cow's milk as well as a goat's milk infant formula and a cow's milk infant formula

References

Buchan, A. M. J. (1999). Nutrient tasting and signalling mechanisms in the gut III, Endocrine cell recognition of luminal nutrients. *American Journal of Physiology*. 277(40): G1103-G1107.

Fox, M., Goergi, G., Boehm, G., Menne, D., Fried, M. & Thumshirn, M. (2004). Dietary protein precipitation properties have effects on gastric emptying in healthy volunteers. *Clinical Nutrition*. 23: 641-646

Furness, J. B., Kunze, W. A. A. & Clerc, N. (1999). Nutrient tasting and signalling mechanisms in the gut II. The intestine as a sensory organ: neural, endocrine, and immune responses. *American Journal of Physiology*. 277(40): G922-G928

Guyton, A. C. & Hall, J. E. (2001). *Medical physiology* (10th Ed.). London: W. B. Saunders Company. pp. 728-737

Hall, W.L., Millward, D.J., Long, S.J., & Morgan, L.M. (2003). Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *The British Journal of Nutrition*. 89 (2): 239-248

Jenness, R. Composition of Milk. In: Wong P. (Editor). (1988) *Fundamentals of Dairy Chemistry 3rd Edition*. Gaithersburg. Aspen Publishers, Inc. pp. 231

MarineBio.org. Orca (Killer Whale) – Orcinus orca. Retrieved Sunday, November 27, 2005 from: http://marinebio.org/species.asp?id=84

Miller, M.J., Witherly, S.A. & Clark, D.A. (1990). Casein: a milk protein with diverse biologic consequences. *Proceedings of the Society for Experimental Biology and Medicine*. 195: 143-159

Park, Y.W. & Haenlein, G.F.W. (2006). Handbook of Milk of Non-Bovine Mammals. Ames: Blackwell Publishing. pp. 1

Rolls, B. (2000). The role of energy density in the over consumption of fat. *Journal of Nutrition*. 130: 268S-271S

Southgate, D.A. & Durnin, J.V. (1970). Calorie conversion factors. An experimental reassessment of the factors used in the calculation of the energy value of human diets. *British Journal of Nutrition*. 24(2): 517-535.

Webb, B.H., A.H. Johnson and J.A. Alford. 1974. Fundamentals of Dairy Chemistry. Second Ed. AVI Publishing Co., Westport, CT., Chap. 1. pp. 17

Westphal, S. , Kastner, S. , Taneva, E. , Leodolter, A. , Dierkes, J. & Luley, C. (2004). Postprandial lipid and carbohydrate responses after the ingestion of a casein-enriched mixed meal. *American Journal of Clinical Nutrition*. 80: 284-290.

Chapter 2

Review of the literature relating to gastric emptying

2.1 Introduction

Gastric emptying is an outcome of the interaction of a number of physiological processes. Different foods engender significantly different rates of gastric emptying according to their macronutrient composition. The stomach appears to allow meals to pass into the small intestine at a rate that is optimum for absorption and digestion of nutrients (Read & Houghton, 1989; Brooks, 1985; Minami & McCallum, 1984). Gastric emptying also has an impact on the availability of nutrients for metabolism. For example, different foods have different glycemic indices (Brand-Miller *et al.*, 2002) i.e. foods with the same load of available carbohydrate result in different glycemic responses. There are a number of physiological systems that regulate gastric emptying; these systems are designed to smooth the delivery of nutrients to the duodenum.

Ingested food moves promptly through the oesophagus to the stomach but exits from there less promptly. The stomach thus functions as a holding reservoir for food before it transits to the small intestine for digestion and subsequent absorption of the digestive products. The stomach increases in volume to accommodate the ingested food (gastric accommodation).

The stomach also acts as a processor of food, breaking the food bolus down into particles small enough to pass through the pylorus to the small intestine at the proximal end of the stomach. This is achieved through physical action of the muscular walls as well as by chemical degradation from the action of hydrochloric acid and various gastric enzymes. The small intestine is the main site of enzymatic digestion and absorption in most vertebrates. The absorptive efficiency of the small intestine (made up of the duodenum, jejunum and ileum) depends on the appropriate rate of delivery of nutrients by the stomach. The functions of the compartments of the digestive system are therefore highly connected through negative feedback mechanisms to regulate rate of passage of foods from the stomach to the duodenum (the proximal end of the small intestine) (Guyton & Hall, 2001).

The rate of appearance of absorbed nutrients in the blood is thus related to the rate of gastric emptying. This link has important implications for human health. In diabetes mellitus the rate of disappearance of glucose from the blood following ingestion of a glucose load is central to diagnosis of the disease. The appearance rate of certain metabolites in the blood following the ingestion of a meal is also related to the speed of onset of satiety. This, in turn, has implications for the control of body weight in adults.

This review of literature will cover the physiology of gastric emptying so as to give an understanding of the many factors that may influence the speed of gastric emptying following a meal. The gastric emptying of a number of different foods will then be described. As with most aspects of nutritional physiology, the measurement of gastric emptying is a challenge as the stomach is an organ that is enclosed within the abdomen and thus not easily accessible. A number of techniques that have been used to assess gastric emptying over the years will be reviewed as well as the more recent technique used in this study. Finally, the compositional differences between the two types of milk, cow's and goats' milk, will be discussed with the aim of gaining an appreciation of the impact these two milks may have on the rate of gastric emptying in consumers, e.g. infants.

2.2 Anatomy and Histology of the stomach

In man, the stomach is a sac-like and somewhat bean-shaped organ that can be divided into three main functional units, the proximal stomach, the distal stomach, and the pylorus (West, 1990). Anatomically, the proximal stomach comprises the fundus, and a portion of the corpus (or body) (Figure 2A). The distal stomach comprises the distal half of the corpus and the antrum (Figure 2A). The pyloric sphincter comprising a distinct thickening of the circular layer of muscle is the "gatekeeper" of the stomach through which gastric chyme (semi fluid mixture of food with gastric secretion) passes to the small intestine (West, 1990).

Food enters the stomach from the esophagus through the esophageal sphincter at the proximal end of corpus in the erect position. The fundus is situated with its upper limit lying above and to left of the esophageal sphincter. Its circumferential contractions act to propel the fluid phase distally. The main storage region is the corpus, which leads to the more distal antrum that is responsible for most of the grinding or trituration. After trituration, the gastric chyme passes distally from the antrum through the pyloric sphincter into the duodenum.

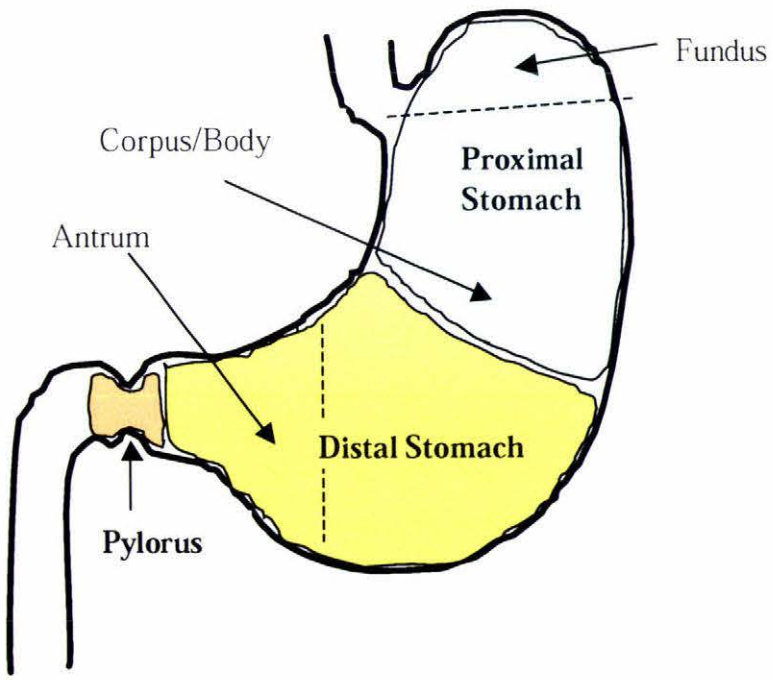


Figure 2A. Functional and anatomical regions of the stomach.

The stomach is lined with mucosal folds called rugae. The stomach wall comprises four tissue layers, as does the remainder of the gastro-intestinal tract. From outer most to inner most these layers are: the serosa, the muscular layer, the submucosa, and the mucosa (Guyton & Hall, 2001). The gastric mucosa comprises a layer of simple columnar epithelium that has two types of tubular glands: oxyntic (or gastric) glands and pyloric glands. The oxyntic glands secrete hydrochloric acid, pepsinogen, intrinsic factor, and mucus. The pyloric glands secrete mainly mucus for protection of the pyloric mucosa but also gastrin and some pepsinogen (Guyton & Hall, 2001).

2.3 Gastric Emptying

The emptying of meals from the stomach involves interplay between the three main functional units of the stomach; the proximal stomach, the distal stomach and the pylorus. The proximal and distal stomach force food towards the pylorus through increased muscular tone or contraction and the pylorus is regulated so as to resist the through flow of digesta to a certain degree.

When a meal is ingested, the tone of the muscular walls of the proximal stomach is reduced allowing the stomach to accommodate greater volumes of food (Guyton & Hall, 2001; West, 1990). This process is called gastric accommodation, and will be discussed in a later section. Following the cessation of eating, muscular tone is gradually restored, forcing food into the distal stomach (West, 1990).

The muscle of the distal stomach exhibits rhythmic peristalsis; ring contractions of the circular layer of muscle develop near the middle of the gastric body and sweep toward the pylorus (West, 1990). The contractions move with increasing force and acceleration, propelling the gastric contents toward the pylorus.

The outflow of gastric chyme from the stomach is tightly regulated. Chyme flows out of the distal opening of the stomach, the muscular pylorus (pyloric sphincter), which is linked to the peristaltic motions of the distal stomach (West, 1990). The operation of the pylorus in cooperation with antral peristalsis is thus the mechanism through which most regulation of gastric emptying occurs. As peristaltic contractions move towards the pylorus, it contracts and seems to remain slightly tonically contracted until the next wave begins (West, 1990), allowing only water and other fluids to empty from the stomach with ease. This contraction prevents passage of larger food particles until they have been reduced in size sufficiently to be suspended in the chyme.

Thus, the rate of gastric emptying depends on the rate that proximal stomach tone is restored, the contractions of the muscle of the distal stomach, and the degree of functioning of the pyloric sphincter.

There are essentially two main controls for the rate of gastric emptying, neural and hormonal (Figure 2B). The pylorus is under neural and humoral control from both the stomach and duodenum.

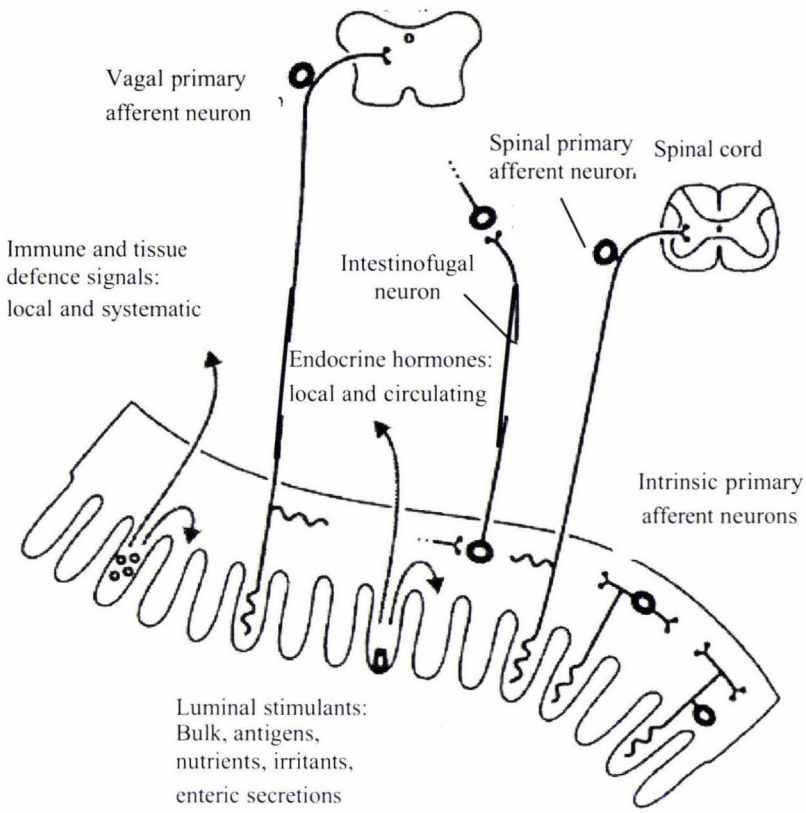


Figure 2B. Neural and hormonal signalling from the gut. Hormones are released from cells in the mucosal epithelium. They can enter the circulation and thus act at remote sites (e.g. stomach) but can also act locally (e.g. nerve endings). The neural sensations are conveyed by extrinsic and intrinsic primary afferent neurons, which connect the gut to the higher centres of control such as the brain and spinal cord (taken from Furness *et al.*, 1999).

2.3.1 Neural Regulation of Gastric Emptying

Gastric emptying is negatively regulated by neural feedback mechanisms that sense changes in the stomach and duodenal digestive environment and adjust the rate of gastric emptying accordingly. Figure 2C illustrates the four hierarchical systems of control that interact to provide this regulation.

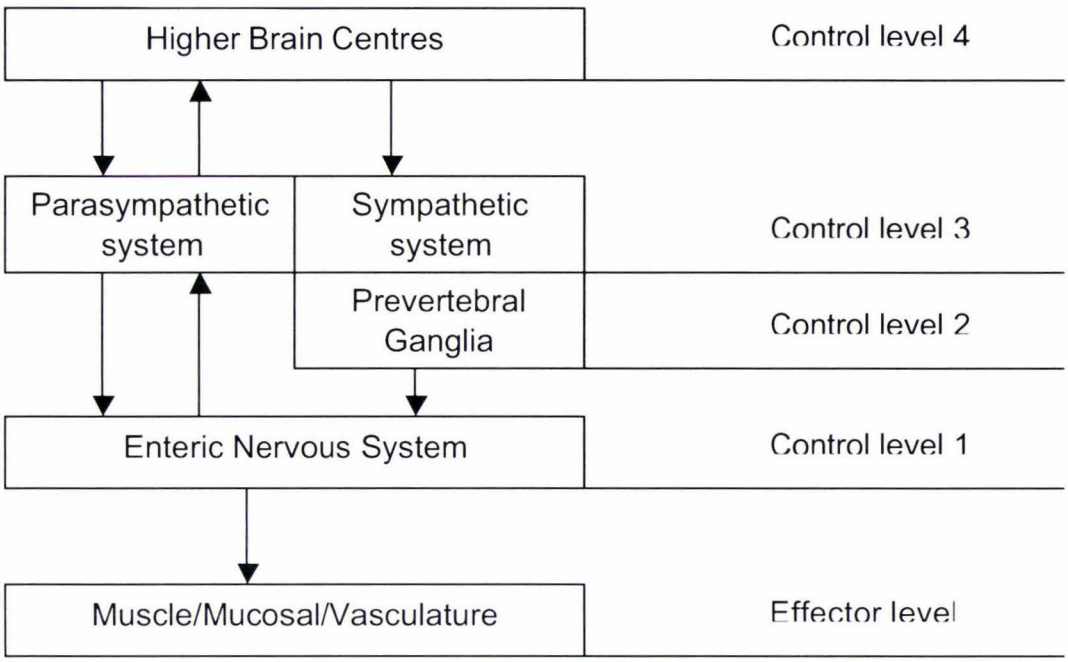


Figure 2C. The four neural control levels of the gut. The first level is the enteric nervous system, which behaves like a local minibrain. The second level is in the prevertebral sympathetic ganglia. The third and forth levels are within the central nervous system (CNS). Sympathetic and parasympathetic signals to the GI tract originate at level three and represent the final common pathways for outflow of information from the CNS to the gut. The forth level includes higher brain centres that provide input for integrative functions at level three (adapted from Wood *et al.*, 1999).

The stomach has a system that allows it to accommodate sudden increases in volume, as is the case when a meal is ingested. Mechanical stimulation of the throat or distension of the oesophagus induces receptive relaxation through vagally mediated reflexes, thereby reducing intragastric pressure (Jansson, 1969). Food entering the stomach induces gastric accommodation, which is mediated by a series of tension receptors in the body of the stomach. The tension receptors detect changes in volume and signal via afferent vagal fibres. These signals subsequently induce inhibition of

efferent discharge to the proximal stomach, resulting in an increase in gastric volume by inducing relaxation of the smooth muscle (Grundy & Scratcherd, 1982).

Upon reception of ingesta, the proximal stomach changes from periodic to relatively regular phasic contractions, which propagate towards the pylorus (Wingate *et al.*, 1994). Distension of the stomach is a natural stimulus that increases gastric emptying by stimulating peristalsis through the myenteric plexus and the vago-vagal reflex (Grundy & Scratcherd, 1982).

The motor activity of the stomach is also influenced by a series of reflexes initiated in the duodenum. Thus the chyme passing into the duodenum stimulates receptor mechanisms that trigger afferent discharges (Stanghellini *et al.*, 1994). These reflexes travel via three routes: 1) from the duodenum to the stomach via the enteric nervous system in the gut wall, 2) via afferent extrinsic nerves that travel to the prevertebral sympathetic ganglia and return via efferent inhibitory sympathetic nerve fibres to the stomach, and 3) via the vagus nerves to the brain stem, where they inhibit the normal excitatory signals transmitted to the stomach through the vagi (Hall *et al.*, 2003; Sarna, 1985). A number of non-nutrient stimuli may generate these reflexes (Guyton and Hall, 2001), such as:

- Distension of the duodenum
- Irritation of the duodenal mucosa
- Acidity of the duodenal chyme
- High osmolality of the chyme

In addition, the presence of certain absorbable nutrients in the chyme, including breakdown products of proteins and complex carbohydrates, and perhaps to a lesser extent fats may also inhibit nervous reflex (Guyton & Hall, 2001).

2.3.2 Hormonal Regulation of Gastric Emptying

Hormones are released into the vascular portal system in response to the presence of chyme in the stomach or duodenum. These hormones then inhibit gastric emptying. The hormonal control of gastric emptying has been described in detail (Lal *et al.*, 2004; Moran, 2004; Tschop *et al.*, 2001; McLaughlin *et al.*, 1999; White *et al.*, 1999; Read, 1994; Raybould *et al.*, 1994; Stanghellini *et al.*, 1994). One of the main theories concerning their mechanism of action is that the duodenum contains

"receptors" that detect specific macronutrients, causing a release of hormones into the blood. The hormones are then transported via the blood to the stomach where they inhibit activity of the pylorus.

Five main hormones have been described that regulate motility of the gastrointestinal tract: cholecystokinin (CCK), glucagon-like peptide 1, motilin, peptide YY (PYY) and more recently, ghrelin (Moran, 2004; Schneeman, 2002).

Cholecystokinin

One of the most influential groups of hormones that controls gastric emptying are the CCK's. Specialized mucosal endocrine cells found mainly in the proximal small intestine release CCK in response to stimulation. It is thought that CCK stimulates gallbladder emptying, pancreatic enzyme secretion, gastric emptying and that it inhibits food intake, i.e. increases satiety (McLaughlin *et al.*, 1999).

It has been suggested (McLaughlin *et al.*, 1999) that the proximal gut possesses a recognition system that is able to differentiate between particulate fatty acid molecules that differ by a single carbon atom in the length of their acyl chain (McLaughlin *et al.*, 1999). Thus differing chain lengths of ingested fat could generate entirely different patterns of endocrine cell and target organ response. There also appears to be a threshold chain length necessary to induce a CCK response; all fatty acids over 12 carbon atoms long were equipotent for CCK secretion (McLaughlin *et al.*, 1999). This has a bearing on any potential differences between milks from various species of mammal. For example goats' milk has a larger proportion of C8:0 and C:10 fatty acids than cow's milk, and this could elicit a different gastric emptying response.

Glucagon-like peptide 1

Glucagon-like-peptide-1 (GLP-1) has multiple actions throughout the body and has, as a result of this, been seen as a possible therapeutic agent (Meier & Nauck, 2005). A glucose-dependent insulinotropic effect has been reported for this hormone, and as such it has been postulated to act primarily as an incretin, i.e. to augment insulin secretion following food ingestion (Meier & Nauck, 2005). It thus plays an important role in glucose homeostasis, and has an additional influence over glucose levels through its action in decreasing the rate of gastric emptying through inhibition of vagal activity (Meier & Nauck, 2005). Additionally, GLP-1 is known to increase

satiety (Meier & Nauck, 2005; Hellstrom & Naslund, 2001) and has received attention for this property in the last few years owing to the rising obesity rates in the western world.

Motilin

Motilin, a polypeptide, stimulates the contraction of the smooth muscles of the gastrointestinal tract under physiological conditions Thus it accelerates gastric emptying and also increases lower oesophageal sphincter pressure (Netzer *et al.*, 2002). Rat studies (Martin *et al.*, 2005) suggest that melatonin, which is released in response to ileal lipids, exerts a modulatory influence that decreases the inhibitory effects of the ileal brake on gastric emptying of nutrients. The ileal break is a mechanism initiated by the presence of lipids in the ileum that regulates the gastric emptying of chyme (Martin *et al.*, 2005).

Peptide YY.

Peptide YY (PYY) is expressed mainly in the endocrine cells in the lower bowel (Sheikh, 1991) in response to the ingestion of a meal (Taylor, 1993). It is a powerful modulator of gastrointestinal function that slows gastric emptying and transit of digesta through the small intestine (Taylor, 1993; Savage *et al.*, 1987). It also has a role in blood flow, being a potent vasoconstrictor in many vascular beds (Sheikh, 1991). Taylor (1993) maintained that the net effect of PYY secretion was increased efficiency of digestion and absorption of nutrients.

The mechanism whereby PYY inhibits gastric emptying is thought to be related to the "ileal brake", which is where a nutrient (such as fat) induces a response that suppresses the digestive activities of the upper digestive tract (Moran *et al.*, 2005, Lin *et al.*, 2003).

Ghrelin

Ghrelin has been termed "a missing link between enteric nutrition and central regulation of energy balance and growth" (Inui *et al.*, 2004). It is said to function as an orexigenic (appetite-stimulating) signal from the stomach to the hypothalamus when an increase in metabolic efficiency is necessary (Inui et al., 2004; Asakawa *et al.*, 2001). Ghrelin has been reported to stimulate food intake and induce weight gain following peripheral or central administration (Tschop *et al.*, 2001; Wren *et al.*, 2000).

Although ghrelin's release is modulated by direct nutrient contact, its major action seems to be in meal initiation rather than affecting how much is eaten (Moran, 2004), which would appear to be contradictory.

Ghrelin stimulates gastric emptying (Inui *et al.*, 2004), similar to motilin (Asakawa *et al.*, 2001). Tschop *et al.* (2001) found a correlation between gastric emptying half time and fasting plasma ghrelin levels, and concluded that ghrelin appeared to provide feedback signalling between nutrient intake, gastric motor function and the central nervous system.

Tack *et al.* (2005) reported that ghrelin induces a premature gastric phase III of the migrating myoelectric complex (MMC). The MMC is a series of contractions, which effectively evacuates the intestine (West, 1990) making way for more contents to be emptied from the stomach. In addition, animal studies have shown that ghrelin stimulates upper gastrointestinal motility through the vagus and through the enteric nervous system (Tack *et al.*, 2005).

2.4 Gastric Emptying of the Different Phases of a Meal

Meals usually comprise a mixture of ingredients in different physical states i.e. solids or liquids. The different components empty from the stomach at differing rates. For example, solids take longer to empty from the stomach than liquids with the same nutrition content due to the presence of a "lag phase" prior to the commencement of gastric emptying (Fox *et al.*, 2004). The gastric emptying of oils has also been included in this section given that oils have been shown to empty differently to the aqueous phase of a meal yet they cannot be classified as solids either.

2.4.1 Solids

The gastric sinus (greater curvature of the stomach) forms a trap for particulate contents following the ingestion of a mixture of solids and liquids (Brown *et al.*, 1993). The gastric emptying of solids is significantly slower than that of liquids as solid food particles must be 'triturated', i.e. ground up into smaller particles, before they are able to pass through the pyloric sphincter. The period of time that solids spend in the stomach prior to transit through the pylorus is known as the lag phase, during which food is processed to particles small enough to be suspended in liquid (Christian, 1991; Siegel *et al.*, 1988). The muscular walls of the stomach grind the food particles up by retropulsion through a narrow segment of the antrum. The stomach contents are first driven towards the pylorus by the deeply segmented contractions that travel distally along the stomach walls (Schiller, 1983). The muscular pylorus contracts so that it is only partially open, thus antral contents on the perimeter of the lumen are carried forward and food matter (solid particles) that lies at the core is forced back towards the proximal end of the stomach (West, 1990). The solid masses are retropelled before the antral peristaltic contraction reaches the terminal antrum (West, 1990) and repetition of this cycle results in the solid particles being ground up until they are small enough to pass through the pylorus.

2.4.2 Liquids

The major difference between the gastric emptying of liquids and solids is the lack of a lag or delay phase in liquid gastric emptying i.e. liquids do not need to undergo any processing before exiting the stomach (Gonzalez *et al.* 2000; Coulie, 1997; Braden *et al.*, 1995; Maes *et al.*, 1994). In the process of retropulsion previously described, as

solids are retropelled back into the antrum, it is mainly liquids that are carried forward to the pylorus by the terminal antral contraction (West, 1990).

When a mixed meal of solids and liquids is consumed, the solid particles (that have a higher specific gravity) settle in the gastric sinus, allowing the liquid phase of the meal to wash over them and in a way be "decanted" over the "brim" of the pylorus as the gastric ring contractions move chyme towards the pylorus (Brown *et al.*, 1993).

Liquids have a characteristic gastric emptying pattern as follows: once a quantity of liquid has entered the stomach, there is an initial rapid exponential emptying phase followed by a slower linear phase of emptying that delivers calories to the duodenum at a constant rate over a range of caloric concentrations up to a maximum limit (Moran *et al.*, 1999a; Hunt *et al.*, 1979; McHugh & Moran, 1979). The rapid emptying in the initial phase depends mainly on the high volume of liquid but duodenal feedback related to the nutrient concentration of the chyme also has a role (Moran *et al.*, 1999a, 1999b; Lin *et al.*, 1992; McCann & Stricker, 1986; McHugh *et al.*, 1982; Moberg & Carlberger, 1974). In contrast to this, the major regulator of the slower linear phase is intestinal feedback based on nutrient concentration and type, and gastric volume has less influence over this phase (Moran *et al.*, 1999a,b; Maerz *et al.*, 1994; Lin *et al.*, 1992; McCann & Stricker, 1986; McHugh & Moran, 1979).

2.4.3 Oils and Fats

For the purposes of this discussion, oils and fats have been separated from solids and liquids, as they seem to have a pattern of gastric emptying that is unique. It is, however, important to note that the emptying pattern of different fats varies substantially making this area somewhat more difficult to define compared to that for solids and liquids (Meyer, 1987). For example, triglyceride has a similar emptying pattern to solid foods (an initial lag phase followed by a linear phase) whereas indigestible lipid empties more like a liquid (without a lag phase) (Meyer, 1987). In addition, Maes *et al.* (1998a) found that the chemical structure of the oil phase had an influence over gastric emptying when an equicaloric amount of olive oil containing more unsaturated fatty acids was more effective at slowing gastric emptying than a margarine containing more saturated fatty acids.

It is generally accepted that fats empty more slowly than nutrient aqueous liquids of a meal (Jian *et al.*, 1982, Chang *et al.*, 1968) but there is disagreement about the

mechanisms responsible for this (Edelbroek *et al.,* 1992). Some theorize that the slower emptying of fat is due to a "layering" effect that takes place in the stomach whereby the oil "floats" on top of the aqueous phase like cream on top of milk or because of the time that it takes to form a stable fat-water emulsion (Edelbroek *et al.,* 1992; Meyer, 1987). Maes *et al.* (1998a) found that the liquid phase of a meal emptied in the same manner in the presence of a solid phase as in the presence of an oil phase, whereas the oil phase emptied more slowly with liquids than with solids.

Research suggests that lipid is sensed in the wall of the intestine triggering the release of cholecystokinin, which inhibits gastric emptying via activation of extrinsic vagal afferent nerve terminals (Raybould, 1999; Raybould *et al.*,1994). It is postulated that when fat comes into contact with "receptors" in the small intestine, it triggers a reduction in antral contractions, and the induction of pyloric pressure waves and duodenal contractions, all of which contribute to control of the gastric emptying of fat (Raybould, 1999, 1994; Maes *et al.,* 1998; Heddle *et al.,* 1988).

2.4.4 Conclusion

When we ingest a meal, the food ingested can be in various physical forms, i.e. liquid or solid. The physical form of food determines how it will pass (or flow) through the rest of the intestine, which is basically a hollow tube. Liquids can flow through the intestine easily, but solids need to first be broken down and mixed with gastric juices in the stomach so that they too can pass through the intestine without complication. Thus, the stomach acts as a sieve, retaining the solids for trituration to a smaller particle size, and allowing liquids to flow through at a much faster rate. This seems to be linked to how fast the small intestine can cope with the digestive process.

The mechanisms responsible for the slower gastric emptying of fat compared to nutrient aqueous liquid meals are poorly understood and controversial; it could be to do with oil's lower density and higher viscosity than water, or because of oil's physical composition (Edelbroek *et al.,* 1992). Perhaps, the slower emptying of oils and fats is an evolutionary adaptation that enables the small intestine to more efficiently process the high-energy content associated with dietary fat intake.

2.5 Other Factors That Influence the Rate of Gastric Emptying

A number of food properties may influence how fast a particular food is emptied from the stomach. Chemical components of a food act at a molecular level to directly elicit hormonal or neuronal responses in the gut. In addition, there are factors relating to the individual that also have an influence over gastric emptying. Age, sex, health status, whether or not the person is a smoker and medication use can all influence the rate of gastric emptying.

2.5.1 Nutrient Factors

The energy density of a meal has a major influence on the rate of gastric emptying; the greater the energy density, the slower the rate of gastric emptying (Peracchi *et al.*, 2000; Calbet and Maclean, 1997; Hunt *et al.*, 1985). Calbet and Maclean (1997) demonstrated a linear relationship between gastric emptying rate and the caloric density of an ingested meal. Moreover, they concluded that the source of calories only played a minor role in determining the rate of gastric emptying in humans.

In contrast, Moukarzel and Sabri (1996) maintained that the type of nutrient does have a major effect on how quickly food exits the stomach, with foods rich in carbohydrates exiting the stomach more slowly than protein-rich foods, and lipid-containing foods emptying most slowly. These workers also showed that the gastric emptying rate of carbohydrate beverages was primarily determined by the volume, caloric content, and osmolality of the fluid ingested. Additionally, they found that isocaloric beverages containing different types of carbohydrates had different emptying rates (e.g. gastric emptying was faster for fructose than it was for glucose).

The type of dietary protein also influences the rate of gastric emptying. Whey proteins empty faster than caseins for example (Hall *et al.*, 2003), but it is difficult to determine whether this results from differences in the physical properties (e.g. coagulation) of the principal proteins or from feedback mechanisms stimulated by ingestion of the dietary proteins. Fox *et al.* (2004) thought that dephosphorylated casein would empty from the stomach quicker than unmodified casein on the grounds that smaller aggregates of protein would form in the stomach and thus empty quicker. However, gastric emptying of unmodified casein turned out to be faster than that of dephosphorylated casein (Fox *et al.*, 2004). Thus, they concluded that factors other

than the size of the protein aggregates determine the rate of gastric emptying of milk-based formula.

Not only type of food, but also the manner in which the particular food is prepared can influence the rate of gastric emptying. One study determined the effect of heat-treated fats on gastric emptying by measuring the rate of gastric emptying following the ingestion of two meals identical in contents but cooked differently (fats fried or not). These researchers found that total gastric emptying was significantly delayed after the fried meal (Benini *et al.*, 1994).

2.5.2 Volume of food ingested

The quantity of food ingested also influences how quickly the meal exits from the stomach as previously discussed. Gastric emptying of a liquid nutrient volume is characterised by an initial rapid exponential phase followed by a slower linear phase of emptying that delivers calories to the duodenum at a constant rate (Moran *et al.*, 1999; Hunt *et al.*, 1985, 1979; McHugh & Moran, 1985). The rate of emptying in the quicker initial phase depends primarily on the volume of fluid so that the higher the volume the faster the rate of gastric emptying (Moran *et al.*, 1999a,b; Lin *et al.*, 1992; McCann & Stricker, 1986; McHugh *et al.*, 1982; Moberg & Carlberger, 1974). The slower phase which follows the initial faster phase is more dependent on the nutrient character and concentration than on volume (Maerz *et al.*, 1994; Meyer *et al.*, 1988; Brener *et al.*, 1983; Hunt *et al.*, 1979; McHugh & Moran, 1979).

The volume of digesta that flows out of the distal end of the stomach is influenced by feedback cycles initiated in the duodenum and taking effect on mechanisms controlling the rate of gastric emptying in the stomach. Moran *et al.* (1999a) found that the rate of gastric emptying of liquid nutrients represents an interaction between gastric volume and nutrient-induced duodenal feedback. For optimum delivery rates of energy to be achieved, there first needs to be sufficient emptying of nutrients into the duodenum resulting in an increase in the magnitude of duodenal feedback and an concomitant inhibition of gastric emptying (Moran *et al.*, 1999b). Moran *et al* (1999b) also showed that the same controls (gastric volume and duodenal negative feedback) contribute to gastric emptying while the stomach is filling up (Moran *et al.*, 1999b).

Doran *et al.* (1998) showed that volume of an ingested meal also influences the gastric emptying rate of a solid meal. These workers found that a threefold increase in the volume of a solid meal was associated with a reduction in the duration of the lag phase and acceleration of the post lag emptying rate.

2.5.3. Viscosity

The gut is basically a hollow tube through which food, chyme and digesta flow. Thus, it is logical to think that viscosity of the gut contents will have a profound influence on the velocity at which this material is able to flow through the gut. A highly viscous meal would be expected to reduce flow out of the stomach given a constant force (Marciani *et al.*, 2000). However, results of experiments on the effects of viscosity on gastric emptying reported in the literature vary.

Several studies have shown that the addition of viscous soluble fibres to test meals as well as fibre naturally present in food reduces gastric emptying rate (Darwhich *et al.*, 2003; Marciani *et al.*, 2001; Benini *et al.*, 1995; Leclere *et al.*, 1994, Schwartz *et al.*, 1988; Blackburn *et al.*, 1984; Ray *et al.*, 1983).

In contrast to those results reporting delayed gastric emptying with increased viscosity, there are studies that have found no differences in gastric emptying with meals of different viscosity (Vivatvakin & Buachum, 2003; Vesa *et al.*, 1997).

Marciani *et al.* (2000) suggest that the stomach responds to the ingestion of a highly viscous meal by rapid intragastric dilution with gastric juices, which rapidly reduces the viscosity of the ingested meal. Thus large differences in the viscosity of ingested meals result in proportionately smaller differences in gastric emptying rates. Although it is logical to assume high viscosity will slow down gastric emptying rate, from the literature it appears that the stomach is able to adjust to viscosity to a certain extent.

2.5.4. Consistency of a Meal

The physical state of a meal i.e. whether it is given as a blend or as separate whole foods also affects the rate of gastric emptying. Santangelo *et al.* (2004) compared gastric emptying after a meal that was made up of solids and liquids with the same one that had been homogenised. The meal included vegetables, cheese, croutons,

olive oil and water. The rate of gastric emptying of the homogenised meal was slower than that of the 'intact meal'.

Essentially, the homogenization of the meal would have resulted in two influencing factors of gastric emptying being changed. Firstly, the particle size of the food would have been decreased, resulting in an increased surface area on which gastric enzymes could operate. This would have resulted in increased liberation of effector nutrients such as sugars and fats, which would have decreased the rate of gastric emptying through nutrient signalling (Furness *et al.,* 1999). Secondly, the decreased particle size would have resulted in a change in flow characteristics of the chyme. There would have been more, smaller particles resulting in it being more difficult for the fluid phase to pass through the solid phase (Santangelo *et al.,* 2004). When there are fewer, larger particles, it is easier for the fluid phase to pass through the solid phase. However more trituration is required for the larger solid particles. It is uncertain in the study of Santangelo *et al.* (2004) which of these two effects was most likely to be responsible for the results.

2.5.5. Disease

Gastric emptying has been shown to be abnormal in several diseased states including diabetes mellitus. Studies (Samsom *et al.*, 2003; De Block *et al.*, 2002; Horowitz *et al.*, 2002) have shown that 30 – 50 % of people with long-standing Type 1 or Type 2 diabetes have abnormally slow gastric emptying of solid, or nutrient liquid meals i.e. gastroparesis (this is due to sympatheic neuropathy i.e. impairment of the regulation of gastric emptying by the sympathetic nervous system). The abnormal gastric emptying may also be related in part to high blood glucose levels as Horowitz *et al.* (2002) found that gastric emptying was slower during periods of hyperglycemia than during periods of euglycemia, and was accelerated during periods of hypoglycaemia in diabetics (Horowitz *et al.,* 2002). There is, therefore, an application for therapies that use gastric emptying rate to improve postprandial glycemic control by modulating the rate of delivery of nutrients to the small intestine as maintained by Horowitz *et al.* (2002). In addition to therapies, there is potential for food manufacturers to produce products that cater for individuals with certain conditions. For example, Liljeberg & Bjorck (1996) showed that blood glucose and insulin responses after bread ingestion could be lowered if lactic acid or sodium propionate were added to the bread. They reported that the reason for the lowered metabolic responses was probably a lowered

gastric emptying rate. A bread of this nature would enable diabetics to have more control over blood glucose levels.

2.5.6 Gender

Several studies have shown that gastric motility varies with gender (Gandhi *et al.*, 2004; Sadik *et al.*, 2003; Hutson *et al.*, 1989; Datz *et al.*, 1987). Females generally have a slower gastric emptying time than males for both solids and liquids. These differences may be related to the effects of female sex hormones, primarily progesterone and estradiol, on the gastrointestinal tract. However, the mechanism by which gastric emptying is slowed in females is not known (Datz *et al.*, 1987). Also, women generally have smaller stomachs than men and thus proportionately smaller pyloric sphincters through which food must pass.

2.5.7 Conclusion

In summary, numerous factors have an influence on the speed gastric emptying of foods, and it is likely that a number have not yet been fully elucidated. All of these factors must conjointly influence gastric emptying and little work has been done subsequently on the potency of the different influences. It is important to bear this in mind when studying the gastric emptying of a food.

2.6 The Gastric Emptying of Milk.

The gastric emptying of milk is a complex process that is dictated in part by the milk's composition. Milk contains lipids in emulsified globules coated with a membrane, proteins in colloidal dispersion as micelles, minerals mostly in solution, and lactose, all of which are in true solution (Jensen *et al.*, 1991).

The milk proteins can broadly be categorized as being either caseins or whey proteins (Lonnerdal & Atkinson, 1995). Each type of protein also has a number of subclasses. Upon coming into contact with the acidic environment of the stomach, caseins precipitate out of solution within a few minutes resulting in delayed gastric emptying as a solid phase; whereas whey proteins remain soluble, emptying more quickly with the liquid phase (Gaudichon *et al.*, 1994; Mahe *et al.*, 1994). Thus milk with a lower ratio of casein to whey protein should empty at a faster rate due to the higher proportion of non-clotting water soluble whey components.

Boirie *et al.* (1997) introduced the concept of "slow" and "fast" dietary proteins referring to the time of appearance of metabolites in the blood following the ingestion of different proteins. These workers maintained that the speed of absorption of dietary amino acids by the gut varies with the type of dietary protein that is ingested. Amino acids appeared in the blood sooner after the ingestion of whey than after ingestion of casein. They postulated that one of the reasons for the differences in rate of appearance in the blood was the rate of gastric emptying of the two different proteins. Hall *et al.* (2003) have subsequently published results that support this.

Although cow's milk is produced for the nourishment of the calf, many humans consume cow's milk throughout their lives. However, the digestive system of a calf has features that allow for optimum digestion of cow's milk whereas the digestive system of humans does not have these features. The digestion of casein in calves is linked to the action of the enzyme rennet, which acts on the a and ? casein fractions of cows milk in the acidic environment of the abomasum (Miller *et al.*, 1990). Upon coming into contact with rennet, casein in cow's milk forms a firm, irreversible curd. The curd can then be slowly released into the small bowel where it is rapidly digested. Human milk does not contain a- casein and the human stomach is not known to secrete rennet (Miller *et al.*, 1990). Thus, when cow's milk is acidified in the stomach of the human, precipitation of the casein micelles occurs, but this process is reversible

upon alkalinization, i.e. a true curd is not formed (Miller *et al.*, 1990). Miller *et al.* (1990) pointed out that there is a possibility that casein from cow's milk may be delivered to the small intestine of human infants in particular, in quantities that may exceed the capabilities of a digestive process that is designed for human milk, i.e. casein from cow's milk is not slowed down in the human stomach as is the case in the stomach of the calf.

Thus the feeding of cow's milk to human infants may adversely influence or modify human infant nutrition through an altered pattern of nutrient supply. Schreiner *et al.* (1982) reported that at that time, as many as 6.6% of low birth weight infants (< 2kg) developed a lactobezoar when fed a bovine casein-predominant formula. It was thought that bovine casein contributed to lactobezoar formation because of its insolubility under acidic conditions leading to the precipitate forming the nucleus of the lactobezoar (Miller *et al.*, 1990). Nevertheless, infant formulae have a wide range of casein to whey ratios, and both casein-dominant and whey dominant formulas are commercially available (DuPont, 2003).

A number of studies have compared the rate of gastric emptying of infant formulae with that of human milk in infants (Table 2A).

Table 2A: *Comparative results of previous studies in infants comparing the rate of gastric emptying*[1] *of breast milk with infant formula*[2].

		Half emptying time (min)	
Reference	Infant age	Breast Fed	Formula Fed
Cavell, 1979	1-9 wk	25	51
Cavell, 1981	4 wk - 6 mo	48	78
Billeaud, 1990	0-12 mo	61	76
Ewer, 1994	0-4 wk	36	72
Van Den Driessche, 1999	1-10 wk	47	65

[1] As represented by half emptying time

[2] Adapted from Van Den Driessche *et al.*, 1999.

In all the studies reported in Table 2A, gastric emptying of human milk was faster than that of the formula. Billeaud *et al.* (1990) attributed the differences in rate of gastric emptying to protein composition and content in recognition of the fact that human milk has not only much less protein (1.05 ± 0.02g/100ml vs. ±1.5g/100ml in milk-based formulas (Garza *et al.*, 1993) but also a different whey to casein ratio. Thus, the higher the protein content of a milk, and the more casein the milk contains, the slower that milk's gastric emptying appears to be (Billeaud *et al.*, 1990).

Fermented milk also appears to empty from the stomach at a slower rate than normal milk (Gaudichon *et al.*, 1994), a behaviour that has been attributed to its overall higher viscosity, a property thought to influence gastric emptying (Houghton *et al.* 1987). However, this may not be the true explanation for this behaviour as other workers (Vesa *et al.* 1997) have reported that the overall viscosity of milk does not affect the rate of gastric emptying. However, viscosities were altered by the addition of varying proportions of rice starch and maltodextrin to a basic milk formula (Vesa *et al.* 1997). Thus it is likely that the outcome was influenced by nutrient density.

In light of the fact that differences in rates of gastric emptying of infants have been related to type of milk (Van Den Driessche *et al.* 1999; Billeaud *et al.*, 1990), and that milk composition varies between species, it is likely that infant formulas based on milk from different species will elicit different rates of gastric emptying in infants. The rate of gastric emptying of goat's milk from the human stomach is not known. Goat's milk-based infant formula is being advocated as a popular substitute for cow's milk based infant formula because of its hypo-allergenicity (Chandan *et al.*, 1992), although others have refuted these claims (Pessler & Nejat, 2004; Dean *et al.*, 1993). Goat's milk has been postulated to have other nutritional benefits compared to cow's milk such as improved mineral bioavailability (Alferez *et al.*, 2003, Barrionuevo *et al.*, 2002) and being easier to digest (Alferez *et al.*, 2001). A piglet study (Murry *et al.*, 1999) showed that piglets fed goat's milk had greater bone mineral density and less body fat compared to piglets fed cow's milk.

To develop this discussion further a more detailed description of the differences in composition of goat's milk and cow's milk is therefore warranted.

2.7 Composition of Goat's and Cow's Milk

Goat's milk is broadly similar in composition to cow's milk (Table 2B). However, a few differences are noteworthy. Goat milk fat has ~35% short and medium chain fatty acids compared to 17% for cow's milk fat (Haenlein, 1992). The relevance of this difference is that there is a fatty acid chain length threshold for eliciting a CCK response in the small intestine (McLaughlin *et al.*, 1999). McLaughlin *et al.*, (1999) showed that only fatty acids with a chain length of greater than twelve carbon atoms elicit the negative feedback response that results in a decrease in rate of gastric emptying.

It has been reported that the fat globules in goat's milk are smaller than those in cow's milk (Attaie & Richter, 2000). However, this is of greater relevance for the processing of liquid milk into other dairy products. Fluid milk is generally homogenised commercially, and this process dramatically reduces the size of fat globules regardless of what species the milk is from. Goat milk lacks "agglutinin", which is a component that causes fat globules in cow's milk to cluster when cooled (Jenness, 1980). As a result goat's milk takes longer to cream than cow's milk (Attaie & Richter, 2000).

Table 2B: *Composition of goat's and cow's milk (units / 100g of liquid whole milk)*[1].

Component	Unit	Cow's Milk	Goat's Milk
Water	g	87.7	88.8
Energy	kJ	280	240
	kcal	67	58
Protein	g	3.28	2.93
Total fat	g	4	3.3
Available Carbohydrate	g	4.7	4.4
Lactose	g	4.7	4.4
Ash	g	0.71	0.78
Cholesterol	g	11.7	10
Calcium	mg	114	102
Phosphorus	mg	87	89
Retinol	ug	80	250
B-Carotene	ug	23	<1
Vit E	mg	0.11	0.06
Vit C	mg	1.4	1.1
Niacin	mg	0.11	NA
Vit B12	mg	0.35	0. 04

[1](From Visser *et al.*, 1991)

With regards to protein composition, goat milk lacks a homolog of the α_{S1}casein that is the most abundant protein in cow's milk (Jenness, 1980). There are also differences in caseinate micelles; those of goat milk contain more calcium and inorganic phosphorus; are less solvated and less heat stable; and lose β-casein more readily than bovine micelles (Jenness, 1980). These differences in protein composition may cause differences in the way the milk coagulates in the acidic environment of the stomach.

Park (1991) reported that a specific type of goats' milk (Nubian goat milk) has a higher buffering capacity than Jersey and Holstein milk i.e. the ability of a solution to resist change in pH through the addition or loss of alkali or acid (Park, 1991). Luminal acidity can be sensed by the small intestine and gastric emptying is slowed as acidity increases (Schiller, 1983), thus the buffering capacity of milk could possibly affect that rate that it is emptied from the human stomach.

In a separate study, Park (1994) suggested that goat's milk proteins may be more readily digested and their amino acids absorbed more efficiently than those of cow's milk. It has been said that goat's milk is considered to form a softer, more friable curd when acidified compared to cow's milk (Park, 1994).

2.8 Measuring the Rate of Gastric Emptying

There are a number of factors that need to be considered when evaluating techniques used to measure gastric function. The ideal technique for measuring gastric function would be non-invasive, widely available, cheap, convenient, and reliable (i.e. have a low coefficient of variation) and would have low potential to damage the subject (Schwizer, 2003). In recent years a number of techniques have been used to measure the rate of gastric emptying (Table 2C). These include fluoroscopy, scintigraphy, manometry, electrogastrography, breath tests and ultrasound.

A number of these techniques were evaluated by Aktay *et al.* (2003). Fluoroscopy was found to be unreliable and not quantitative, and manometry invasive but quantitatively accurate in measurement of contractile activity. Electrogastrography was still considered experimental at the time of Aktay's evaluation. These researchers considered the use of radio labelled solid meals to monitor gastric emptying time as the mainstay in the evaluation of gastric function in clinical practice because of its qualitative and physiologic attributes (Aktay *et al.*, 2003).

Table 2C: *Techniques both historically, and currently used to assess gastric emptying*[1].

Intubation techniques	Gastric aspiration
	Gastric dye dilution
	Duodenal dye dilution
Imaging techniques	Radiology
	Scintigraphy
	Ultrasonography
	Magnetic Resonance Imaging
Indirect techniques	Blood tests
	Breath tests

[1](From Vantrappen, 1994)

2.8.1 Radiological Methods

The quantitative assessment of gastric emptying with barium contrast radiography is technically challenging because it is difficult to assess the specific percentages of barium that have emptied from the stomach over time from a two dimensional image. The most useful measure that can be obtained from this method is the time taken to completely empty an entire meal. A further problem with this method is uncertainty over the relationship between the time of emptying of the barium and that of food i.e. barium may empty separately from the food (Schiller, 1983). The test is useful, however, for separating out subjects with extremely fast or slow gastric emptying but more qualitatively sensitive tests are needed to distinguish small differences in the emptying of solids from that of liquids (Schiller, 1983).

2.8.2 Gastric Intubation

Gastric intubation has been widely used in research practice and involves the ongoing measurement of gastric volume by repeated aspiration along with (in some cases) an assessment of dilution by gastric secretions using an indicator dye (Schiller, 1983). A known volume of a liquid test meal is infused into the stomach and then aspirated after a period of time. The difference between the infused and the recovered volumes is assumed to represent the amount that has exited the stomach (Schiller, 1983).

To increase the accuracy of the test, some researchers also use the dilution of markers to obtain a measurement of how significantly gastric juices have contributed to the volume of aspirate. A non-absorbable marker of known concentration is ingested and allowed an amount of time for mixing with gastric juices. The concentration of the marker in an aspirate of gastric contents is then used to calculate how much gastric juice has diluted the ingesta. From this, gastric volume can be calculated and thus gastric emptying (Schiller, 1983).

The disadvantage of this method is that it is only suitable for measuring the gastric emptying of liquids and not solids. Also, in addition to causing discomfort for the subjects, the presence of a tube in the GI tract may affect normal gastric functioning, . The main advantage of this method is that the equipment required is relatively inexpensive (Vantrappen, 1994).

2.8.3 Ultrasonography

Ultrasonography measures changes in volume of the gastric antrum over time demonstrating antropyloroduodenal motility and flow of gastric contents (Vantrappen, 1994). The gastric emptying rate described by this method correlates well with the emptying of the liquid phase but not with emptying of the solid phase (Scarpignato, 1990).

The appeal of this method is that it allows visualisation of contractions in the gastro-duodenal wall, and the actual movement of the gastric contents in addition to providing a means of measuring the rate and pattern of gastric emptying (Vantrappen, 1994).

One disadvantage of this method is that only changes in volume of the gastric antrum are recorded (not the fundus and corpus) because air /fluid interfaces disrupt the ultrasound beam when assessing the whole gastric volume (Marciani *et al.*, 2000). The main disadvantage, however, is that it is time consuming and requires an experienced and skilful operator (Vantrappen, 1994), limiting both experimental and routine use.

2.8.4 Magnetic Resonance Imaging

Gastric emptying assessment by magnetic resonance imaging (MRI) involves repeated volumetric calculations based on transaxial image stacks covering the gastric region, following the ingestion of a test meal (Schwizer *et al.*, 2003). Total volume of the gastric lumen can be identified by distinct positive contrasts. Corrections are made for gastric secretions into the stomach by doping meals with paramagnetic contrast markers. Ultimately, the technique provides a time plot of corrected gastric volume, which in turn provides a direct assessment of the half emptying time and lag time (Schwizer *et al.*, 2003).

The main advantage of this technique is that it is possible to simultaneously measure gastric emptying and the total volume of gastric contents (gastric secretion, saliva and duodenal reflux) (Vantrappen, 1994). Although MRI seems to be a good method for measuring gastric emptying, it requires expensive equipment and trained operators.

2.8.5 Scintigraphy

Gamma camera scintigraphy is considered the gold standard for measuring the rate of gastric emptying and has been used extensively in clinical practice and research for some time (Vantrappen, 1994). As such, it has been used to validate other methods for measuring gastric emptying. Following a 12-hour fast, subjects are fed a mixed solid and liquid test meal with one or more of the components labelled with a radioisotope. Some of the most commonly used isotopes are ^{99m}Tc-sulfur colloid, ^{111}In-DTPA, $^{111}InCl_3$, and ^{99m}Tc-ovalbumin (Camilleri *et al.* 1998). A large field-of-view gamma camera is used to determine the dose of isotope in a delineated regions of the stomach and repeated observations allow the development of an emptying curve, with the decline of this curve over time being corrected for isotope decay and tissue attenuation (Sleisenger & Fordtran 1993). Gamma camera counts need to be corrected for isotope decay and tissue attenuation (i.e. depth related changes in the detected activity). A gamma camera image is taken immediately following the ingestion of the test meal and at various intervals for up to four hours. Each isotope and thus each phase i.e. solid and liquid can be imaged separately (Fisher & Malmud, 1985).

The original method determined the time taken for 50% of the isotope to empty from the stomach. More recently it has been used to estimate the lag time (time before initiation of gastric emptying) following the consumption of a meal, and for estimating relative proportions of isotope remaining in the stomach at certain time points (Camilleri *et al.*, 1998).

The disadvantage of scintigraphy lies in the use of radioactive isotopes. It is therefore not suitable for use on children and pregnant women, or when frequently repeated measurements are required (Schwizer *et al.*, 2003). The low level of temporal and spatial resolution can also be a problem. Changes in shape, position, and distribution of gastric contents during emptying can lead to errors in measurement (Schwizer *et al.*, 2003). As is the case with MRI and ultrasonography, this method also requires expensive equipment and trained operators.

2.8.6 Paracetamol (Acetaminophen) Absorption Test

The paracetamol test is used to assess gastric emptying of liquids (Kim *et al.*, 2000). The test is based on the principle that gastric emptying is the rate-limiting step in the

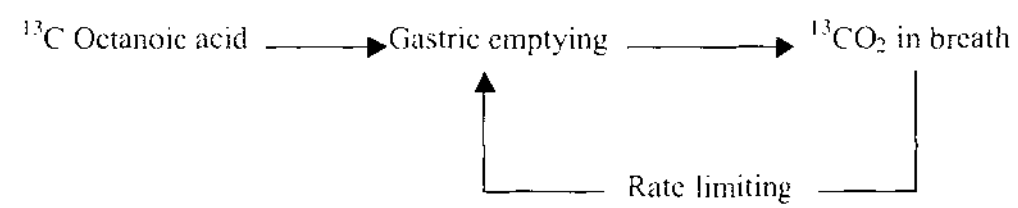
absorption of paracetamol in the small intestine. The test involves subjects ingesting a liquid drink containing paracetamol followed by repeated blood sampling at regular intervals. Serum or plasma paracetamol concentrations are measured and the concentration curves from these measures are used to derive gastric emptying parameters (Kim et al., 2000).

The main advantage of the paracetamol test is that it is simple and well tolerated by subjects (Kim et al., 2000). In addition, it has been shown to correlate with certain parameters measured by scintigraphy, the "gold standard" (Medhus *et al.*, 1999).

The disadvantage of this method is that it is an indirect quantification of gastric emptying rate and is only validated for the gastric emptying of liquids. The repeated blood sampling may also be problematic when investigating gastric emptying in young children (Kim *et al.*, 2000).

2.8.7 Breath Tests

Breath tests for measuring gastric emptying offer an indirect measurement that is non-invasive and reliable. The method involves feeding subjects a food with a substrate that bears the functional group in which a normally present ^{12}C atom has been replaced by the stable isotope ^{13}C, i.e. the substrate is labelled with ^{13}C. Following ingestion of the meal, the marker is absorbed on exit from the stomach, i.e. in the small intestine, and promptly metabolised. The $^{13}CO_2$, a by-product of the substrate metabolism, mixes with the body pool of CO_2- and HCO_3- and is subsequently exhaled as labelled $^{13}CO_2$. The rate of excretion of the carbon label in the breath reflects the rate that labelled food has emptied from the stomach. The main principle on which the $^{13}CO_2$ breath test is based is that gastric emptying is the rate-limiting step in the appearance of $^{13}CO_2$ in the breath as indicated by the following schematic:



(Taken from Ghoos *et al.*, 2002)

The ratio of labelled $^{13}CO_2$ to normal $^{12}CO_2$ in expired air is determined by ratio-isotope mass spectrometry. Man at rest produces a roughly constant amount of CO_2 per unit of time and the total amount of CO_2 excreted per unit time is determined from a nommogramme based on body surface area (Haycock *et al.*, 1978).

There are several advantages to the $^{13}CO_2$ breath test method. The main ones being that the rate of gastric emptying can be determined over the course of time and that it is reliable (Ghoos *et al.*, 2002). In addition, the method is not invasive for the patient/subjects and easy to use; patients can even perform this method at home (Ghoos *et al.*, 2002).

The disadvantage of this method is that it is an indirect measurement and not a real time measurement. Thus, it is necessary to make certain assumptions when converting data from the breath measurements, e.g. that time for metabolism of the marked substrate is constant.

The ^{13}C-Octanoic Acid Breath Test

This technique was first reported by Ghoos *et al.* (1993) who validated its use in healthy subjects for testing gastric emptying of solids (Ghoos *et al.*, 1993a). The same group subsequently developed several applications for the technique, including pathophysiological and pharmacological studies (Galmiche *et al.* 1998). In the past few years it has become a popular technique in the medical field and in research.

Octanoic acid is a medium chain fatty acid that is rapidly absorbed in the duodenum and metabolised in the liver (Havel, 1997;Bach & Babayan, 1982, McGarry & Foster, 1980). For this test, one carbon atom in the molecule is a labelled ^{13}C non-radioactive isotope. Following metabolism in the liver the labelled carbon is excreted as $^{13}CO_2$ in the breath at a level that can be easily measured by isotope radio mass spectrometry (Maes *et al.*, 1994, 1998b, Ghoos *et al.*, 1993a,b).

Given that a correction factor is available that can be applied to correct for the time that the octanoic acid takes to be metabolised and that ^{13}C-octanoic acid is absorbed rapidly in the duodenum, the only rate-limiting factor in the appearance of the ^{13}C in the breath is assumed to be the rate of gastric emptying (Maes *et al.*, 1994). The rate of gastric emptying of a meal can therefore be calculated by correcting for the time that it takes for the ^{13}C to be metabolised by the liver (Ghoos *et al.*, 1993a,b).

The ^{13}C octanoic acid breath test (OABT) has been shown to correlate well with the gold standard, radioscintigraphy (Ghoos *et al.,* 1993a). In comparison to the other methods that have been reviewed here, the ^{13}C OABT is easy to perform with the least amount of patient/subject discomfort (Maes *et al.,* 1994). Additionally, several tests can be conducted in a relatively short period of time and breath samples can be collected at a site remote from the mass spectrometry facility, making it very practical (Maes, 1994). Above all else, it is a safe, reliable and non-invasive method (Ghoos *et al* 1993a, Van Den Driessche *et al.,* 999).

The ^{13}C OABT was initially validated for measurement of gastric emptying rate of solids, but has also been used effectively to measure the rate of gastric emptying of milk (Pozler *et al.,* 2003, Omari *et al.,* 2002; Van Den Driessche *et al.,* 1999), and liquid meals (Ritz *et al.,* 2001; Debreceni, 1999).

The disadvantage of the ^{13}C method breath test is that it only provides an indirect measurement of gastric emptying and at this stage an optimal algorithm for relating the outcome of this to data obtained using other methods remains controversial (Schwizer *et al.,* 2003). A particular problem is the delay resulting from post gastric processing of octanoic acid prior to the recovery of the expired ^{13}C in CO_2. These limitations come from the obvious delay caused by the post gastric processing of octanoic acid in the recovery of ^{13}C in the breath, as was proven by Wyse *et al.* (2003). However, a constant correction factor can be applied to correct for the delay (Braden *et al.,* 1995; Ghoos *et al.,* 1993a,b). When comparing the rates of gastric emptying of two foods, the correction factor becomes less important.

2.9 Conclusion and Inferences from the Review of Literature

Milk proteins undergo changes in their physical state on exposure to gastric acid, usually resulting in the precipitation of casein proteins out of the aqueous phase. This could have an influence on how milk empties from the stomach given that solids follow a different pattern of gastric emptying compared to liquids. Variation in the protein composition of milk from different species, and specifically the types and amounts of casein and whey proteins could therefore influence the rate of emptying from the stomach.

Whole cow and goat's milk differ slightly in composition, which could cause differences in their rates of gastric emptying. Numerous studies have been done on gastric emptying in infants, and it is generally accepted that whey predominant formulas promote more rapid gastric emptying than ones with a higher proportion of casein.

Studying the gastric functions of humans is technically challenging given that the stomach is a relatively inaccessible internal organ. A number of techniques have been used to measure the rate of gastric emptying but the ^{13}C octanoic acid breath test appears to be the most feasible for the current study. It is safe, non-invasive, practical and reliable method and has been used previously to measure the rate of gastric emptying of milk, albeit in infants (Pozler *et al.*, 2003, Omari *et al.*, 2002; Van Den Driessche *et al.*, 1999). The study of infant gastric function is more challenging given the small size of the stomach and the size of the meals consumed. However, the effect of differences in milk composition (which can affect gastric emptying) on gastric emptying can more easily be studied in the adult, assuming that any differences in gastric emptying in the adult would similarly occur in the infant.

According to the literature, there are a number of factors that influence the rate at which a food is emptied from the stomach. As a food, milk can vary widely in composition from mammalian species to mammalian species. This led to the hypothesis that milk from different species may elicit different rates of gastric emptying, and as such may have a different impact on the postprandial appearance of metabolites in the blood. Thus, the purpose of this study was to compare the rates of

gastric emptying of milk from different species, bovine and caprine, and to establish what affect this may have on the appearance of metabolites in the blood.

2.10 References

Aktay, A.N., Werlin, S.L., & Hellman, R.S. (2003). The impact of solid-phase gastric emptying studies in the management of children with dyspepsia. *Clinical Paediatrics*. 42(7): 621 –625

Alferez, M.J., Lopez Aliaga, I., Barrionuevo, M. & Campos, M. S. (2003). Effect of dietary inclusion of goat milk on the bioavailability of zinc and selenium in rats. *Journal of Dairy Research*. 70(20): 181-187

Alferez, M.J., Barrionuevo, M., Lopez, A.I., Sanz-Sampelayo, M.R., Lisbona, F., Robles, J.C. & Campos, M.S. (2001). Digestive utilization of goat and cow milk fat in malabsorption syndrome. *Journal of Dairy Research*. 68(3): 451-461

Asakawa, A., Inui, A., Kaga, T., Yuzuriha, H., Nagata, T., Ueno, N., Makino ,S., Fujimiya, M., Niijima, A., Fujino, M.A. & Kasuga, M. (2001). Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology*. 120: 337-345

Attaie, R. & Richter, R.L. (2000). Size distribution of fat globules in goat milk. *Journal of Dairy Science*. 83: 940-944

Bach, A.C. & Babayan, V.K. (1982). Medium-chain triglycerides: an update. *American Journal of Clinical Nutrition*. 36: 950-962

Barrionuevo, M., Alferez, M.J., Lopez, A.I., Sanz, S.M. & Campos, M.S. (2002). Beneficial effect of goat milk on nutritive utilization of iron and copper in malabsorption syndrome. *Journal of Dairy Science*. 85(3): 657-664

Benini, L., Brighenti, F., Castellani, G., Brentegani, M.T., Casiraghi, M.C., Ruzzenente, O., Sembenini, C., Pellegrini, N., Caliari, S. & Porrini, M. (1994). Gastric emptying of solids is markedly delayed when meals are fried. *Digestive Diseases and Sciences*. 39 (11): 2288-2294

Benini, L., Castellani, G. & Brighenti, F. (1995). Gastric emptying of a solid meal is accelerated by the removal of dietary fibre naturally present in food. *Gut*. 36(6): 825 –830

Billeaud, C., Guillet,J., & Sandler, B (1990). Gastric emptying in infants with or without gastro-oesophageal reflux according to the type of milk. *European Journal of Clinical Nutrition*. 44: 577-583

Blackburn, N.A., Holgate, A.M. & Read, N.W. (1984). Does guar gum improve postprandial hyperglycaemia in humans by reducing small intestinal contact area: *British Journal of Nutrition*. 52 (2): 197-204

Boirie, Y., Dangin, M., Gachon, P., Vasson, M-P., Maubois, J-L., & Beaufrere, B. (1997). Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proceedings of the National Academy of Sciences, USA*. 94: 14930-14935

Braden, B., Adams, S., Duan, L-P., Orth, K-H., Maul, F.D.& Lembcke, B. (1995). The [^{13}C] acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. *Gastroenterology*. 108: 1048-1055

Brand-Miller, J., Holt, S. H. A., Pawlak, D.B. & McMillan, J. (2002). Glycemic index and obesity. *American Journal of Clinical Nutrition*. 76: 281S-285S

Brener, W., Hendrix, T.R. & McHugh, P.R. (1983). Regulation of gastric emptying of glucose. *Gastroenterology*. 85: 76-82

Brooks, F.P. (1985). Physiology of the stomach. In Bockus, H.L. (ed). Gastroenterology, Vol I. Philadelphia, W.B. Saunders. pp. 874–896

Brown, B.B., Schulze-Delrieu, K., Schrier, J.E. & Abu-Yousef, M. (1993). The configuration of the human gastroduodenal junction in the separate emptying of liquids and solids. *Gastroenterology*. 105: 433-440

Calbet, J.A.L., & MacLean, D.A. (1997). Role of caloric content of gastric emptying in humans. *Journal of Physiology – London*. 498 (2): 553-559

Camilleri, M., Hasler, W.L, Parkman, H.P., Quigley, E.M.M., & Soffer, E. (1998). Measurement of gastrointestinal motility in the GI laboratory. *Gastroenterology, 115*(3): 747-762.

Cavell, B. (1979). Gastric emptying in preterm infants. *Acta Paediatrica Scandinavia.* 68: 725–730

Cavell, B. (1981). Gastric emptying in infants fed human milk or infant formula. *Acta Paediatrica Scandinavia.* 70: 639–641

Chandan, R.C., Attaie, R. & Shamani, K.M. (1992). Nutritional aspects of goats' milk. Proceeding V International Conference Goats' New Delhi. 1002, Vol. II Pt1, pp399–402

Chang, C.A., McKenna, R.D. & Beck, I.T. (1968). Gastric emptying rate of the water and fat phases of a mixed test meal in man. *Gut.* 9(4): 420-424

Christian, P.E., Datz, F.L. & Moore, J.G. (1991). Confirmation of short solid-food lag phase by continuous monitoring of gastric emptying. *Journal of Nuclear Medicine.* 32(7): 1349-1352

Coulie, B. & Camelleri, M. Interactions between gastric emptying rate and fat absorption. In: Armand, B. & De Vries, S. (Editors) (2000). *Fat Digestion and Absorption.* AOCS Press. Illinois: Champaign. pp. 65-67

Coulie, B, Tack, J., Maes, B., Geypens, B., De Roo, M. & Janssens, J. (1997). Sumatriptan, a selective 5-HT1 receptor agonist, induces a lag phase for gastric emptying of liquids in humans. *American Journal of Physiology.* 272(4): G902-G908

Datz, F. L., Christian, P. E., & Moore, J. (1987). Gender-related differences in gastric-emptying. *Journal Of Nuclear Medicine, 28*(7), 1204-1207

Darwhich, G., Bjorgell, O. & Almer, L. (2003). The addition of locust bean gum but not water delayed the gastric emptying rate of a nutrient semisolid meal in healthy subjects. *BioMed Central Gastroenterology.* 3(1): 12

Dean, T. P., Adler, B. R., Ruge, F. & Warner, J. O. (1993). *In vitro* allergenicity of cow's milk substitutes. *Clinical & Experimental Allergy.* 23(3): 205-210

De Block, C. E. M., De Leeuw, I. H., Pelckmans, P. A., Callens, D., Maday, E., & Van Gaal, L. F. (2002). Delayed gastric emptying and gastric autommunity in type 1 diabetes. *Diabetes Care, 25*(5), 912-917

Debreceni, A., Abdel-Salam, O.M.E., Figler, M., Juricskay, I., Szolcsanyi, J. & Mozsik, G. (1999). Capsaicin increases gastric emptying rate in healthy human subjects measured by ^{13}C-labeled octanoic acid breath test. *Journal of Physiology (Paris)*. 93: 455-460

Doran, S., Jones, K.L., Andrews, J.M., & Horowitz, M. (1998). Effects of volume and posture on gastric emptying of solids and appetite. *American Journal of Physiology*. 275 (44): R1712–R1718

DuPont, C. (2003). Protein requirements during the first year of life. *American Journal of Clinical Nutrition*. 77 (suppl): 1544S-1547S

Edelbroek, M., Horowitz, M., Maddox, A., & Bellen, J. (1992). Gastric-emptying and intragastric distribution of oil in the presence of a liquid or a solid meal. *Journal Of Nuclear Medicine*. 33(7): 1283-1290

Ewer, A.K., Durbin, G.M., Morgan, M.E. & Booth, I.W. (1994). Gastric emptying in preterm infants. *Archives of Diseases in Children*. 71: F24-F27

Fisher, R. S. & Malmud L.S. (1985). Radionuclide scintigraphy. In J. E. Berk (Ed.), *Gastroenterology, 4th edition* (Vol. 1, pp. 502-511). London: W.B.Saunders Company. pp. 43-45

Fox, M., Goergi, G., Boehm, G., Menne, D., Fried, M. & Thumshirn, M. (2004). Dietary protein precipitation properties have effects on gastric emptying in healthy volunteers. *Clinical Nutrition*. 23: 641-646

Furness, J.B., Kunze, W.A.A. & Clerc, N. (1999). Nutrient tasting and signalling mechanisms in the gut II. The intestine as a sensory organ: neural, endocrine, and immune responses. *American Journal of Physiology*. 277(40): G922-G928

Galmiche, J.P., Delbende, B., Perri, F. & Andriulli, A. (1998). ^{13}C Octanoic acid breath test. *Gut*. 43 (suppl3): S28-S30

Gandhi, M., Aweeka, F., Greenblatt, R. M., & Blaschke, T. F. (2004). Sex differences in pharmacokinetics and pharmacodynamics. *Annual Review of Pharmacology and Toxicology, 44*, 499-523

Garza, C., Butte, N.F. & Goldman, A.S. (1993). Human Milk and Infant Formula. In: Suskind, R.M. & Lewinder-Suskind, L. (Eds). *Textbook of Pediatric Nutrition.* New York. Raven Press, Ltd. pp. 27

Gaudichon, C., Roos, N., Mahe, S., Sick, H., Bouley, C., & Tome, D. (1994). Gastric-emptying regulates the kinetics of nitrogen absorption from n-15-labeled milk and n-15-labeled yogurt in miniature pigs. *Journal Of Nutrition, 124*(10), 1970-1977

Ghoos, Y.F., Geypens, B. & Rutgeerts, P. (2002). Stable isotopes and $^{13}CO_2$ breath tests for investigating gastrointestinal functions. *Food and Nutrition Bulletin.* 23 (3S): 166-168

Ghoos, Y.F., Maes, B.D., Geypens, B.J., Mys, G., Hiele, M.I., Rutgeerts, P.J. & Vantrappen, G. (1993a). Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. *Gastroenterology.* 104(6): 1640-1647

Ghoos, Y., Geypens, B., Maes, B., et al. (1993b). Breath tests in gastric emptying and transit studies: Technical aspects of $^{13}co_2$-breath tests. In J. Janssens (Ed.), *Progress in understanding and management of gastro-intestinal motility disorders.* Kuleaven. pp. 169 - 180

Gonzalez, A, Mugueta, C., Parra, D., Labaten, I., Martinez, A., Varo, N., Monreal, I., & Gil, M.J. (2000). Characterisation with stable isotopes of the presence of a lag phase in the gastric emptying of liquids. *European Journal of Nutrition.* 39: 224-228

Grundy, D. & Scratcherd, T. (1982). A splanchno-vagal component of the inhibition of gastric motility by distention of the intestin. *In*: Weinbeck, (Ed). *Motility of the Digestive tract.* New York: Raven Press. pp. 39-43

Guyton, A.C., & Hall, J.E. (2001). *Medical physiology* (10th ed.). London: W.B.Sauders Company. pp. 728-737

Haenlein, G.F.W. (1992). Role of goat meat and milk in human nutrition. Proc.V. Intl. Conf. Goat. New Delhi, India. Vol II: Part 1. pp. 575-580

Hall, W.L., Millward, D.J., Long, S.J., & Morgan, L.M. (2003). Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *The British Journal of Nutrition*. 89 (2): 239-248

Haycock, G.B., Schwartz, G.J. & Wisotsky, D.H. (1978). Geometric method for measuring body-surface area - height-weight formula validated in infants, children, and adults. *Journal Of Pediatrics*. 93(1), 62-66

Havel, R.J. (1997). Postprandial lipid metabolism: an overview. Guest Lecture. *Proceedings of the Nutrition Society*. 56: 659-666

Hellstrom, P.M. & Naslund, E. (2001). Interactions between gastric emptying and satiety, with special reference to glucagon-like pepetide-1. *Physiology and Behaviour*. 74(4-5): 735-741

Heddle, R., Dent, J., Read, N.W. (1988). Antropyloroduodenal motor responses to intraduodenal lipid infusion in healthy volunteers. *American Journal of Physiology*. 254: G671-G679

Horowitz, M., O'Donovan, D., Jones, K.L., Feinle, C., Rayner, C.K., & Samsom, M. (2002). Gastric emptying in diabetes: clinical significance and treatment. *Diabetic Medicine*. 19 (3): 177-194

Houghton, L. A., Hickson, F., & Read, N. W. (1987). Effect of food consistency on gastric-emptying in man. *Gut, 28*(12), 1584-1588

Hunt, J.N., Smith, J.L. & Jiang, C.L. (1985). Effect of meal volume and energy density on the gastric emptying of carbohydrates. *Gastroenterology*. 89: 1326-1330

Hunt, J.N., Smith, J.L. & Jiang, C.L. (1979). The volume and energy content of meals as determinants of gastric emptying. *Journal of Physiology*. 245: 209-225

Hutson, W. R., Roehrkasse, R. L., & Wald, A. (1989). Influence of gender and menopause on gastric-emptying and motility. *Gastroenterology, 96*(1), 11-17

Inui, A, Asakawa, A., Bowers, C.Y., Mantovani, G., Laviano, A., Meguid, M.M. & Fujimiya, M. (2004). Ghrelin, appetite, and gastric motility: the emerging role of the stomach as an endocrine organ. *FASEB Journal*. 18: 439-456

Jansson, G. (1969). Vago-vagal reflex relaxation of the stomach in the cat. *Acta Physiology Scandinavia.* 75(1): 245-252

Jeness, R. (1980). Composition and characteristics of goat milk: Review 1968-1979. *Journal of Dairy Science.* 63: 1605-1630

Jensen, I.G., Ferris, A.M. & Lammi-Keefe, C.J. (1991). The composition of milk fat. *Journal of Dairy Science.* 74: 3228-3243

Jian, R., Vigeron, N., Najean, Y. & Bernier, J.J. (1982). Gastric emptying and intragastric distribution of lipids in man: A new scintigraphic method of study. *Digestive Diseases and Sciences.* 27(8): 705-711

Kim, D-Y., Myung, S-J. & Camilleri, M. (2000). Novel testing o human gastric motor and sensory function: Rationale, Methods, and Potential Application in Clinical Practice. *The American Journal of Gastroenterology.* 95(12): 3365-3373

Lal, S., McLaughlin, J., Barlow, J., D'Amato, M., Giacovelli, G., Varro, A., Dockray, G. & Thomson, D.G. (2004). Cholecystokinin pathways modulate sensations induced by gastric distension in humans. *American Journal of Physiology.* 287(1): G72-G79

Leclere, C.J., Champ, M., & Boillot, J. (1994). Role of viscous guar gums in lowering the glycemic response after a solid meal. *American Journal of Clinical Nutrition.* 59 (4): 914-921

Liljeberg, H. G. M., & Bjorck, I. M. E. (1996). Delayed gastric emptying rate as a potential mechanism for lowered glycemia after eating sourdough bread: Studies in humans and rats using test products with added organic acids or an organic salt. *American Journal Of Clinical Nutrition.* 64(6): 886-893

Lin, H.C., Neevel, C. & Hai Chen, J. (2003). Slowing intestinal transit by PYY depends on serotonergic and opioid pathways. *American Journal of Physiology.* 286: G558-G563.

Lin, H.C., Elashoff, J.D., Gu, Y.G. & Meyer, J.H. (1992). Effect of meal volume on gastric emptying. *Journal of Gastrointestinal Motility.* 4: 157-163

Lonnerdal, B. & Atkinson, S. (1995). Nitrogenous Components of Milk. A. Human Milk Proteins. In: Jensen, R.G. (Ed). Handbook of Milk Composition. New York. Academic Press. pp. 64-69

Maerz, L.L., Sankaran, H., Scharpf, S.J. & Deveney, C.W. (1994). Effect of caloric content and composition of a liquid meal on gastric emptying in the rat. *American Journal of Physiology* 267: R1163-R1167

Maes, B.D., Ghoos, Y.F., Rutgeers, P.J., Hiele, M.I., Geypens, B. & Vantrappen, G. (1994). [*C] Octanoic acid breath test to measure gastric emptying rate of solids. *Digestive Diseases and Sciences*. 39(12): 104S-106S

Maes, B.D., Hiele, M.I., Geypens, B.J., Ghoos, Y.F. & Rutgeerts, P.J. (1998a). Gastric emptying of the liquid, solid and oil phase of a meal in normal volunteers and patients with Billroth II gastrojejunostomy. *European Journal of Clinical Investigation*. 28: 197-204

Maes, B.D., Mys, G., Geypens, B.J., Evenpoel, P., Ghoos, Y.F. & Rutgeerts, P.J. (1998b). Gastric emptying flow curves separated from carbon-labeled octanoic acid breath test results. *American Journal of Physiology*. G169-G175

Mahe, S., Marteau, P., Huneau, J-F., Thuillier, F. & Tome, D. (1994). Intestinal nitrogen and electrolyte movements following fermented milk ingestion in man. *British Journal of Nutrition*. 71: 169-180

Marciani, L., Gowland, P.A., Spiller, R.C., Manoj, P., Moore, R.J., Young, P. & Fillery-Travis, A.J. (2001). Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. *American Journal of Physiology*. 280: G1227-G1233

Marciani, L., Gowland, P.A., Spiller, R.C., Manoj, P., Moore, R.J., Young, P., Al-Sahab, S., Bush, D., Wright, J. & Fillery-Travis, A.J. (2000). Gastric response to increased meal viscosity assessed by echo-planar magnetic resonance imaging in humans. *Journal of Nutrition*. 130: 122-127

Martin, T., Azpiroz, F. & Malagelada, J.R. (2005). Melatonin as a modulator of the ileal brake mechanism. *Scandinavian Journal of Gastroenterology*. 40(5): 559-563

McCann, M.J. & Stricker, E.M. (1986). Gastric emptying of glucose loads in rats: effects of insulin-induced hypoglycemia. *American Journal of Physiology*. 251: R609-R613

McGarry, J.D. & Foster, D.W. (1980). Regulation of hepatic fatty acid oxidation and ketone body production. *Annual Reviews in Biochemistry*. 49: 395-420

McHugh, P.R. & Moran, T.H. (1979). Calories and gastric emptying: a regulatory capacity with implications for feeding. *American Journal of Physiology*. 236: R254-R260

McHugh, P.R., Moran, T.H. & Wirth, J.B. (1982). Postpyloric regulation of gastric emptying in rhesus monkeys. *American Journal of Physiology*. 243: R408-R415

McLaughlin, J., Luca, M.G., Jones, M.N. & D'amato, M. (1999). Fatty acid chain length determines cholecystokinin secretion and effect on human gastric motility. *Gastroenterology*. 116: 46-53

Medhus, A.W., Sandstad, O., Bredesen, J. & Husebye, E. (2001). Delay of gastric emptying by duodenal intubation: sensitive measurement of gastric emptying by the paracetamol absorption test. *Alimentary pharmacology & therapeutics*. 13(5): 609-620

Meier, J.J. & Nauck, M.A. (2005). Glucagon-like peptide 1 (GLP-1) in biology and pathology. *Diabetes/Metabolism Research and Reviews*. 21: 91-117

Meyer, J.H., Go, Y.G., Jehn, D. & Taylor, I.L. (1988). Intragastric vs. intraintestinal viscous polymers and glucose tolerance after liquid meals of glucose. *American Journal of Clinical Nutrition*. 48: 260-266

Meyer, J.H. (1987). Motility of the stomach and gastroduodenal junction. In: Johnson, L.R. (Ed). Physiology of the gastrointestinal tract. Volume 1. 2nd Ed. New York: Raven. pp. 613–629

Miller, M.J., Witherly, S.A. & Clark, D.A. (1990). Casein: a milk protein with diverse biologic consequences. *Proceedings of the Society for Experimental Biology and Medicine*. 195: 143-159

Minami, H. & McCallum, R.W. (1984). The physiology and pathophysiology of gastric emptying in humans. *Gastroenterology*. 86: 1592–610.

Moberg, S. & Carlberger, G. (1974). The effect on gastric emptying of test meals with various fat and osmolar concentrations. *Scandinavian Journal of Gastroenterology*. 9: 29-32

Moran, T.H., Smedh, U., Kinzig, K. P., Scott, K.A., Knipp, A. & Ladenheim, E.E. (2005). Peptide YY(3-36) inhibits gastric emptying and produces acute reductions in food intake in rhesus monkeys. *American Journal of Physiology*. 288: 384-388

Moran, T.H. (2004). Gut peptides in the control of food intake: 30 years of ideas. *Physiology and Behaviour*. 82: 175–180

Moran, T.H., Wirth, J.B., Schwartz, G.J., & McHugh, P.R. (1999a). Interactions between gastric volume and duodenal nutrients in the control of liquid gastric emptying. *American Journal of Physiology*. 276(45): R997-R1002

Moran, T.H., Knipp, S., & Schwartz, G.J. (1999b). Gastric and duodenal features of meals mediate controls of liquid gastric emptying during fill in rhesus monkeys. *American Journal of Physiology*. 277(46): R1282-R1290

Moukarzel. A.A., & Sabri, M.T. (1996). Gastric physiology and function: effects of fruit juices. *Journal of the American College of Nutrition*. 15(5): 18S-25S

Murry, A.C.Jr., Gelaye, S., Casey, J.M., Foutz, T.L., Kouakou, B. & Arora, D. (1999). Type of milk consumed can influence plasma concentrations of fatty acids and minerals and body composition in infant and weanling pigs. *Journal of Nutrition*. 129(1): 132-138

Netzer, P., Schmitt, B. & Inauen, W. (2002). Effects of ABT-229, a motilin agonist, on acid reflux, oesophageal motility and gastric emptying in patients with gastro-oesophageal reflux disease. *Alimentary Pharmacology & Therapeutics*. 16(8) : 1481-1490

Omari, T.I., Barnett, C.P., Benninga, A., Lontis.R., Goodchild, L., Haslam, R.R., Dent,J., and Davidson, G.P. (2002). Mechanism of gastro-oesophageal reflux in preterm and term infants with reflux disease. *Gut*. 51: 475-479

Peracchi, M., Gebbia, C., Ogliari, C., Fraquelli, M., Vigano, R., Baldassarri, A., Bianchi, P.A. & Conte, D.(2000). Influence of caloric intake on gastric emptying of solids assessed by C-13-octanoic acid breath test. *Scandanavian Journal of Gastroenterology*. 35(8): 814–818

Park, Y W. (1994). Hypo-allergenic and therapeutic significance of goat milk. *Small Ruminant Research* 14: 151-159

Park, Y.W. (1991). Relative buffering capacity of goat milk, cow milk, soy-based infant formulas, and commercial nonprescription antacid drugs. *Journal of Dairy Science*. 74: 3326-3333

Pessler, F. & Nejat, M. (2004). Anaphylactic reaction to goat's milk in a cow's milk-allergic infant. *Pediatric Allergy and Immunology*. 15(2): 185-185

Pozler, O., Neumann, D., Vorisek, V., Bukac, J., Bures, J., and Kokstein, Z. (2003). Development of gastric emptying in premature infants: use of the C-13-octanoic acid breath test. *Nutrition*. 19(7): 593-596

Raybould, H. E. (1999). Nutrient tasting and signaling mechanisms in the gut I. Sensing of lipid by the intestinal mucosa. *American Journal of Physiology*. 277(40) : G751-G755

Raybould, H., Zittel, T., Holzer, H., Kent Lloyd, K.C. & Meyer, J.H. (1994). Gastroduodenal sensory mechanisms and CCK in inhibition of gastric emptying in response to a meal. *Digestive Diseases and Sciences*. 39(12): 41S-43S

Ray, T.K., Mansell, K.M., Knight, L.C., Malmud, L.S., Owen, O.E. & Boden, G. (1983). Long-term effects of dietary fiber on glucose tolerance and gastric emptying in noninsulin-dependent diabetic patients. *American Journal of Clinical Nutrition*. 37(3): 376-381

Read, N.W. (1994). Feedback Regulation and Sensation. *Digestive Diseases and Sciences*. 39(12): 37S-40S

Read, N.W. & Houghton, L.A. (1989). Physiology of gastric emptying and pathophysiology of gastroparesis. *Gastroenterology Clinics of North America*. 18: 359- 373

Ritz, M.A., Fraser, R., Edwards, N., Di Matteo, A., Chapman, M., Butler, R., Cmielewski, P., Tournadre, J-P., Davidson, G.& Dent, J. (2001). Delayed gastric emptying in ventilated critically ill patients: measurement by ^{13}C-octanoic acid breath test. *Critical Care Medicine*. 29(9): 1744-1749

Sadik, R., Abrahamsson, H., & Stotzer, P. O. (2003). Gender differences in gut transit shown with a newly developed radiological procedure. *Scandinavian Journal Of Gastroenterology, 38*(1), 36-42

Samsom, M., Vermeijden, J. R., Smout, A., van Doorn, E., Roelofs, J., van Dam, P. S., et al. (2003). Prevalence of delayed gastric emptying in diabetic patients and relationship to dyspeptic symptoms - a prospective study in unselected diabetic patients. *Diabetes Care, 26*(11), 3116-3122

Santangelo, A., Peracchi, M., Conte, D., Fraquelli, M., & Porrini, M. (2004). Physical state of meal affects gastric emptying, cholecystokinin release and satiety. *British Journal of Nutrition*. 80(6): 521-527

Sarna, S.K. (1985). Cyclic motor activity; migrating motor complex. *Gastroenterology*. 89: 894-913

Savage, A.P., Adrian, T.E., Carolan, G., Chattenjee, V.K. & Bloom, S.R. (1987). Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers. *Gut*. 28: 166-170

Scarpignato, C (1990). Gastric emptying measurements in man. In: Scarpignato, C. & Bianchi Porro (Eds): *Clinical Investigation of Gastric Function. Frontiers in Gastrointestinal Research,* Vol 17. Basel. Karger. pp. 198-246

Schiller, L.R. (1983). Motor function of the stomach. In M. H. F. Sleisenger, J. S. (Ed.), *Gastrointestinal disease, pathophysiology, diagnosis, management. 3rd edition*. London: W. B. Saunders Company. pp. 521 537

Schreiner, R.L. Brady, M.S., Ernst, J.A. & Lemons, J.A. (1982). Lack of lactobezoars in infants given predominantly whey protein formulas. *Archives of pediatrics & adolescent medicine*. 136(5): 437-439

Schneeman, B.O. (2002). Gastrointestinal physiology and functions. *British Journal of Nutrition*. 88: S159-S163

Schwartz, S.E., Levine, R.A., Weinstock, R.S., Petokas, S., Mills, C.A. & Thomas, F.D. (1988). Sustained pectin ingestion: effect on gastric emptying and glucose tolerance in non-insulin-dependent diabetic patients. *American Journal of Clinical Nutrition*. 48(6): 1413-1417

Schwizer, W., Fox, M., & Steingotter, A. (2003). Non-invasive investigation of gastrointestinal functions with magnetic resonance imaging: Towards an "ideal" investigation of gastrointestinal function. *Gut,* 52(6), 34

Sheik, S.P. (1991). Neuropeptide Y and peptide YY: major modulators of gastrointestinal blood flow and function. *American Journal of Physiology*. 261(5): G701-G715

Siegel, J. A., Urbain, J. L., Adler, L. P., Charkes, N. D., Maurer, A. H., Krevsky, B., et al. (1988). Biphasic nature of gastric-emptying. *Gut, 29*(1), 85-89

Sleisenger, M.H. & Fordtran, J.S. (Eds). (1993). *Gastrointestinal Disease 1.* Philadelphia: W.B.Saunders Company. pp. 321-329

Stangellini, V., Borovicka, J. & Read, N.W. (1994). Feedback regulation and sensation. *Digestive Diseases and Sciences*. 39(12): 124S-127S

Tack, J., Depoortere, I., Bisschops, R., Delporte, C., Coulie, B., Meulemans, A., Janssens, J. & Peeters, T. (2005). Influence of ghrelin on interdigestive gastrointestinal motility in man. *Gut*. Oct (ahead of print)

Taylor, I.L. (1993). Role of peptide YY in the endocrine control of digestion. *Journal of Dairy Science*. 76: 2094-2101

Tschop, M., Wawarta, R., Riepl, R.L., Friedrich, S., Bidlingmaier, M., Landgraf, R. & Folwaczny, C. (2001). Postprandial decrease of circulating human ghrelin levels. *Journal of Endocrinological Investigation*. 24(6): RC19–RC21

Van Den Driessche, M., Peeters, K., Marien, P., Ghoos, Y., Devlieger, H., & Veereman-Wauters, G. (1999). Gastric emptying in formula-fed and breast-fed infants measured with the C-13-octanoic acid breath test. *Journal Of Pediatric Gastroenterology And Nutrition, 29*(1): 46-51

Vantrappen, G. (1994). Methods to study gastric emptying. *Digestive Diseases and Science*. 39(12): 91S-94S

Vesa, T.H., Marteau, P.R., Briet, F.B., Flourie, B., Briend, A., & Rambaud, J.C. (1997). Effects of milk viscosity on gastric emptying and lactose intolerance in lactose maldigesters. *American Journal Of Clinical Nutrition.* 66(1): 123-126

Visser, F.R., Gray, I.K. & Williams, M.M.F. (1991). Composition of New Zealand Dairy Products. Wellington. Dairy Advisory Bureau, New Zealand Dairy Board. pp. 13

Vivatvakin, B. & Buachum, V. (2003). Effect of carob bean on gastric emptying time in Thai infants. *Asia Pacific Journal of Clinical Nutrition.* 12(2): 193-197

White, W., Schwartz, G. & Moran, T. (1999). Role of endogenous CCK in the inhibition of gastric emptying by peptone and intralipid in rats. *Regulatory Peptides.* 88: 47–53

West, J.B. (1990). Best and Taylor's Physiological Basis of Medical Practice, 12th Edition. London. Williams & Wilkins. pp. 45-50

Wingate, D.L., Stacher, G., & Kreiss, C. (1994). Role and integration of mechanisms controlling gastric emptying. *Digestive Diseases and Sciences.* 39(12): 120S-123S

Wood, J.D., Alpers, D.H. & Andrews, P.L.R. (1999). Fundamentals of neurogastroenterology. *Gut.* 45: 6-16

Wren, A.M., Small, C.J., Ward, H.L., Murphy, K.G., Dakin, C.L. Taheri, S., Kennedy, A.R., Roberts, G.H., Morgan, D.G., Ghatei, G.A. & Bloom, S.R. (2000). The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology.* 141: 4325-4328

Wyse, C.A., Yam, P.S., Slater, C., Cooper,J.M., & Preston, T. (2003). A comparison of the rate of recovery of $^{13}CO_2$ in exhaled breath with ^{2}H in body water following ingestion of [$^{2}H/^{13}C$] octanoic acid in a dog. *Research in Veterinary Science.* 74(2):123-127

Chapter 3

Comparison of the effectiveness of two types of container used for the collection of expired air samples in the ^{13}C octanoic acid breath test

Prepared for submission to a methodology journal

The ^{13}C octanoic acid breath test requires expired air samples to be collected in evacuated containers. There are a number of containers that can be used for this purpose. The aim of the experiment reported in this chapter was to compare two commonly used container types for their ability to provide a suitable environment for the collection of uncontaminated (by residual air) breath samples.

3.1 Abstract

The ^{13}C octanoic acid breath test involves the collection of breath samples for analysis by ratio isotope mass spectrometry for the ratio of ^{13}C to ^{12}C. Air samples are usually collected into sealed test tubes that contain a vacuum. Two types of tube are widely available for this purpose; Exetainer® (Labco Ltd, High Wycombe, UK) and Vacutainer® (BD Vacutainer Sytems, Plymouth, UK). The aim of this experiment was to evaluate the integrity of the vacuum in each brand so that the most appropriate container could be used for the ^{13}C octanoic acid breath test. Integrity of vacuum was tested in ten containers from each brand by piercing the seal of the container with the needle of a syringe containing enough water to fill the entire container. The amount of vacuum displaced by water was determined by weighing and related to the total amount of water that the container would hold.

The test showed that the Exetainer® tubes had a greater vacuum compared to the Vacutainer® ($P<0.001$), and were thus better suited for collection of breath samples when using the ^{13}C octanoic acid breath test.

3.2 Introduction

The ^{13}C-Octanoic acid breath test has been used extensively as a tool for assessing the rate of gastric emptying in both clinical and research settings (Pozler *et al.*, 2003; Omari *et al.*, 2002; Van Den Driessche *et al.*, 1999, Maes *et al.*, 1998). The method is based on the ratio of ^{13}C to ^{12}C present in expired air is collected from subjects for analysis by ratio isotope mass spectrometry. It is often necessary to despatch the samples to an off-site location for analysis. This necessitates the use of suitable containers for transit of breath samples. It is technically difficult to produce a container that has a complete vacuum, i.e. has no residual air, and is easy to pierce with a needle. A number of problems may occur with the containers currently used. Air may either enter between the glass and the bung during storage, or may enter by leakage through the needle puncture in the stopper, which is made during sampling. Entry of air via the sides of the bung may also be facilitated by its loosening from the loss of vacuum as a result of sampling.

A number of researchers have opted to use evacuated containers that are normally used for the collection of blood samples; one commonly used such brand is the

Vacutainer® (Becton Dickinson Vacutainer Systems, Plymouth, United Kingdom), (see Figure 3A). A male fitting rubber bung preserves the vacuum in Vacutainer® tubes. Another container, the Exetainer® (Labco Ltd, High Wycombe, United Kingdom, 838W), (see Figure 3A) differs from the Vacutainer® in that the male fitting rubber bung is retained by an encircling plastic collar, which prevents loosening when the vacuum is lost. Detailed enquiries to the manufacturers of both these containers revealed that no data was available regarding the extent of vacuum that was initially applied, the volume of air that was withdrawn, any variation in this volume or the extent to which the contained vacuum was reduced over time. This information is essential in order to standardize for dilution of the breath samples with residual air in these tubes.

The purpose of this technical note is to describe work conducted to assess the volume of residual air present in both Vacutainer® and Exetainer® tubes, and the variation between tubes.

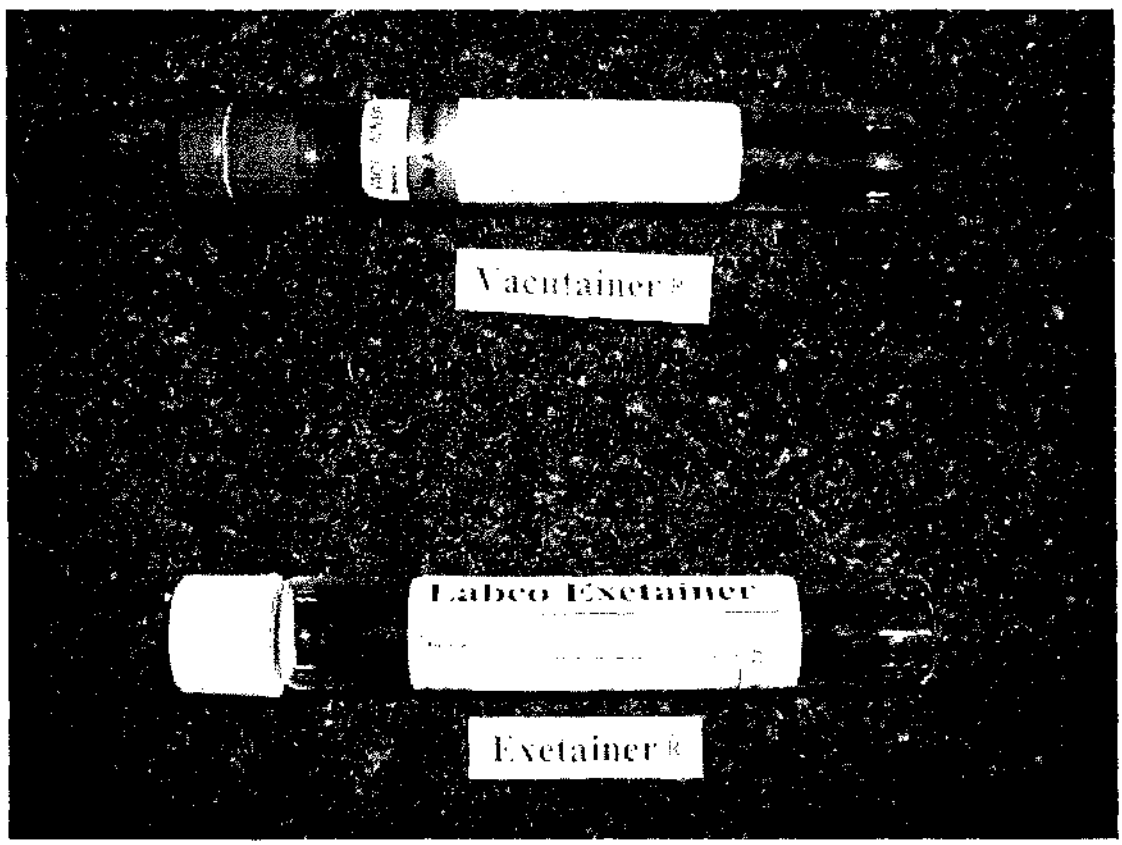


Figure 3A. Two brands of commonly used gas collection vials. Vacutainer® (top) and Exetainer® (bottom).

3.3 Method

The extent of vacuum in a set of randomly selected tubes was tested by determining the volume of water drawn from a syringe (Becton Dickinson, Drogheda, Ireland) loaded with 20ml of water at room temp following puncture of the tube bung with a 0.8 x 25 mm needle (Becton Dickinson, Precision Glide 21G 1 TW, Singapore). The results from ten randomly selected Exetainers were compared to those from ten randomly selected Vacutainers.

The selected containers of both types were equilibrated for ten minutes at 23 °C. Fifty ml of distilled water was drawn up in a syringe via the needle from a water bath maintained at 23 °C. Each container was weighed (average (±SE) of 13.51 ± 0.009g and 9.05 ± 0.002g for Exetainers and Vacutainers respectively). The needle of the syringe was inserted through the rubber bung of the container and the container left for one min so as to allow water from the syringe to be drawn into the container. Each container was then re-weighed (Initial Volume).

Following this procedure, each container was filled completely with water at 23.5 °C so as to abolish any air space when the bung had been reinserted. This gave the Total Volume of water that could be contained in each tube (room temperature was also recorded at 22.25°C). The Initial Volume drawn up was subtracted from Total Volume. The difference between the two weights was approximately equal to the volume of air that had been removed to create the vacuum, thus the amount of water that was drawn into the tube was inversely proportional to the amount of air remaining in the tubes when the vacuum was dissipated.

The amount of water spontaneously drawn up from the syringe (Initial Volume) into each tube was expressed as a percentage of the Total Volume.

A Student's t test was used to statistically compare the percentages of water drawn up by each group of containers.

3.4 Results

The mean percentage (± SE) of the total vacuum volume as calculated from the amount of water spontaneously drawn up from the syringe was as follows:

- Exetainer® 92.9 ± 0.43%*
- Vacutainer® 85.1 ± 0.74%*.

* These results were statistically different – $P<0.001$ (Student's *t* test)

The amount of dead space in each of the types of tubes was calculated (from the completely filled volume) to be as follows:

- Exetainer® 0.85 ± 0.05 ml
- Vacutainer® 1.80 ± 0.11 ml

3.5 Conclusion

Both container types contained a significant amount of residual air following breach of the vacuum, which would dilute any breath samples drawn into the tube. The amount of residual air was lower in the Exetainer® than the Vacutainer® (0.85 ± 0.05ml versus 1.80 ± 0.11ml respectively). This experiment showed that if Vacutainer® tubes are used for the collection of breath samples, approximately 15% of the sample would be contaminated by the residual air in the collection tube, whereas using Exetainer® tubes would reduce that percentage to 7%.

Based on these results, the suitability of the Vacutainer® tube as a collection container for breath samples destined for ratio isotope mass spectrometry is questionable. The Vacutainer® is designed for "determinations requiring serum" (Packaging label, Becton Dickinson Vacutainer Systems, Plymouth, United Kingdom). Thus, the tube is specifically designed to draw up a specific volume of serum only, which is stated on the packaging as 10 ml. When collecting expired air samples, it is more important (for the test to be accurate) to collect a maximum amount (as close to 100% of the volume of the tube as possible) rather than a specific amount.

The Exetainer®, however, is specifically designed for the collection of breath samples, and had an observed vacuum that was closer to 100% of the available space (92.9% ± 1.8). Thus, by using the Exetainer® for the collection of breath samples, there is less dilution of the breath sample by air already present in the container.

In conclusion, the Exetainer® brand of container is more appropriate for the collection of breath samples for the ^{13}C octanoic acid breath test.

3.6 References

Maes, B.D., Hiele, M.I., Geypens, B.J., Ghoos, Y.F. & Rutgeerts, P.J. (1998). Gastric emptying of the liquid, solid and oil phase of a meal in normal volunteers and patients with Billroth II gastrojejunostomy. *European Journal of Clinical Investigation*. 28: 197-204

Omari, T.I., Barnett, C.P., Benninga, A., Lontis, R. Goodchild, L., Haslam, R.R., Dent,J., and Davidson, G.P. (2002). Mechanism of gastro-oesophageal reflux in preterm and term infants with reflux disease. *Gut*. 51: 475-479

Pozler, O., Neumann, D., Vorisek, V., Bukac, J., Bures, J., and Kokstein, Z. (2003). Development of gastric emptying in premature infants: use of the C-13-octanoic acid breath test. *Nutrition*. 19(7): 593-596

Van Den Driessche, M., Peeters, K., Marien, P., Ghoos, Y., Devlieger, H. & Veereman-Wauters, G. (1999). Gastric emptying in formula-fed and breast-fed infants measured with the ^{13}C-octanoic acid breath test. *Journal of Pediatric Gastroenterology and Nutrition*. 29: 46-51

Chapter 4

Gastric emptying of whole milks and infant formulas derived from either the goat or cow in healthy adult men

Prepared in paper format for submission to a peer-reviewed journal.

The gastric emptying of goat's milk compared to cow's milk has not been studied. In addition, studies comparing the rates of gastric emptying of infant formula and human breast milk in infants have not used a goat's milk-based infant formula. The aim of the experiment in the following study was to compare the rates of gastric emptying of whole goat's milk with whole cow's milk; and goat's milk infant formula with cow's milk infant formula in healthy men.

4.1 Abstract

The rate of gastric emptying and coagulating properties of goats' milk was compared with that of cow's milk by the ^{13}C octanoic acid breath test (^{13}C OABT) following consumption of doped test milks by men. In a cross-over study design 15 fasting healthy young males each received four test milks, namely whole cow's milk (WC), whole goats' milk (WG), cow's milk infant formula (CIF), and goats' milk infant formula (GIF) in random sequence. Milks were administered (in a scaled dose according to metabolic body mass) at intervals of at least seven days to allow baseline levels of ^{13}C to return to normal.

The rate of gastric emptying of CIF was significantly faster ($P<0.05$) than that of GIF ($t_{1/2}$=120min vs. 159min), but there were no differences in the rate of emptying of WC and WG. CIF had the lowest $t_{1/2}$ (120 min), followed by WC (141 min), then WG (150 min), with GIF having the highest $t_{1/2}$ (159 min).

Vastly different coagulation properties were observed on *in vitro* acidification of milk samples to gastric pH (3). WC formed large curds with a clear separation between the whey-containing liquid and the curd whereas the WG and the GIF were more homogenous with finer curds and considerably less clear fluid. CIF exhibited the least difference in consistency with only a very fine curd visible.

Thus the slower GE rate of GIF is likely to be related to the higher percentage of casein compared to the CIF (81% vs. 46%) resulting in more coagulant being formed in the stomach and thus slowing gastric emptying.

Key words: milk; goat; cow; gastric emptying rate

4.2 Introduction

Milk from domesticated animals has been an important component in the human diet for centuries. However, there are clear differences between species in milk composition (Webb et al., 1974). Taking into account the fact that the macronutrient composition of a meal can influence the rate of gastric emptying (Moukarzel and Sabri, 1996), it is possible that milks from different species would empty from the human stomach at different rates. A substantial amount of work has compared the gastric emptying (GE) of various milks (human breast milk vs. cow's milk infant formula) in the infant (Pozler *et al.*, 2003; Omari *et al.*, 2002; Van Den Driessche *et al.*, 1992; Billeaud *et al.*, 1990) whereas little comparison of GE has been made in adults. Most studies involving the GE of milk in adults have been comparisons between fluid milk and fermented milk (Sanggaard *et al.*, 2004; Mahe *et al.*, 1994).

A variety of factors influence the rate of gastric emptying, e.g. physical state (solids or liquid) and volume of the meal, macronutrient character, and pH (Schneeman, 2003; Moran, 1999; Lin 1992; Hunt & Stubbs, 1979). Whilst the GE of solids, liquids and fats has been separately described, that of milk (which coagulates in the stomach) has not been adequately described. Milk is a complex food consisting of an aqueous fluid with proteins, fat globules, sugars and minerals suspended in it (Jensen, 1991). Moreover, once milk comes into contact with the acidic environment of the stomach, its consistency changes. The casein precipitates out of solution forming a coagulant with fragments of insoluble protein suspended in the aqueous phase, whereas the whey remains in solution (Fox *et al.*, 2004; Hall *et al* ., 2003).

Whilst the constituents of milk remain similar across species, proportions of these constituents vary. For example, the proportions of fat in whale milk (53% fat for the Gray whale) is significantly higher than that found in human breast milk (2% fat) (ACS, 2004). Cow's milk is of broadly similar composition to goats' milk but there are specific differences that may affect the rate of GE. For example, goat's milk has a higher percentage of medium chain triglycerides (8 – 12 carbons) and triglycerides over a chain length of 12 carbons are known to induce cholecystokinin (CCK) secretion which inhibits GE (McLaughlin *et al.*, 1999).

The purpose of this work was to compare the rate of gastric emptying of whole goats' milk (WG) to whole cow's milk (WC) using the ^{13}C octanoic acid breath test (^{13}C

OABT). As a positive control a goats' milk infant formula was compared with a whey predominant cow's milk infant formula. It would be expected that the whey predominant formula would empty more quickly than the casein predominant goats' milk formula as previous studies have shown whey to empty from the stomach at a faster rate compared to casein (Fox *et al.*, 2004; Hall *et al.*, 2003).

4.3 Materials and Methods

In vitro acidification

In vitro acidification was carried out on all four milks in order to determine how the physical characteristics would change in the gastric environment. Samples (100ml) of each of the test milks were mixed into a 150ml beaker and warmed to 37°C. Hydrochloric acid (1 molar) was added to the milk, with constant stirring, until the milk dropped to pH 3. One ml of prepared pepsin (Merck 64271, Darmstadt, Germany) was added and the mixture incubated at 37.5°C for 30 min in constant motion on a shaker board. After 30 minutes, the appearance of the mixtures was noted and photographs were taken.

Rheometric testing of the acidified milks was attempted, but the coagulated milk did not offer sufficient resistance for the rheometer to register a reading.

Gastric Emptying

Given that the adult stomach allows for greater effects to be detected because of the greater amounts of fluid that they can ingest at one time (this is particularly important in light of the fact that the comparison was between foods with such similar compositions) it was decided to test for differences in GE rates in adult men. The ^{13}C OABT has been used in a number of studies to measure gastric emptying of milk in infants (Pozler *et al.*, 2003; Omari *et al.*, 2002; Van Den Driessche *et al.*, 1992) and is considered safe, non-invasive and practical (Ghoos *et al.*, 1993a).

Fifteen healthy men between 18 and 40 years of age with a Body Mass Index (BMI) between 19 and 30 were recruited for the trial. All subjects gave their informed written consent to take part in the trial, and the trial was approved by the Massey University Human Ethics Committee (Protocol No. 04/19). The subjects had no history of diabetes mellitus or previous gastrointestinal surgery; they were not using

any medication that could affect gastric motility; were non-smokers; and did not suffer from lactose intolerance or any known milk allergy.

The study was conducted as a randomized double-blind four-way crossover. Thus each subject received all four milks in a unique, random sequence on a series of test days. Each test day was followed by a wash-out period of at least seven days to allow residual ^{13}C from the test drinks to be eliminated.

The four milks tested were: Whole cow's milk (WC) (Anchor Instant Milk Powder, New Zealand Dairy Foods, Ltd, Auckland, New Zealand), whole goats' milk (WG) (Dairy Goat Co-operative (N.Z) Ltd., Hamilton, New Zealand), goats' milk infant formula (GIF) (Nanny, Dairy Goat Co-operative (N.Z) Ltd., Hamilton, New Zealand), and whey modified cows milk infant formula (CIF) (S-26, Wyeth Nutritionals Inc. Georgia, USA). Each of the four milks was reconstituted from powder on the morning of each test day according to manufacturers' instructions and warmed in a water bath to a mean temperature of 34.7°C (SD 0.94) for approximately fifteen minutes. The volume of milk that was administered was standardized according to the metabolic body weight of each subject so as to account for differences in stomach size (Moughan *et al.*, 1992; Kleiber, 1975). Thus, each subject received 9.8 ml of milk per kg metabolic body weight (BW to the power of 0.75); a 75-kg man would thereby receive 250 ml of milk. Each test drink was doped with 100µL of 99-atom % octanoic-1-^{13}C acid (Isotec, Miamisburg, OH, USA) after which it was shaken then returned to the warm water bath for ten minutes before being given to the subjects to drink.

The nutrient composition of the test milks was determined at Massey University's IANZ-accredited Nutrition Laboratory (see Table 4A). The whey and casein ratios were determined by an independent laboratory (Fonterra Research Centre, Palmerston North, New Zealand) using the SDS PAGE method. The gross composition and ingredients of the four test milks as per package labelling are shown below.

Table 4A. *Nutrient composition of test milks[1,2] (units per 100ml) to be used in a gastric emptying study with adult men.*[2]

Component	CIF	GIF	WC	WG
Energy (kJ)	281	290	262	262
Energy (cal)	67	69	63	62.5
Protein (g)	1.5	1.5	3.4	3.3
Carbohydrate (g)	7.2	7.6	4.6	4.4
Fat (g)	3.6	3.6	3.4	3.5
Calcium (mg)	46	66	120	116
Sodium (mg)	18	24	42	31.3

[1] when reconstituted as advised by manufacturer (CIF: 14. 0g + 100ml water, GIF: 14. 8g + 100ml water, WC: 11. 33g + 100ml water; WG: 12. 5g + 100ml water)

[2] CIF = Cow's Milk Infant Formula (S-26, Wyeth Nutritionals Inc., Georgia, USA); GIF = Goat's Milk Infant Formula (Nanny, Dairy Goat Co-operative of New Zealand, Hamilton, New Zealand); WC = Whole Cow's Milk (Anchor Instant Milk Powder, New Zealand Dairy Foods, Ltd. Auckland, New Zealand); WG = Whole Goat's Milk (Dairy Goat Co-operative of New Zealand: Hamilton, New Zealand)

Table 4B. *Ingredients of test milks used in a gastric emptying study in adult men.*

Milk*	Ingredients
CIF	Skimmed milk; reduced minerals whey; vegetable oils [palm, soybean, coconut and oleic (safflower or sunflower)]; lactose; soy lecithin; L-cysteine; taurine; NUCLEOTIDES (cytidine-5'-monophosphate; disodium uridine-5'-monophosphate; adenosine-5'-monophosphate; disodium inosine-5'-monophosphate; disodium guanosine-5'-monophopshate); calcium chloride; potassium bicarbonate; sodium and potassium citrates; potassium hydroxide; ferrous sulphate; sodium chloride; zinc sulphate; calcium hydroxide; copper sulphate; potassium chloride; manganese sulphate; potassium iodide; sodium selenite; Vitamin C; vitamin E; calcium pantothenate; vitamin A; vitamin B2; Vitamin B1; vitamin B6; natural beta-carotene; folate; vitamin K; biotin; vitamin D3; vitamin B12.
GIF	Goat milk solids, lactose, vegetable oils, choline chloride, taurine, tri-sodium citrate, calcium hydroxide, ferrous sulphate, copper sulphate, tri-potassium citrate, zinc sulphate, manganese sulphate, sodium selenite, potassium iodide, citric acid, ascorbic acid, dl-alpha-tocopheryl acetate, retinyl acetate, vitamin K1, cholecalciferol, niacinamide, calcium d-pantothenate, thiamin hydrocholirde, pyridonxine hydrocholoride, riboflavin-5-phosphate, folic acid, biotin, cyanocobalamin..
WC	Dried whole cow's milk (99.4%), lecithin (0.6%)
WG	Dried whole goat's milk.

* As advised by manufacturers; CIF = Cow's Milk Infant Formula (S-26, Wyeth Nutritionals Inc. Georgia, USA); GIF = Goat's Milk Infant Formula (Nanny, Dairy Goat Co-operative of New Zealand, Hamilton, New Zealand); WC = Whole Cow's Milk (Anchor Instant Milk Powder, New Zealand Dairy Foods, Ltd, Auckland, New Zealand); WG = Whole Goat's Milk (Dairy Goat Co-operative of New Zealand; Hamilton, New Zealand)

No water was consumed for two hours prior to the test or during the test. Subjects were asked to refrain from eating foods that contained naturally high proportions of ^{13}C (such as corn and confectionery-based products) for three days before each test day so as to minimise baseline levels of ^{13}C in the body.

All tests commenced at 8 am in the morning following an overnight fast of at least 8h. The room temperature was maintained at 24°C and exchange of room air with fresh air was continued throughout the experimental period so as to remove expired ^{13}C from the air.

Subjects were asked to consume the milk in one minute. Time zero was taken as the time the subject had consumed the entire drink. For the duration of the test, subjects

were seated in comfortable chairs or at a desk; they were allowed to stand or go to the toilet if required.

Breath samples were collected in evacuated containers (Exetainer®,Labco Ltd, High Wycombe, UK) every ten minutes for the first two hours after consumption of the milks, and every fifteen minutes for the following two hours. Each subject's exhaled air was collected in a 4L rubber anaesthetic bag (Jorgen Kruuse, Marslev, Denmark, 600140) via a mouthpiece and a one-way valve system (Instrumentation Industries, Inc., Bethal Park, USA, BE 117). Collection was continued until the bag was perceived to be full. Three separate samples were removed via a needle port in the bag; the first was discarded so as to flush the collecting system, and the two subsequent samples were retained for analysis.

The ratio of ^{13}C to ^{12}C in the expired air samples was determined by ratio isotope mass spectrometry (Europa Scientific Ltd, Crewe, UK) carried out by the Waikato University Stable Isotope Laboratory. The delta values (that relate the actual ratio of ^{13}C to ^{12}C in the sample to an international standard which is the ratio of ^{13}C to ^{12}C in Pee Dee Belemnite Limestone) were used to calculate the percentage of administered dose recovered per hour as done by Ghoos et al (1993a,b; 1988). The CO_2 production of subjects was assumed to be 300mmol/m^2 of body surface area per hour based on literature values (Ghoos *et al*, 1993a,b; 1988). Body surface area was calculated using the weight-height formula of Haycock *et al* (1978).

Curve fitting for the variation of percentage dose recovered with time was carried out in the NLIN sub routine of Systat using two equations developed by Ghoos *et al.* (1993a). The two equations were as follows:

$$y = at^b e^{-ct} \qquad (A)$$

where y is the percentage of dose recovered in breath per hour, t is the time in minutes, and a, b and c are constants.

$$y = mk\beta e^{-kt}(1-e^{-kt})^{\beta-1} \qquad (B)$$

where y is the percentage of ^{13}C excretion in breath per hour, t is time, k and ß are constants and m is the total cumulative ^{13}C recovered when time is infinite. Equation B is the first derivative of the modified power exponential formula of Siegel *et al.* (1988).

In all instances, the best fitting curve according to the value of the corrected R^2 was used for further calculations. Curves with unacceptable fits ($R^2 < 0.7$) were excluded. The values of the constants obtained from this curve fitting procedure were then used to calculate gastric emptying parameters. These parameters were the time to maximum excretion (t_{max}) and the gastric emptying half time ($t_{1/2}$) which is the estimated time it would take for half the dose of ^{13}C to be excreted (half the area under the curve) (Ghoos *et al.*, 1993a,b). Other researchers have applied a correction factor of 60 min to account of the time taken for metabolism of labelled octanoic acid bound to solids (Ghoos *et al.*, 1993a). Braden *et al* (1995) used a correction factor of 55 min for semisolids and 49 mins for liquids when using the ^{13}C acetate breath test. In the current study, there was insufficient information available to make a decision as to whether a correction factor was necessary, or indeed, which correction factor would have been most appropriate. Consequently, no correction factor was applied to the current set of results. Given that the results were to be compared in a relative manner, the need for a correction factor was lessened.

The physiological parameters were derived as follows: $t_{1/2}$= GAMMAINV[1] (0.5;b+1;1/c) (Using Microsoft Excel XP) i.e. the area under the fitted curve until half of the dose of ^{13}C is excreted when time is infinite. T_{lag} = b/c (A) or (lnß)/k (B) depending on which equation was used.

The gastric half-emptying time and t_{max} were determined by the shape of the curve and were assumed to be independent of endogenous CO_2 production (Maes *et al.*, 1998).

4.4 Results

The results of compositional analyses of the four milk powders are shown in Table 4C. Analyses were conducted on the milks in powder form. The values shown in the table are for the milks as they would have been in the reconstituted state (as the subjects would have received them). Both infant formulas had higher energy content than the whole milks (due to the higher lactose content, as well as higher fat contents).

[1] GAMMAINV is a function in Microsoft Excel

Table 4C: *Compositional analysis of test milks[1] (units / 100ml) when reconstituted according to manufacturers' instructions[2]*

Milk[2]	Energy (kJ/100ml)	Lactose (g/100ml)	Fat (g/100ml)	Crude Protein (g/100ml)	Ash (g/100ml)
CIF	294.43	6.5	3.49	1.56	0.33
GIF	282.89	6.45	3.16	1.45	0.45
WC	235.99	3.39	2.75	2.64	0.57
WG	251.44	3.64	3.00	2.89	0.33

1 - CIF=Cow's Milk Infant Formula, GIF=Goat's Milk Infant Formula, WC=Whole Cow's Milk, WG=Whole Goat's milk.

2 - CIF: 14. 0g + 100ml water, GIF: 14. 8g + 100ml water, WC: 11. 33g + 100ml water; WG: 12. 5g + 100ml water

CIF was a whey dominant infant formula, and as such had a greater percentage of whey compared to the other three milks tested (Table 4D). GIF, WC and WG all had similar ratios of whey to casein (~20:80).

Table 4D. *Determined[1] percentage of whey and casein in the test milks[2] used in a gastric emptying study conducted with adult men.*

Milk[2]	% Whey	%Casein
CIF	53.8	46.2
GIF	19.2	80.8
WC	18.4	81.6
WG	20.3	79.7

1 - As determined by SDS Page method (Fonterra Research Centre, Palmerston North, New Zealand).

2 - CIF=Cow's Milk Infant Formula, GIF=Goat's Milk Infant Formula, WC=Whole Cow's Milk, WG=Whole Goat's milk.

The fatty acid composition of the four test milks is detailed in Table 4E. WG and GIF had higher percentages of capric acid (C10) than both of the cow's milks (Table 4E). WG had a higher percentage of medium chain fatty acids compared to WC, especially those of chain length C8 and C10. CIF had a higher percentage of C12 fatty acids compared to the other three milks.

Table 4E. *Fatty acid composition (expressed as percentage of total fatty acid content[2]) of test milks[1] used in a gastric emptying study with adult men.*

	Percentage of Total Fatty Acid Content			
Fatty Acids	CIF	GIF	WC	WG
C 4:0 Butyric	5.1	4.7	5.7	5.5
C 6:0 Caproic	0.0	2.0	2.9	3.3
C 8:0 Caprylic	1.8	2.0	1.6	3.2
C 10:0 Capric	1.3	6.3	3.4	9.9
C 12:0 Lauric	10.2	3.0	4.1	4.8
C 14:0 Myristic	4.2	6.5	12.6	10.5
C 16:0 Palmitic	19.7	18.3	32.7	26.2
C 18:0 Steric	3.6	8.5	11.0	11.7
C 18:1-cis Oleic	33.4	26.2	17.8	18.5
C18:2 -cis Linoleic	18.5	18.0	1.5	2.2
C 18:3 linolenic	1.9	2.2	1.3	1.3
% < C 11	91.4	84.5	85.7	77.8
% < C 12	8.2	15.3	14.0	22.2

[1] - CIF Cow's Milk Infant Formula, GIF Goat's Milk Infant Formula, WC Whole Cow's Milk, WG Whole Goat's milk. -

[2] - As determined by the Massey University Nutrition Laboratory in 100g dry powder.

In vitro testing

The physical properties and appearance of the milks changed when the pH dropped below the isoelectric point of various proteins contained in the milk. WC displayed the most dramatic change with large curds forming surrounded by a clear fluid. GIF also coagulated with finer curd formation surrounded by cloudy fluid in contrast to the clear surrounding fluid of WC. The WG also coagulated but to a lesser extent than WC and GIF, and the curds were of a much finer nature. The CIF showed the least amount of coagulation with a fine curd forming.

Interestingly, the curd of WC did not float; instead remaining at the bottom of the beaker, whereas curd from the GIF and WG floated (there was a small amount of clear fluid at the bottom of the beaker). Also the visible proportion of clear fluid in WC was much larger than in the two goat milks (there was no separate clear fluid in CIF). The changes in appearance can be observed in Figure 4A-C

Figure 4A. From left to right: Cow's milk infant formula, whole cow's milk, goat's milk infant formula and whole goat's milk following addition of 1M HCl and pepsin, and incubation at 37.5°C for 30 min.

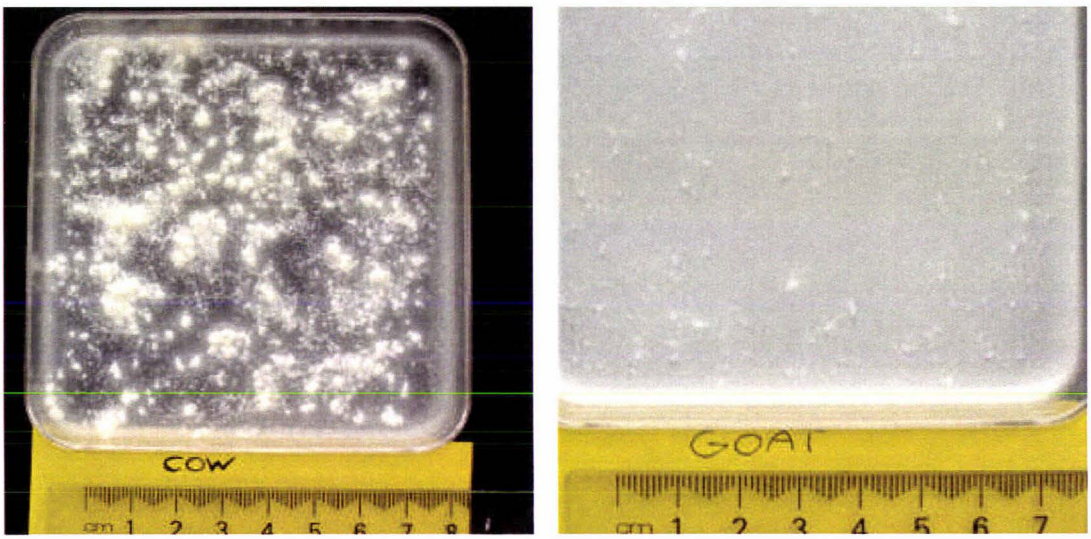


Figure 4B. One mm deep layer (to show the extent of casein precipitation) of whole cow's milk (left) and whole goat's milk (right) following addition of 1M HCl and pepsin, and incubation at 37.5° C for 30 min.

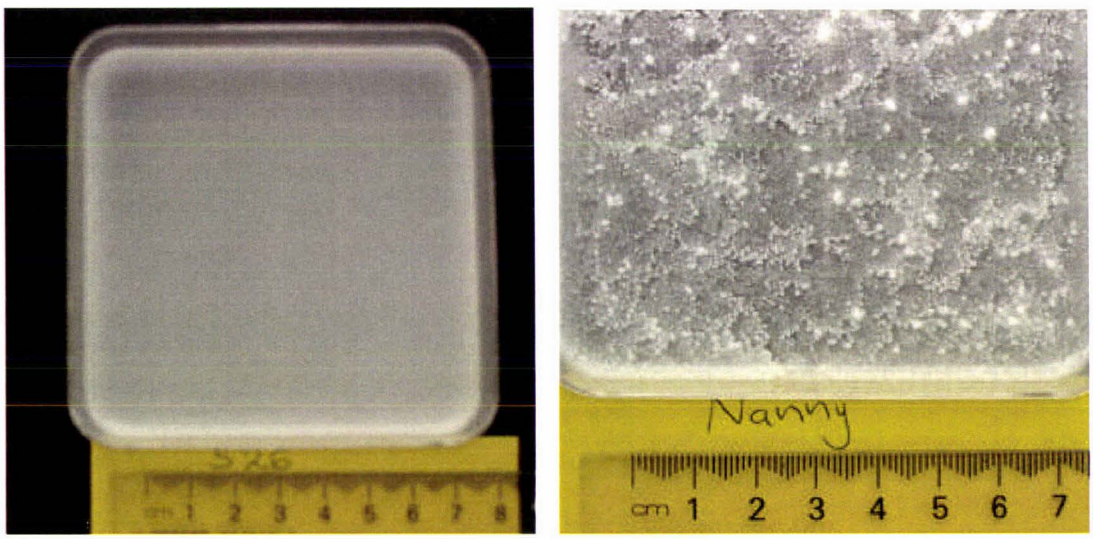


Figure 4C. One mm deep layer (to show the extent of casein precipitation) of cow's milk infant formula (left) and goat's milk infant formula (right) following addition of 1M HCl and pepsin, and incubation at 37.5°C for 30 min.

Gastric emptying

Subjects (n = 15) consumed each of the four test drinks in the requested one-minute time limit, with the exception of one who took five minutes to consume one of the beverages. No adverse effects were noted by any of the participants. Subjects acted as their own control (repeated measures). The data of six subjects was excluded from the statistical analysis. Two subjects received the incorrect dosage of octanoic acid on a test day, which meant the results from that day had to be disregarded. Two subjects were excluded based on an R square value of less than 0.7. An R square of less than 0.7 indicates that the model used did not accurately fit the data (>30% unexplained variability). Gastric emptying parameters obtained from such curves would be inaccurate. Two subjects were excluded based on their calculated $t_{1/2}$ values being greater than the duration of the experiment, i.e. four hours (one was greater than three standard deviations from the mean, the other greater than 1.7 standard deviations from the mean). As a consequence of the trial being a repeated measures design, if one day's data was excluded, then all data for that subject was excluded from the statistical analysis.

There were no significant differences in the rate of gastric emptying between WC and WG, either in $t_{1/2}$ or t_{max}. There were, however, significant differences in $t_{1/2}$ between the infant formulas (Table 4F). The $t_{1/2}$ of CIF was significantly lower than GIF (120 and 159 min respectively, P = 0.01). There was no difference in t_{max} for the two infant formulas.

Table 4F. *Gastric emptying half time ($t_{1/2}$*) and time to maximum excretion rate (t_{max}*) for the four test milks (mean ± SEM†).*

Parameter	CIF	GIF	WC	WG
t_{max} (min)	75 ± 4.63[a]	79 ± 5.10[a]	71 ± 4.40[a]	82 ± 7.16[a]
$t_{1/2}$ (min)	120 ± 6.52[a]	159 ± 11.02[b]	141 ± 11.56[ab]	150 ± 11.10[ab]

n = 9. * t_{max} = time to maximum $^{13}CO_2$ excretion; $t_{1/2}$ = time for excretion of half the dose of ^{13}C or half area under the $^{13}CO_2$ excretion curve. No correction factor has been applied to account for time taken to metabolize the ^{13}C octanoic acid.

† Means with different superscripts are significantly different (P<0.01).

The curves for the mean gastric emptying rates following the ingestion of the GIF and CIF are shown in Fig.4D. The lines were obtained by curve fitting of the $^{13}CO_2$ excretion data, expressed as percentage of ^{13}C dose excreted per hour. The gastric emptying curves for WG and WC were similar in shape to that of the curve of GIF.

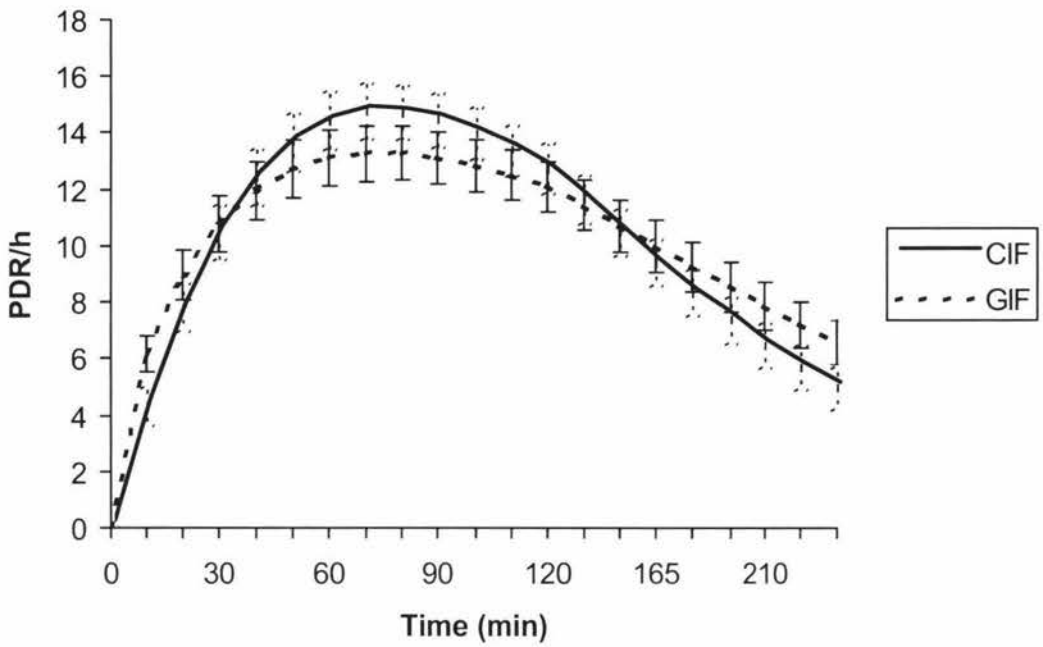


Figure. 4D. Fitted curves (with standard error bars) for Mean Percentage of administered dose of ^{13}C recovered in breath per hour for the two infant formulas (CIF = cow's milk infant formula, GIF = goats' milk infant formula). PDR/h = Percentage Dose of ^{13}C Recovered per hour. N=9

Although CIF had a higher peak excretion rate than GIF, the time it took for both of the infant formulas to reach peak excretion rate (t_{max}) was not significantly different (Table 4D). It is apparent from the shape of the curves that GIF had a quicker initial rate of gastric emptying compared to CIF, i.e. from zero to forty minutes; after which the excretion rate of CIF exceeded that of the GIF. However, at around 170 minutes, the rate of CIF once again dropped to below that of GIF, and remained lower until completion of the trial.

The fitted curves for mean percentage of dose of ^{13}C recovered per hour for WC and WG are presented in figures 4E and 4F.

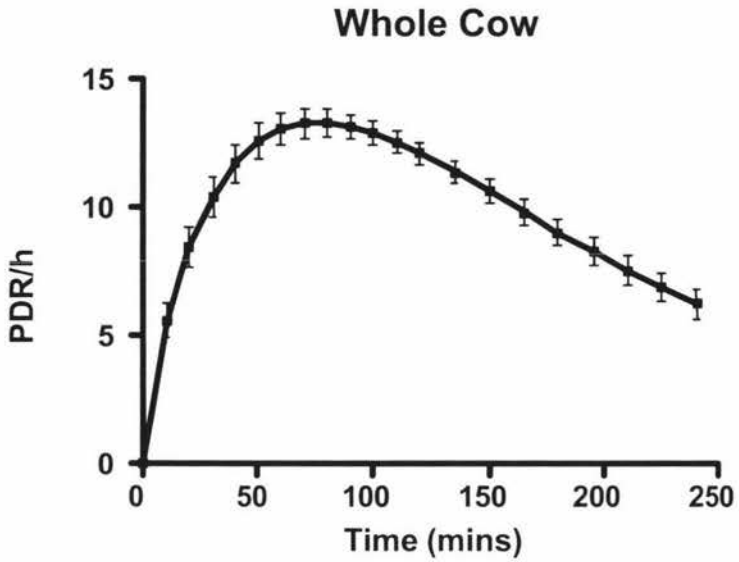


Figure 4E. Fitted curve (with std error bars) for Mean Percentage of administered dose of ^{13}C recovered in breath per hour for whole cow's milk (WC). PDR/h = Percentage Dose of ^{13}C Recovered per hour. N=9

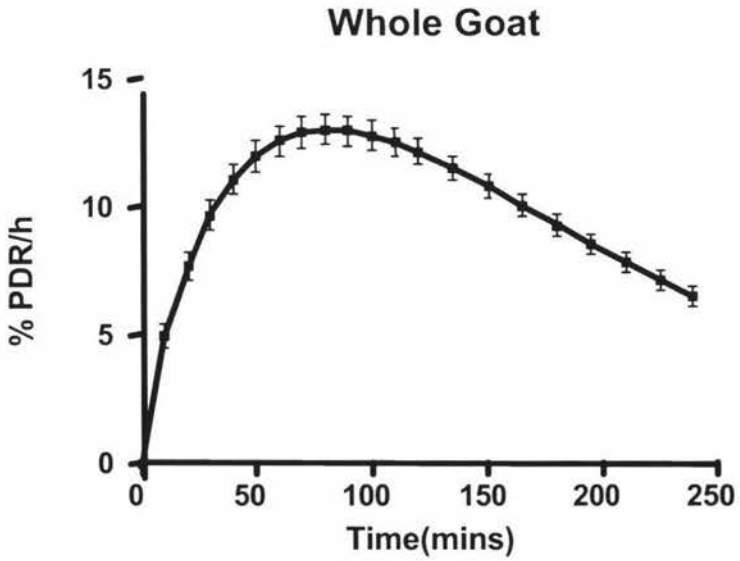


Figure 4F. Fitted curves(with std error bars) for Mean Percentage of administered dose of ^{13}C recovered in breath per hour for whole goat's milk (WG). PDR/h = Percentage Dose of ^{13}C Recovered per hour. N=9

4.5 Discussion

The main aim of this study was to compare the parameters describing GE of whole goats' milk and whole cow's milk. The same parameters for goats' milk based infant formula and cow's milk based infant formula were measured to test method sensitivity, i.e. as a positive control. The study showed that there was no difference in the rate of GE of the whole milks but confirmed significant differences between the two infant formulas. Differences in composition of the whole milks, such as a higher percentage of medium chain fatty acids and higher percentage total fat for WG, did not appear to be sufficient to affect GE.

The infant formulas differed slightly in energy content (CIF, 294kJ/100ml; GIF, 283kJ/100ml) and total fat (CIF, 3.49g/100ml, GIF, 3.16g/100ml). The major difference between the two infant formulas, however, was that the GIF had a much larger percentage of casein in relation to whey (81% for GIF vs 46% for CIF). Casein is known to coagulate in the acidic environment of the stomach (Hall *et al.*, 2003; Billeaud *et al.* 1990; Miller *et al.*, 1990) resulting in a slower rate of gastric emptying compared to whey protein.

The photographs taken of the *in vitro* experiment offer visual support of the coagulation phenomenon. After being subjected to *in vitro* testing conditions, CIF maintained a homogenous consistency with no visible separation between the aqueous and coagulated phases whereas the GIF clearly separated into two distinct phases, a coagulated phase floating above a clear layer of fluid. The absolute amount of casein present in the GIF compared to the CIF may have been responsible for these differences and possibly for the differences in rate of GE found between the infant formulas. Further, and given that pylorus is a powerful regulator of gastric emptying acting as the gatekeeper at the junction between the stomach and the duodenum (Brown *et al.*, 1993), these differences (in GE) are likely to be more significant in the stomach of the infant as the size of the infant's pyloric sphincter is much smaller than that of an adult male and they would presumably face the same physical challenge of emptying the casein-based curds.

The digestion of bovine casein in calves involves gastric rennet (acting on the α and κ fractions) promoting irreversible formation of curds. Curd formation in calves ensures the slow release of casein into the small intestine, where it is rapidly digested

(Miller *et al.,* 1990). When humans, who are not known to secrete rennet, ingest bovine milk, however, the acid in the stomach merely precipitates casein out of the emulsion and this process is reversible upon alkalinization (Miller *et al.,* 1990); it is not technically speaking a true curd. Because human milk does not contain α-casein, and the infant does not secrete rennet in the stomach, it is possible that the precipitate that forms during digestion of a cow's milk-based infant formula could be delivered to the small intestine at a rate that exceeds the infant's digestive capacity (Miller *et al.,* 1990). Thus, it is possible that influx of milk proteins into the small intestine results in a temporary metabolic burden, which may be especially relevant in the infant gut.

To date there appear to be no other studies that have compared the GE of goats' milk with that of cow's milk. The infant studies reported in the literature have all focused on comparison of infant formula to breast milk. Also, the volume of the test meal has such a large effect on gastric emptying studies that it is difficult to compare results across studies when the volumes ingested are not the same.

It should be noted that the type of marker used to measure the GE rate of milk is also not standardized. Sanggaard *et al.,* (2004) used ^{13}C-acetate as a marker for their breath test, which (as stated in their paper) would have marked more of the aqueous phase of the milk due to its high solubility. The ^{13}C OABT has been used in numerous studies to measure the GE of milk, mainly infant formula versus breast milk, in infants (Pozler *et al.,* 2003; Omari *et al.*, 2002; Van Den Driessche *et al.*, 1999). ^{13}C-octanoic acid has a solubility of 68mg/100g at 20°C in water and would presumably partition into the milk fat globules when added to test drinks. This is important, as it is an established fact that the liquid, solid and oil phase of meals empty from the stomach differently (Maes *et al.,* 1998; Brown *et al.,* 1993; Edelbroek *et al.,* 1992). The question then has to be asked, does the ^{13}C OA get trapped in the casein coagulant, or exit from the stomach with the aqueous phase? This could not be determined in the present study.

A limitation of the method used to measure the rate of GE in this study was the assumption that all subjects produce CO_2 at a rate of 300mmol/m^2 body surface area per hour (pg 65). Real CO_2 production may fluctuate (King & Toskes, 1981), and the test is therefore semiquantitative (Ghoos *et al.*, 1988). The digestion, absorption and metabolism of food requires energy; thus when food is ingested it is accompanied by a subsequent increase in metabolic rate and in heat production by the body known as

the thermic effect of food (Crovetti *et al.*, 1997; Tai et al., 1991). Proteins are known to have the highest thermogenic effect (20-30%) followed by carbohydrate (5-10%) and fat (0-3%) (Raben *et al.*, 2003). This has implications for this study in that the test milks had different macronutrient compositions (Table 4A, pg 63), and therefore could have elicited different thermic effects in the subjects, i.e. could have also influenced the rate of carbon dioxide production. This is likely to have introduced some error into the results, especially in an absolute sense. However, comparisons were made within the different treatments which had similar macronutrient compositions, i.e. GIF vs. CIF or WC vs. WG, so a relative interpretation of the results is still valid.

With regards to other limitations of the study; unfortunately, the statistical approach associated with excluding subjects limited the power of the study and has highlighted a weakness in the design that would need to be modified in future experiments.

One aspect of this study that differed from other studies of gastric emptying using the ^{13}C-OABT is that the amount of milk each subject received was adjusted according to their metabolic body weight (body weight raised to the power of 0.75). The size of the stomach increases in proportion to body size (Moughan *et al.*, 1992). Therefore, to control for volume (which has a large effect on GE), the amount of milk that each subject ingested had to be standardised on a body weight basis.

The present study showed that the protein profile of milk does affect the rate at which it empties from the stomach. It was expected that other factors such as the fatty acid profile and energy content might have stronger influences over the rate of GE. In this instance, however, the physical form of gastric chyme was a more important modulator of gastric emptying than the nutrient composition or energy density. In conclusion, there was no difference in rate of gastric emptying between whole cow's milk and whole goats' milk. Therefore, any nutritional differences that might exist between goats' milk and cow's milk are not likely to be linked to the rate of gastric emptying. The same cannot be said for the infant formula based on either goats' or cow's milk, and specifically when comparing casein versus whey predominant formulas, as it is clear from this study that whey predominant cow's milk infant formula has a faster rate of GE compared to goats' milk infant formula in healthy adult males.

4.6 References

American Cetacean Society Fact Sheet. (Internet) http://www.acsonline.org/factpack/grayWhale/grayWhale.pdf

Billeaud, C., Guillet,J., & Sandler, B (1990). Gastric emptying in infants with or without gastro-oesophageal reflux according to the type of milk. *European Journal of Clinical Nutrition.* 44: 577-583

Braden, B., Adams, S., Duan, L-P., Orth, K-H., Maul, F.D.& Lembcke, B. (1995). The [^{13}C] acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. *Gastroenterology.* 108: 1048-1055

Brown, B.P., Schulze-Delrieu, K., Schrier, J.E. & Abu-Yousef, M.M. (1993). The configuration of the human gastroduodenal junction in the separate emptying of liquids and solids. *Gastroenterology.* 105: 433-440

Crovetti, R., Porrinin, M., Santangelo, A. & Testolin, G. (1997). The influence of thermic effect of food on satiety. *European Journal of Clinical Nutrition.* 52: 482-488

Edelbroek, M., Horowitz, M., Maddox, A. & Bellen, J. (1992). Gastric emptying and intragastric distribution of oil in the presence of a liquid or a solid meal. *Journal of Nuclear Medicine.* 33: 1283-1290

Fox, M., Goergi, G., Boehm, G., Menne, D., Fried, M. & Thumshirn, M. (2004). Dietary protein precipitation properties have effects on gastric emptying in healthy volunteers. *Clinical Nutrition.* 23: 641-646

Ghoos, Y.F., Maes, B.D., Geypens, B.J., Mys, G., Hiele, M.I., Rutgeerts, P.J. & Vantrappen, G. (1993a). Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. *Gastroenterology.* 104: 1640-1647

Ghoos, Y., Geypens, B., Maes, B., Hiele, M., Vantrappen, G. & Rutgeerts, P. (1993b). Breath tests in gastric emptying and transit studies: Technical aspects of $^{13}CO_2$-Breath Tests. In: Janssens, J. (Ed). Progress in understanding and management of gastro-intestinal motility disorders. pp. 169-180. Belgium. Kuleuven.

Ghoos, Y., Rutgeerts, P., Hiele, M. & Vantrappen, G. (1988). Use of Stable Isotopes in Gastroenterology: $^{13}CO_2$ Breath Tests. Klin. Ern. 34: 52-61

Hall, W.L., Millward, D.J., Long, S.J., & Morgan, L.M. (2003). Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *British Journal of Nutrition.* 89: 239-248

Haycock, G.B., Schwartz, G.J. & Wisotsky, D.H. (1978). Geometric method for measuring body-surface area - height-weight formula validated in infants, children, and adults. *Journal Of Pediatrics.* 93(1), 62-66

Hunt, J.N. & Stubbs, D.F. (1979). The volume and energy content of meals as determinants of gastric emptying. *Journal of Physiology.* 245:209-225

Jensen, I.G., Ferris, A.M. & Lammi-Keefe, C.J. (1991). The composition of milk fat. *Journal of Dairy Science.* 74: 3228-3243

King, C. & Toskes, P. (1981). Alteration of CO_2 production during nonfasting isotopic CO2 breath tests: Concise communication. *Journal of Nuclear Medicine.* 22: 955-958

Kleiber, M. (1975). The fire of life: an introduction to animal energetics. Huntington, New York: R. E. Krieger Pub. Co. pp. 55-57

Lin, H.C., Elashoff, J.D., Gu, Y.G. & Meyer, J.H. (1992). Effect of meal volume on gastric emptying. *Journal of Gastrointestinal Motility.* 4: 157-163

Maes, B.D., Hiele, M.I., Geypens, B.J., Ghoos, Y.F. & Rutgeerts, P.J. (1998). Gastric emptying of the liquid, solid and oil phase of a meal in normal volunteers and patients with Billroth II gastrojejunostomy. *European Journal of Clinical Investigation.* 28: 197-204

Mahe, S., Marteau, P., Huneau, J-F., Thuillier, F. & Tome, D. (1994). Intestinal nitrogen and electrolyte movements following fermented milk ingestion in man. *British Journal of Nutrition.* 71: 169-180

McLaughlin, J., Luca, M.G., Jones, M.N., D'Amato, M., Dockray, G.J. & Thompson, D.G. (1999). Fatty acid chain length determines cholecystokinin secretion and effect on human gastric motility. *Gastroenterology.* 116(1): 46-53

Millller, M.J.S., Witherly, S.A. & Clark, D.A. (1990). Casein: a milk protein with diverse biologic consequences. *Proceedings of the Society for Experimental Biology and Medicine.* 195: 143-159

Moran, T.H., Wirth, J.B., Schwartz, G.J. & McHugh, P.R. (1999). Interactions between gastric volume an dduodenal nutrients in the control of liquid gastric emptying. *American Journal of Physiology.* 276: R997-R1002

Moughan, P.J., Birtles, M.J., Cranwell, P.D., Smith, W.C. & Pedraza, M. (1992). The piglet as a model animal for studying aspects of digestion and absorption in milk-fed human infants. *World Review of Nutrition and Dietetics.* 67: 40-113

Moukarzel. A.A., & Sabri, M.T. (1996). Gastric physiology and function: effects of fruit juices. *Journal of the American College of Nutrition.* 15(5): 18S-25S

Omari, T.I., Barnett, C.P., Benninga, A., Lontis.R., Goodchild, L., Haslam, R.R., Dent,J., and Davidson, G.P. (2002). Mechanism of gastro-oesophageal reflux in preterm and term infants with reflux disease. *Gut.* 51: 475-479

Pozler, O., Neumann, D., Vorisek, V., Bukac, J., Bures, J., and Kokstein, Z. (2003). Development of gastric emptying in premature infants: use of the c-13-octanoic acid breath test. *Nutrition.* 19 (7-8): 593-596

Raben, A., Agerholm-Larsen, L., Flint, A., Holst, J.J. & Astrup, A. (2003). Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. *American Journal of Clinical Nutrition.* 77: 91-100

Sanggaard, K.M., Holst, J.J., Rehfeld, J.F., Sandstrom, B.Raben, A., & Tholstrup, T. (2004). Different effects of whole milk and a fermented milk with the same fat and lactose content on gastric emptying and postprandial lipaemia, but not on glycaemic response and appetite. *British Journal of Nutrition.* 92: 447-459

Schneeman, B.O. (2002). Gastrointestinal physiology and functions. *British Journal of Nutrition.* 88(Suppl. 2): S159-S163

Siegel, J. A., Urbain, J. L., Adler, L. P., Charkes, N. D., Maurer, A. H. & Krevsky, B. (1988). Biphasic nature of gastric-emptying. *Gut, 29*(1), 85-89

Tai, M.M., Castillo, P. & Pi-Sunyer, F. X. (1991). Meal size and frequency: effect on the thermic effect of food. *American Journal of Clinical Nutrition*. 54: 783-787

Van Den Driessche, M., Peeters, K., Marien, P., Ghoos, Y., Devlieger, H., & Veeremean-Wauters, G. (1999). Gastric emptying in formula-fed and breast-fed infants measured with the ^{13}C-octanoic acid breath test. *Journal of Pediatric Gastroenterology and Nutrition*. 29: 46-51

Webb, B.H., A.H. Johnson and J.A. Alford. 1974. Fundamentals of Dairy Chemistry. Second Ed. AVI Publishing Co., Westport, CT., Chap. 1. pp. 17

Chapter 5

Plasma glucose levels in response to the ingestion of milks from different species

The results reported in chapter four showed that a whey-dominant cow's milk infant formula emptied from the stomach at a faster rate than a goat's milk infant formula. The rate of appearance of absorbed nutrients in the blood is related, in the first instance, to the rate of gastric emptying. If milk from a particular species had a faster rate of gastric emptying compared to milk from another species in man, then one would expect there to be a concomitant faster initial appearance of glucose in blood plasma. The purpose of this experiment was to observe the change in concentration of plasma glucose levels following the consumption of milk from different species and to relate that to the rates of gastric emptying obtained in chapter four.

5.1 Abstract

Fifteen healthy adults males had an indwelling cannula inserted into a vein in the forearm prior to the ingestion of one of four different test milks: whole goat's milk (WG); whole cow's milk (WC); goat's milk infant formula (GIF); or cow's milk infant formula (CIF). Three blood samples were drawn from the cannula prior to the ingestion of the test milk, and samples were drawn at regular intervals thereafter for a further four hours. Blood samples were analysed for plasma glucose levels. The plasma glucose levels for the four test milks were compared across treatments and in relation to the gastric emptying results from chapter four. The results showed that a faster rate of gastric emptying might be related to a higher initial plasma glucose peak and a lower subsequent drop to below baseline levels.

5.2 Introduction

In this part of the study, temporal variations in blood glucose concentrations were examined in order to relate gastric emptying to the rate of appearance of blood metabolites following the ingestion of food.

Systemic blood glucose levels rise following the digestion and absorption of foods containing available carbohydrate, and subsequently fall in response to the action of insulin (Korach-Andre *et al.*, 2004; Schenk *et al.*, 2003). The digestion of the milk sugar lactose yields glucose and galactose.

It was postulated that if the rate of gastric emptying of goat's milk and cow's milk differed, there might be a consequential difference in the subsequent postprandial rise in systemic plasma glucose concentrations.

5.3 Materials and Methods

Plasma glucose measurements were taken in conjunction with the collection of breath samples in the ^{13}C octanoic acid breath test for gastric emptying described in Chapter Four. Thus, subject selection and the trial protocol were as described in Chapter Four. Subject numbers were reduced from fifteen to nine after six data sets were excluded; two as a result of experimental error and four as a result of non-fitting (based on the

value of R^2) gastric emptying curves. Test meals were consumed at 8 am following an overnight fast so as to ensure minimal chrono-biological differences in the results.

A venous cannula (BD Insyte™ 16 GA 1.7 x 30mm, 220ml/sec Catheter; Becton Dickinson Infusion Therapy Inc., Utah, USA) was inserted into a suitable vein in the forearm under aseptic conditions by a medical practitioner at the start of the experiment. The cannula site was secured to the arm with an adherent cover (Teraderm™ I.V. 7cm x 8.5cm, 3M Health Care, St Paul, MN, USA). Blood samples were drawn from the cannula using a blunt plastic cannula (Becton Dickinson Infustion Therapy Inc., Utah, USA) via a Baxter Interlink® system (Baxter Healthcare Corporation, deerfiled, IL, USA) following cleaning of the sampling port with a MediSwab™ pre-injection swab (Smith & Nephew Pty, Ltd, Auckland, New Zealand).

Prior to a blood sample being drawn, the cannula was flushed with 3ml of saline (Sodium Chloride injection BP 0.9%, Astra Zeneca Ltd, Auckland, New Zealand) and the first 3ml of blood withdrawn from the sampling port subsequently discarded so as to avoid inadvertent dilution with residual saline. A sample was then drawn into a fresh syringe and transferred into a 10ml EDTA tube (BD Vacutainer Sytems, Plymouth, UK) and subsequently stored in a refrigerator at 4°C pending completion of the test.

Three baseline blood samples were drawn via the cannula prior to ingestion of each test drink to establish mean baseline values. Following consumption of each test drink, blood samples were drawn every ten minutes during the first two hours, and every fifteen minutes during the subsequent two hours. All blood samples were centrifuged for ten minutes at 3500 rpm at 4°C, and the supernatant plasma pipetted into Cryos Cellstar® tubes (Greiner bio-one, RayLab, Auckland, New Zealand) and frozen. The glucose concentration in plasma was determined using the hexokinase method (Peterson & Young, 1958). Duplicate samples were analysed on a Cobas Fara II analyser (Hoffman La Roche, Berel, Switzerland) using a commercial diagnostic kit obtained from Roche Diagnostics, New Zealand Ltd.

The mean of the baseline values was subtracted from each plasma glucose value in order to examine any change in plasma glucose concentration relative to baseline values (relative blood glucose concentration). The rate of fall of blood glucose

concentration from the peak value to the minimum value was calculated and compared between treatments. Numerous other aspects of the relative blood glucose plots were investigated and compared, such as the area under the curve above baseline and time to peak, but no meaningful data was obtained. Changes in relative plasma glucose concentrations were instead compared graphically with gastric emptying curves from the ^{13}C octanoic acid breath test (OABT).

5.4 Results

The mean relative blood glucose levels of all subjects showed an initial rise following ingestion of the milks, and a subsequent fall to below baseline levels. The levels remained below baseline for the remainder of the experiment period on each test day, except for those following consumption of the cow's milk infant formula, which periodically returned to baseline (Fig. 5A)

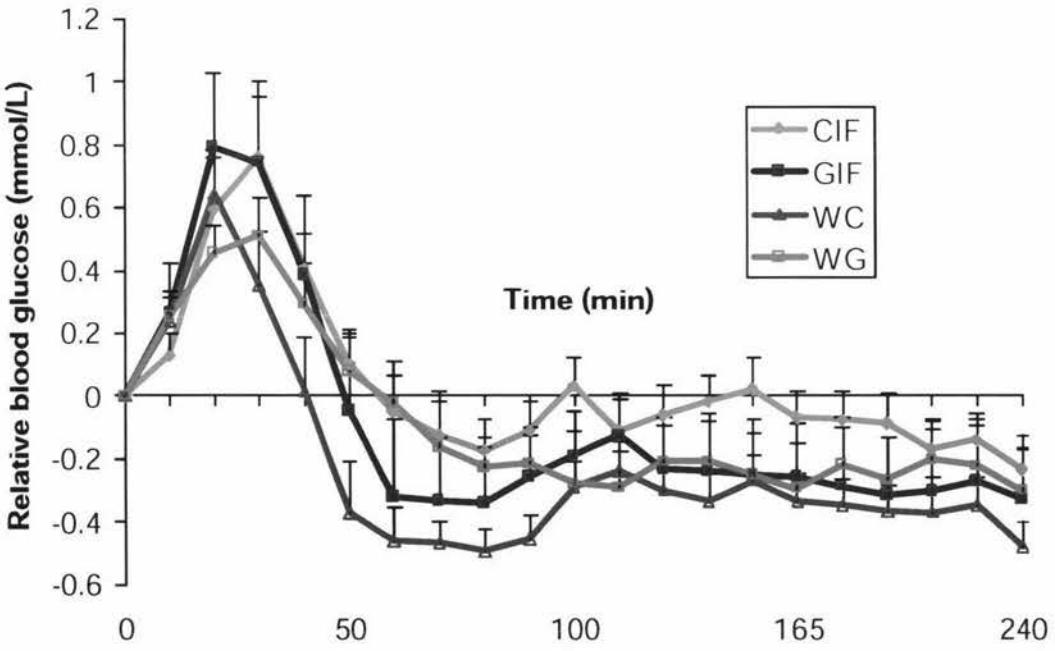


Figure 5A. Mean relative plasma glucose concentration curves (+ SE bars) for four different test milks (CIF = Cow's milk infant formula, GIF=Goat's milk infant formula, WC=Whole cow's milk, WG=Whole goat's milk) consumed in a single meal by adult males after an 8-hour overnight fast. (n =9)

The plasma glucose concentration observed following consumption by adult men of both infant formulas rose to higher peak values than those for whole milk. Plasma glucose concentration peaked around 20 to 30 minutes after ingestion and returned to below baseline values between 40 to 60 minutes post-meal. There was a subsequent slight rise and fall in plasma glucose levels between 80 to 120 minutes for all milks.

The blood glucose concentrations of the subjects fell from the peak value to the minimum value at similar rates, 0.0186 mmol/min for CIF, 0.0189 mmol/min for GIF, 0.0190 mmol/min for WC and 0.0113 mmol/min for WG.

It was not possible to fit curves to the relative blood glucose data (table 5A), eliminating the possibility of directly comparing the effect that the different milks had on blood glucose concentration. The combined plots of the fitted ^{13}C excretion curves and the relative plasma glucose concentration (RPG) plots, used for this comparison are illustrated in Figures 5 B and C on the following pages.

Table 5A. *Mean relative blood glucose concentrations (with standard deviation, standard error of the means and maximum and minimum values) of healthy men (n=9) following the consumption of four test milks*[1].

Time	CIF	GIF	WC	WG
0	0.00	0.00	0.00	0.00
10	0.13	0.26	0.24	0.25
20	0.59	0.79	0.64	0.46
30	0.76	0.74	0.35	0.51
40	0.41	0.38	0.01	0.29
50	0.10	-0.05	-0.37	0.08
60	-0.05	-0.32	-0.47	-0.02
70	-0.13	-0.33	-0.47	-0.16
80	-0.17	-0.34	-0.50	-0.22
90	-0.11	-0.26	-0.46	-0.22
100	0.03	-0.19	-0.29	-0.28
110	-0.11	-0.13	-0.24	-0.29
120	-0.06	-0.23	-0.30	-0.21
135	-0.02	-0.24	-0.34	-0.21
150	0.02	-0.25	-0.27	-0.25
165	-0.07	-0.26	-0.33	-0.29
180	-0.08	-0.29	-0.35	-0.22
195	-0.09	-0.32	-0.37	-0.27
210	-0.17	-0.30	-0.37	-0.20
225	-0.14	-0.27	-0.35	-0.22
240	-0.23	-0.33	-0.48	-0.30
Std Deviation	0.255	0.344	0.304	0.254
Std Error of mean	0.085	0.115	0.101	0.085
Maximum value	0.76	0.79	0.64	0.51
Minimum value	-0.23	-0.34	-0.50	-0.30

[1] - CIF = Cow's milk infant formula, GIF=Goat's milk infant formula, WC=Whole cow's milk, WG=Whole goat's milk.

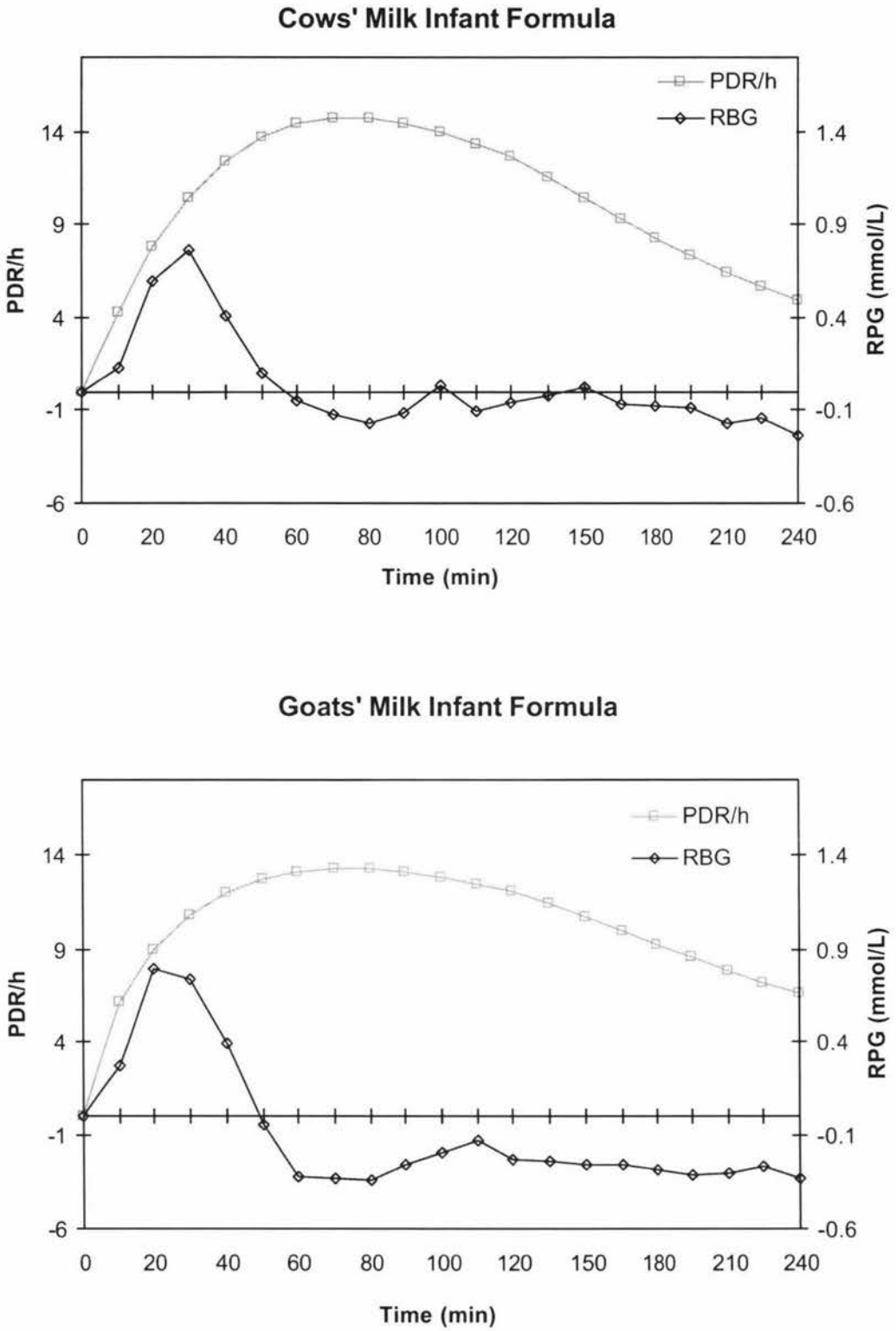


Figure 5B. Combined plots of the ^{13}C excretion curves (?) and relative plasma glucose concentration (?) plots over a 240-minute collection period following ingestion of goat's milk infant formula (top) and cow's milk infant formula (bottom) (n=9). The left y-axis is for the Percentage of Dose of ^{13}C Recovered /hour (PDR/h) and the right y-axis is for the Relative Plasma Glucose Concentration in mmol/litre (RPG (mmol/l)).

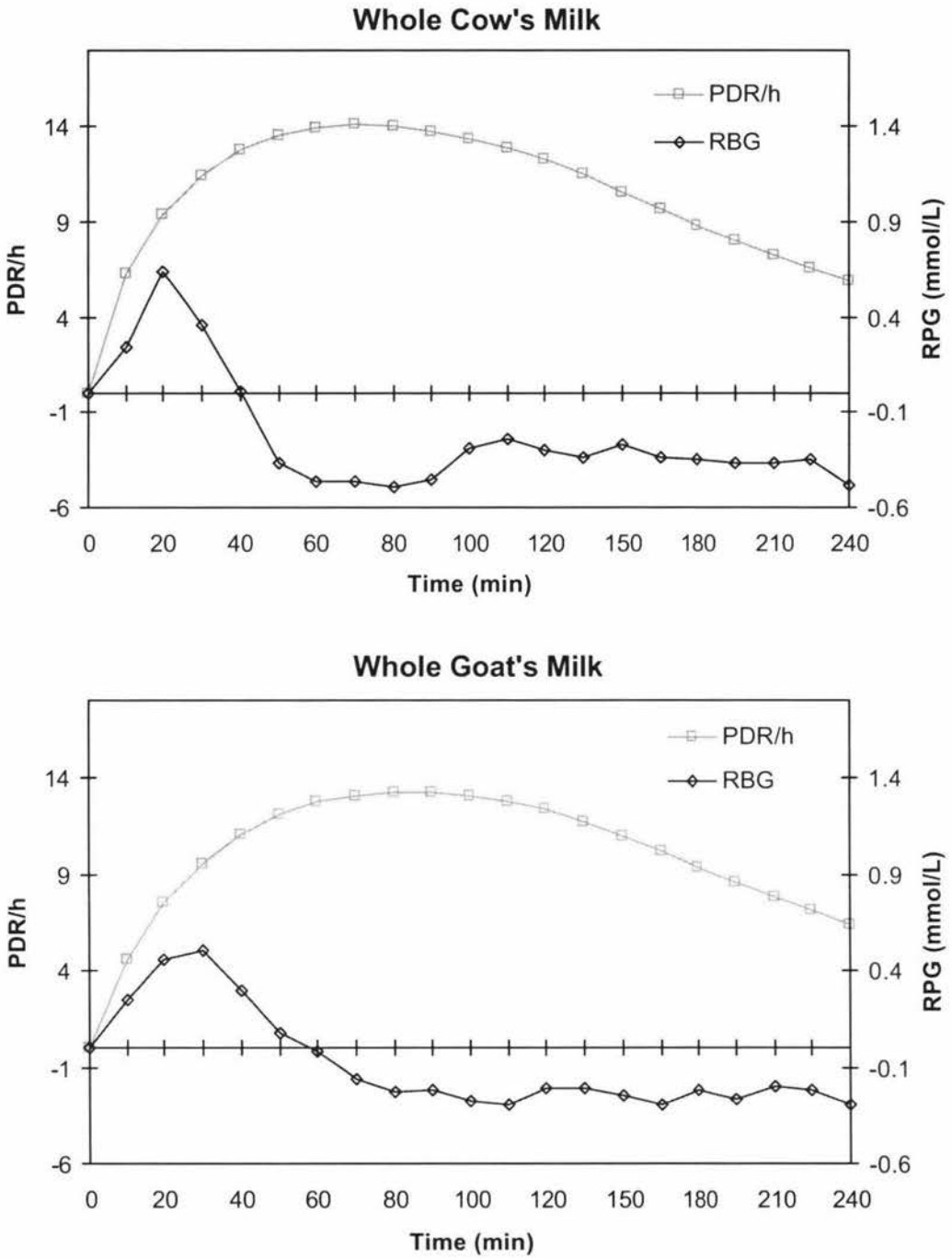


Figure 5C. Combined plots of the ^{13}C excretion curves (?) and relative plasma glucose concentration (?) plots (over a 240-minute collection period following ingestion of whole cow's milk (top) and whole goat's milk (bottom). The left y-axis is for the Percentage of Dose of ^{13}C Recovered /hour (PDR/h) and the right y-axis is for the Relative Plasma Glucose Concentration in mmol/litre (RPG (mmol/l)).

5.5 Discussion

The purpose of this study was to investigate the relationship between the rate of gastric emptying of milk and the subsequent appearance of glucose in blood plasma following the ingestion of various milks.

As there are no published equations that can be fitted to postprandial plasma glucose curves, we were unable to derive any governing parameters that could be used as a basis for comparisons between groups and individuals or for assessment of correlation with gastric emptying rates. Consequently, the analysis was limited to a graphic comparison only.

It should be noted that in the current study subjects received a volume of milk that was standardised according to metabolic body weight so as to give equal gastric emptying rates (see pg 62). Thus, each subject received a different volume of milk and a different dose of lactose. This prevented meaningful comparisons of gastric ejection rate using the area under the relative glucose concentration curves either between subjects or between treatments. Moreover, each infant formula contained approximately twice the quantity of lactose than that found in the whole milks. Thus, comparisons were limited to those within whole milks or within the infant formulas.

If the rates of appearance of glucose in the blood were predominantly influenced by the rate of gastric emptying, it would be expected that the blood glucose curves would have a similar shape to that of the gastric emptying curves. Differences in the shape of the plasma glucose concentration plots compared to those of the gastric emptying curves indicated that they were not of the same form.

Although a direct comparison between rate of gastric emptying and rate of appearance of plasma glucose was not possible, a number of inferences may be drawn from the relationship between the relative plasma glucose plots and the respective gastric emptying curves when infant formulas have been consumed (Fig. 5B, pg 87). Gastric emptying half time of the CIF was significantly faster than the GIF (see Table 4F, pg 71 and fig 4D, pg 72). However, the initial rise of the gastric emptying curve of the GIF was somewhat steeper than the rise for that of the CIF, i.e. there was a faster initial rate of gastric emptying (see Fig 4D, pg 72). This correlates to a steeper initial rate of rise in RPG concentration and an earlier peak following the consumption of GIF compared to that of CIF (Fig 5A, pg 84). The plot for RPG concentration of GIF

peaked 10 minutes prior to that for the CIF (at 20 min) at a time when the CIF RPG concentration was well below that of the GIF (Fig 5A, pg 84). In addition, the glucose level profile of the GIF that produced an earlier glucose peak fell to lower levels than was seen following the ingestion of the CIF which had a later peak. Similarly, plasma glucose of WC peaked earlier than WG and again had a greater subsequent drop, although in this case there were no accompanying differences in rates of GE between these two milks (Table 4D, pg 67). These observations lead to the hypothesis that a faster initial rate of gastric emptying results in more prompt peaking of postprandial plasma glucose concentration and a proportionately greater subsequent fall to below baseline levels. The greater fall to below baseline levels when GIF was consumed is probably due to a greater incretin response (Nauck *et al.*, 1993; Shuster *et al.*, 1988), which is stimulated by the faster initial gastric emptying leading to a greater amount of food coming into contact with the absorptive surface area of the small intestine. Incretin has an insulinotropic effect that decreases postprandial blood glucose levels (Nauck *et al.*, 1993; Creutzfeldt & Nauck, 1992; Creutzfeld, 1979)

A recent study (Sanggaard *et al.*, 2004) also measured plasma glucose levels following the consumption of a much larger quantity of milk or fermented milk (Fig. 5D, pg 91). In their study, subjects were given 1400g of plain cow's milk or fermented milk and they found that gastric emptying of plain cow's milk was significantly faster than that of fermented milk. Whilst no significant differences were found between the two milks in the profile of postprandial glucose concentrations in the Sanggaard *et al.*, (2004) study, the greater volume of milk given (1400g vs 250g/75kg subject in the current study) would be expected to cause more rapid initial gastric emptying than that in the current study on the basis that greater gastric volumes elicit quicker gastric emptying (Moran *et al.*, 1999; Hunt *et al.*, 1985, 1979; McHugh & Moran, 1985). In concordance with the current study's hypothesis, the ingestion of the greater volumes of milk in Sanggaard *et al's* study (and probably faster initial gastric emptying rate) was accompanied by earlier plasma glucose peaks relative to those observed in the current study (15 min vs 20-30 min). Also, Sanggaard *et al.* (2004) observed a far greater subsequent fall below baseline compared to that in the current study (>-1.4mmol/L versus –0.5mmol/L). This would

appear to suggest that a faster rate of gastric emptying is linked to earlier glucose peaks and subsequently more extreme falls in plasma glucose concentrations.

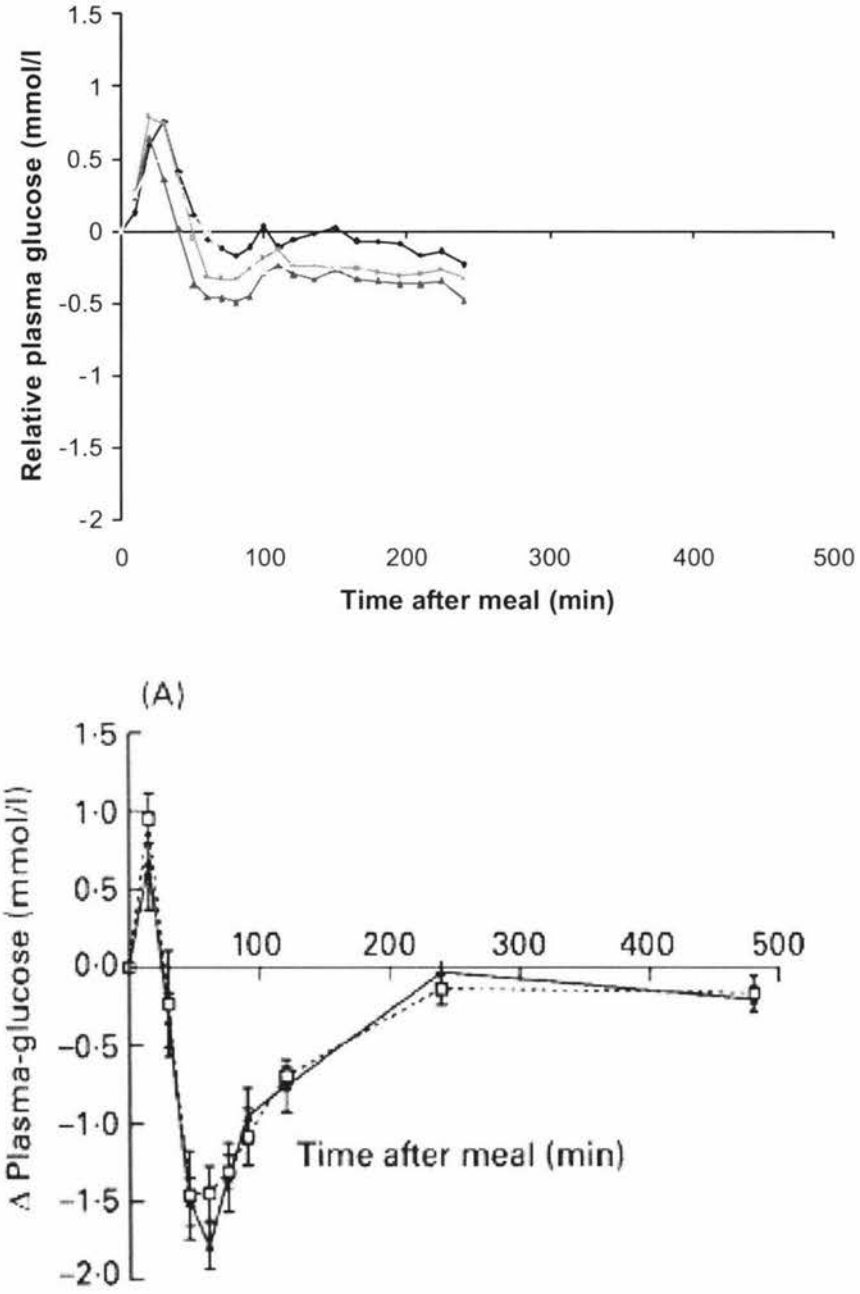


Figure 5D. Plasma glucose levels following the ingestion of milk in the present study (top) and in a similar study with whole milk and fermented milk (bottom) (Sanggaard *et al.*, 2004). The amount of milk fed in B was much higher than in A, yet the form of the curves up to 240 min is similar although glucose levels took longer to near baseline levels in B. Levels have an earlier peak in B and a lower subsequent drop to below baseline levels. This could be related to the amount of insulin that was released in response to the peak levels.

Sanggaard *et al* (2004) fed their subjects whole milk, as occurred in the current study, effectively creating the situation where there are two experiments where subjects received the same food/nutrient density but at different intakes. All of the subjects in Sanggaard *et als'* study received 1400g regardless of body weight, whereas the subjects in the current study received 9.8ml/kg metabolic body weight such that a 75kg man would receive 250ml.

It is reasonable to assume in view of the relationship between gastric volume and gastric emptying rate (Guyton and Hall, 2001; Moran *et al.,* 1999; Hunt *et al.,* 1985, 1979; McHugh & Moran, 1985) that gastric emptying following ingestion of the 1400g dose of milk would have been substantially quicker that that following ingestion of the 9.8ml/kg metabolic body weight dose (around 250ml for a 75kg man). If we hypothesize that 'on flow' would occupy a greater volume of the small intestine following ingestion of the 1400g dose of milk, i.e. the milk would have travelled further down the intestine during the same time interval, this milk would have been in contact with greater absorptive surface area. Under such conditions, it is surprising that plasma glucose levels did not rise to a substantially higher peak following the larger dose of milk. Thus, the incretin response may have been engaged more promptly, a hypothesis that is supported by the greater fall below baseline levels, i.e. a larger "overshoot effect" following consumption of the larger dose of milk.

In conclusion, a direct correlation between the rate of gastric emptying and plasma glucose levels was not possible in the current study, most likely due to relatively small alterations in the rate of gastric emptying between the different milk treatments. When the results are compared with similar work (which used much larger volumes of milk and presumable engendered higher rates of gastric emptying), however, it is evident that the timing of the peaks of plasma glucose levels and subsequent drop varied.

It seems plausible that a faster initial rate of gastric emptying and accompanying change in the rate of on-flow into and through the small intestine elicits a greater incretin induced correction of rising glucose levels.

5.6 References

Creutzfeldt, W. & Nauck, M. (1992). Gut hormones and diabetes mellitus. *Diabetes Metabolism Reviews.* 8: 149-177

Creutzfeldt, W. (1979). The incretin concept today. *Diabetologia.* 16(2): 75-85

Ghoos, Y.F., Maes, B.D., Geypens, B.J., Mys, G., Hiele, M.I., Rutgeerts, P.J. & Vantrappen, G. (1993). Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. Gastroenterology 104: 1640-1647

Guyton, A.C. & Hall, J.E. (2001). *Medical Physiology* (10th ed.). London: W.B.Sauders Company. pp. 728-737

Hunt, J.N., Smith, J.L. & Jiang, C.L. (1985). Effect of meal volume and energy density on the gastric emptying of carbohydrates. *Gastroenterology.* 89: 1326-1330

Korach-André, M., Roth, H., Barnoud, D., Péan, M., Peronnet, F. & Leverve, X. (2004). Glucose appearance in the peripheral circulation and liver glucose output in men after a large ^{13}C starch meal. *American Journal of Clinical Nutrition.* 80(4): 881-886

Moran, T.H., Wirth, J.B., Schwartz, G.J. & McHugh, P.R. (1999). Interaction between gastric volume and duodenal nutrients in the control of liquid gastric emptying. *American Journal of Physiology.* 276: R997 – R1002

McHugh, P.R. & Moran, T.H. (1979). Calories and gastric emptying: a regulatory capacity with implications for feeding. *American Journal of Physiology.* 236: R254-R260

Nauck, M.A., Bartels, E., Orskov, E., Ebert, R. & Creutzfeldt, W. (1993). Additive insulinotropic effects of exogenous synthetic human gastric inhibitory polypeptide and glucagon-like peptide 1 (7-36) amide infused at near-physiological insulinotropic hormone and glucose concentrations. *Journal of Endocrinology and Metabolism.* 76: 912–917

Peterson, J.I. & Young, D.S. (1958). *Annals of Biochemistry.* 23: 301

Sanggaard, K.M., Holst, J.J., Rehfeld, J.F., Sandstrom, B.Raben, A. & Tholstrup, T. (2004). Different effects of whole milk and a fermented milk with the same fat and lactose content on gastric emptying and postprandial lipaemia, but not on glycaemic response and appetite. *British Journal of Nutrition.* 92: 447-459

Schenk, S., Davidson, C.J., Zderic, T.W., O Byerley, L. & Coyle, F. (2003). Different glycemic indexes of breakfast cereals are not due to glucose entry into blood but to glucose removal by tissue. *American Journal of Clinical Nutrition.* 78(suppl): 742-748

Shuster, L.T., Go, V.L. Rizza, R.A. O'Brien, P.C. & Service, F.J. (1988). Incretin effect due to increased secretion and decreased clearance of insulin in normal humans. *Diabetes*. 37(2): 200-203

Chapter 6

General Discussion and Conclusions

The stomach is a highly regulated organ that has been hypothesized to pass the food we eat to the small intestine at a rate that is optimal for the digestion and absorption of nutrients in food (Guyton & Hall, 2001). Rate of gastric emptying is influenced by a range of factors, including physical state (solids or liquid), volume, and macronutrient character of a meal (Schneeman, 2003; Moran, 1999; Lin *et al.*, 1990; Hunt, 1979). However, one of the principal factors affecting gastric emptying is the nutrient composition of the meal, as the small intestine 'tastes' the digested nutrients and back-regulates gastric emptying accordingly (Furness *et al.*, 1999). Neural and hormonal mechanisms react to internal physiological changes to control motility of the stomach (Meyer, 1987).

Milk from different species comprises the same basic components; water, fat, carbohydrate and protein, although the proportions of these differ from species to species. If gastric emptying is highly regulated, then milk from different species should elicit different rates of gastric emptying when given to humans. Based on this, it was hypothesized that milk from different species, i.e. goat and cow, would have different rates of gastric emptying.

The purpose of this thesis was to compare the rate of gastric emptying of goat's milk with that of cow's milk in healthy adult men. The results showed no significant difference in gastric emptying rate of whole goat's milk and whole cow's milk. Thus, the subtle differences in nutrient composition of whole goat and cow's milk were not sufficient to influence gastric emptying rate. However, the results showed that cow's milk infant formula emptied significantly faster than goat's milk infant formula. This may have been due either to the nutrient composition or physical form of the milk in the stomach.

Plasma glucose responses following ingestion of the milks were also measured with the aim of elucidating the relationship between gastric emptying and appearance of digestion end-products in the blood. This study provided evidence that a faster initial rate of gastric emptying results in earlier peak glucose levels as well as a greater subsequent drop to below baseline levels.

The ^{13}C Octanoic acid breath test was used to measure gastric emptying rates. The accurate and uncontaminated collection of breath samples in test tubes is central to the success of this method. There were two kinds of containers available for this purpose, one (more readily accessible and frequently used for this purpose) was a brand initially designed for the collection of blood samples (Vacutainer®); the other specifically designed for collection of breath samples (Exetainer®). In Chapter 3, a technical note describes the validation of container choice, and the decision to use 'Exetainers', which were shown to be the most appropriate and accurate container for the collection of breath samples. This was based on the finding that 'Vacutainers' contained a greater amount of residual air that would contaminate the breath sample by altering the ratio of ^{12}C to ^{13}C in the sample.

The current study was the first to compare the rates of gastric emptying of cow's and goats' milk. A number of studies have compared, using infants, the gastric emptying of infant formulas versus human milk (Veereman-Wauters *et al.*, 2004; Pozler *et al.*, 2003; Van Den Driessche *et al.*, 1999; Thorkelsson *et al.*, 1994; Billeaud *et al.*, 1990; Cavell, 1981) but none, however, have investigated gastric emptying with goat's milk infant formula. Moreover, of the studies of gastric emptying of cow's milk in adults, most compared whole milk with fermented milk, the latter having a much higher viscosity than whole milk (Sanggaard *et al.*, 2004; Gaudichon *et al.*, 1994; Mahe *et al.*, 1994). All found that fermented milk empties more slowly than whole milk, supporting the hypothesis that viscosity influences gastric emptying. A number of studies have investigated the rate of gastric emptying of particular cow's milk proteins (Calbet & Holst, 2004; Fox *et al.*, 2004; Hall *et al.*, 2003) and there is general agreement that gastric emptying of whey proteins is faster than that for caseins. This is due to the fact that casein coagulates in the acidic environment of the stomach, and follows a gastric emptying pattern more similar to that of solids than that of liquids (Calbet & Holst, 2004; Fox *et al.*, 2004; Hall *et al.*, 2003). In the current study, the infant formula with the highest proportion of whey proteins (CIF) emptied more quickly from the stomach than the one with more caseins (GIF).

The *in vitro* acidification experiments in this study showed that in the simulated acidic environment of the stomach casein curds are formed that may be expected to behave to some extent like a solid in the gastric environment. However, it was not possible to measure the viscosity of the coagulated milks by rheometry, as the curd fragments

moved through the fluid phase during rheometric testing. Thus, assessment was only possible by qualitatively examining photographs of the coagulated milks (Figure 4 A, B & C, pg 70).

The GIF had a much higher ratio of casein to whey compared to the CIF (80.8 : 19.2 vs 46.2 : 53.8, respectively). It seems that the lower ratio of casein to whey may have augmented the rate of gastric emptying, as evidenced by the different gastric emptying half times (Table 4F, pg 71). These results raise the question as to which formula would be most beneficial for the human infant, a formula with a higher level of coagulation and lower emptying rate or a formula with low coagulation and higher emptying rate. This question is particularly relevant given that both the formulas tested are available commercially.

Human milk has traditionally been used as the gold standard for infant formulas. Thus, it is reasonable to assume that an optimal infant formula would empty from the stomach at a rate that is similar to that of human breast milk. Studies that have compared the gastric emptying rate of infant formula to breast milk, however, have found that breast milk empties more rapidly (Van Den Driessche *et al.,* 1999; Ewer *et al.,* 1994; Billeaud *et al.,* 1990; Cavell, 1981; Cavell, 1979).

Alternatively, it may be better for a formula based on milk from a foreign species to empty at a slower rate than breast milk in order to allow the infant's small intestine time to fully digest the foreign proteins. In the stomach of the calf, casein from the cow's milk is turned into firm curds via the enzymatic action of rennet so that the casein can be slowly digested. Human infants are not known to secrete rennet (Miller *et al.,* 1990), so it is likely that although a precipitate does form, the same firm curds that form in the stomach of the calf do not form in the stomach of the infant (Miller *et al.,* 1990). It is possible that infant formula empties from the stomach at a rate that exceeds the optimum performance of the infant's digestive and metabolic system. In such an instance it would be better to recommend an infant formula that emptied more slowly from the stomach.

It is worthwhile at this juncture to review the gastric emptying results of the current study. Cow's milk infant formula (CIF) emptied more quickly from the stomach than the goat's milk infant formula, but there was no significant difference between the gastric emptying of whole goat's milk (WG) and whole cow's milk (WC). Also, there

were no differences in gastric emptying rate when the infant formulas were compared with either of the whole milks.

Satiety is also an important consideration in infant well-being, and can be induced by several factors from basic physical distension (Powley & Phillips, 2004) through to metabolic signalling (Moran, 2004). Given that metabolic signalling can induce satiation (Moran, 2004), it may be more beneficial for an infant formula to empty quickly from the stomach, resulting in more prompt appearance of metabolic end products in the system. On the contrary, gastric distension provided by a formula that empties at a slower rate may provide feelings of fullness for a longer period of time between meals leading to a more contented infant.

There are no clear indications in the scientific literature as to which of these two scenarios is more optimal. Consequently, infant formulas that empty rapidly (e.g. whey dominant) or more slowly (e.g. casein dominant) are both available commercially for parents to choose from.

The impact of postprandial plasma nutrient concentrations on overall metabolism is also an important issue to take into consideration. Comparisons of postprandial plasma glucose profiles following the ingestion of the different milks led to the hypothesis that a faster initial rate of gastric emptying leads to a greater "incretin effect" through increased contact with the absorptive surface area of the small intestine. The greater incretin effect augments insulin secretion, which is in turn responsible for a more pronounced drop in plasma glucose levels following peaking of these levels.

It would be useful to study the effect of gastric emptying on the dynamics of plasma glucose concentrations with a larger dose of glucose. The main sugar in milk is lactose, a disaccharide of glucose and galactose. Galactose does not have a direct effect on blood glucose as it first has to be converted to glucose in the liver (Guyton & Hall, 2001). In order to clearly establish the effects of gastric emptying on plasma glucose concentrations arising from dietary glucose, it would be appropriate to use a carbohydrate that had a direct effect on glucose concentrations and a dosage that would provide a substantial response that could be more easily monitored. In addition to work on the precise relationship between gastric emptying and plasma glucose

concentration, further research is needed to ascertain the extent of the role of incretin on the postprandial glucose concentrations.

If the rate of gastric emptying does modulate the magnitude of the incretin effect, with an accompanying lower subsequent drop in plasma glucose levels, and this does indeed lead to lower average blood glucose levels, then it may be worth considering the implication this may have for people with a tendency for elevated blood glucose levels over the course of a day. It may be that fewer, larger meals will lead to lower mean daily blood glucose levels compared to when more, smaller meals are ingested. The effect of the larger meals would be to augment the insulin response thereby ensuring greater drops to below baseline values following a meal. Whether or not this is optimum needs to be elucidated in further research. It would be interesting to repeat the current experiment with larger doses of milk, as higher meal volumes are likely to result in higher rates of gastric emptying. This would accentuate any potential differences in gastric emptying rate between different meals, and the appearance of nutrients in the blood.

In conclusion, the present study compared the gastric emptying of whole milks of similar composition and of infant formulas of differing composition. The lack of difference found between the whole milks negates gastric emptying as a point of difference between goat's and cow's milk and calls for studies at the next possible point of difference, absorption from the small intestine. Further studies in infants would also be of interest in terms of determining whether these findings are also applicable in the infant and if so, which type of milk would be nutritionally better as a base for an infant formula. The results obtained in this study showed how important the physical and chemical characteristics of a food are in affecting the rate of gastric emptying. Also, the physical state of food can change when acted upon by the acid in the stomach thereby affecting how it is emptied from the stomach. Gastric emptying may be closely related to the incretin response to a meal, which has implications for postprandial plasma glucose levels.

References

Billeaud, C., Guillet, J. & Sandler, B. (1990). Gastric-emptying in infants with or without gastroesophageal reflux according to the type of milk. *European Journal Of Clinical Nutrition.* 44(8): 577-583

Calbet, J.A.L. & Holst, J.J. (2004). Gastric emptying, gastric secretion and enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans. *European Journal of Nutrition.* 43:127-139

Cavell, B. (1979). Gastric emptying in preterm infants. *Acta Paediatrica Scandinvia.* 68: 725 - 730

Cavell, B. (1981). Gastric-emptying in infants fed human milk or infant formula. *Acta Paediatrica Scandinavica.* 70(5): 639-641

Ewer, A.K., Durbin, G.M., Morgan, M.E., Booth, I.W. (1994). Gastric emptying in preterm infants. *Archives of Diseases in Children.* 71: F24 - F27

Fox, M., Goergi, G., Boehm, G., Menne, D., Fried, M. & Thumshirn, M. (2004). Dietary protein precipitation properties have effects on gastric emptying in healthy volunteers. *Clinical Nutrition.* 23: 641-646

Furness, J.B., Kunze, W.A.A. & Clerc, N. (1999). Nutrient tasting and signalling mechanisms in the gut II. The intestine as a sensory organ: neural, endocrine, and immune responses. *American Journal of Physiology.* 277(40): G922-G928

Gaudichon, C., Roos, N., Mahe, S., Sick, H., Bouley, C. & Tome, D. (1994). Gastric-emptying regulates the kinetics of nitrogen absorption from N-15-labeled milk and N-15-labeled yogurt in miniature pigs. *Journal of Nutrition.* 124(10): 1970-1977

Guyton, A.C. & Hall, J.E. (2001). *Medical physiology (10th ed.).* London: W.B.Sauders Company. pp. 728-737

Hall, W.L., Millward, D.J., Long, S.J. & Morgan, L.M. (2003). Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *British Journal of Nutrition.* 89(2): 239-248

Hunt, J.N. & Stubbs, D.F. (1979). The volume and energy content of meals as determinants of gastric emptying. *Journal of Physiology.* 245:209-225

Lin, H.C., Elashoff, J.D., Gu, Y.G. & Meyer, J.H. (1992). Effect of meal volume on gastric emptying. *Journal of Gastrointestinal Motility.* 4: 157-163

Mahe, S., Marteau, P., Huneau, J.F., Thuillier, F., & Tome, D. (1994). Intestinal nitrogen and electrolyte movements following fermented milk ingestion in man. *British Journal Of Nutrition.* 71(2): 169-180

Meyer, J.H. (1987). Motility of the stomach and gastroduodenal junction. In: Johnson, L.R.(Ed). *Physiology of the gastrointestinal tract. Volume 1. 2nd Ed.* New York: Raven. pp. 613 – 629

Miller, M.J.S., Witherly, S.A. & Clark, D.A (1990). Casein: a milk protein with diverse biologic consequences. *Proceedings of the Society for Experimental Biology and Medicine.* 195: 143-159

Moran, T.H., Wirth, J.B., Schwartz, G.J., & McHugh, P.R. (1999). Interactions between gastric volume and duodenal nutrients in the control of liquid gastric emptying. *American Journal of Physiology.* 276 (45): R997-R1002

Moran, T.H. (2004). Gut peptides in the control of food intake: 30 years of ideas. *Physiology & Behavior.* 82(1): 175-180

Powley, T.L. & Phillips, R.J. (2004). Gastric satiation is volumetric, intestinal satiation is nutritive. *Physiology & Behaviour.* 82: 69-74

Pozler, O., Neumann, D., Vorisek, V., Bukac, J., Bures, J., & Kokstein, Z. (2003). Development of gastric emptying in premature infants: Use of the c-13-octanoic acid breath test. *Nutrition.* 19(7-8): 593-596

Sanggaard, K.M., Holst, J.J., Rehfeld, J.F., Sandstrom, B.Raben, A., and Tholstrup, T. (2004). Different effects of whole milk and a fermented milk with the same fat and lactose content on gastric emptying and postprandial lipaemia, but not on glycaemic response and appetite. *British Journal of Nutrition.* 92: 447-459

Schneeman, B.O. (2002). Gastrointestinal physiology and functions. *British Journal of Nutrition.* 88(Suppl. 2): S159-S163

Thorkelsson, T., Mimouni, F., Namgung, R., Fernandezulloa, M., Krugwispe, S. & Tsang, R.C. (1994). Similar gastric-emptying rates for casein-predominant and whey-predominant formulas in preterm infants. *Pediatric Research.* 36(3): 329-333.

Van Den Driessche, M., Peeters, K., Marien, P., Ghoos, Y., Devlieger, H., & Veereman-Wauters, G. (1999). Gastric emptying in formula-fed and breast-fed infants measured with the c-13-octanoic acid breath test. *Journal Of Pediatric Gastroenterology And Nutrition.* 29(1): 46-51.

Veereman-Wauters, G., Staelens, S., Van Den Driessche, M.L.M., Barclay, D., Carrie-Faessler, A.E., Haschke, F. (2004). Gastric emptying in newborns fed an intact protein formula, a partially and an extensively hydrolysed formula. *Gastroenterology.* 126(4): A67-A67.

Appendices

A- Individual Percentage Dose Recovery per Hour Data for Cow's milk infant formula.

B - Individual Percentage Dose Recovery per Hour Data for Goat's milk infant formula.

C - Individual Percentage Dose Recovery per Hour Data for Whole Cow's milk.

D - Individual Percentage Dose Recovery per Hour Data for Whole Goat's milk.

E - Gastric emptying parameters for individual subjects (plus mean, std deviation and std error of the means).

F - Individual subject data for plasma glucose concentration (mmol/l) following the ingestion of cow's milk infant formula (CIF) over time.

G - Individual subject data for plasma glucose concentration (mmol/l) following the ingestion of goat's milk infant formula (GIF) over time.

H - Individual subject data for plasma glucose concentration (mmol/l) following the ingestion of whole cow's milk (WC) over time.

I - Individual subject data for plasma glucose concentration (mmol/l) following the ingestion of whole goat's milk (WG) over time.

Appendix A. Individual Percentage Dose Recovered per Hour Data for Cow's milk infant formula

	Subject														
Time	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	1.75	4.10	1.66	3.33	2.41	3.99	4.97	5.52	2.70	2.85	2.47	1.97	5.10	4.37	3.66
20	4.95	8.28	4.31	7.21	6.81	8.53	8.16	12.07	5.72	6.70	5.26	4.46	10.43	10.62	9.57
30	8.12	9.69	6.68	9.04	9.96	12.73	11.85	15.49	9.46	9.43	8.39	6.85	11.83	13.05	11.11
40	14.09	10.80	7.62	12.10	11.97	14.75	14.16	18.53	11.70	11.45	7.37	9.19	12.64	14.86	11.78
50	14.58	12.39	9.28	11.36	12.96	13.48	15.34	20.65	12.88	11.45	11.02	11.12	14.10	15.16	12.49
60	15.55	13.23	10.13	12.44	14.41	16.22	15.88	21.80	13.90	12.06	10.89	12.00	14.40	16.20	12.53
70	15.52	14.00	11.00	11.93	15.20	15.34	14.85	21.84	15.38	12.53	10.78	13.23	15.52	15.45	14.10
80	13.93	14.87	10.51	12.47	16.06	14.55	13.30	20.91	15.86	12.24	10.83	14.07	14.92	15.15	14.80
90	12.85	14.09	10.59	11.50	16.45	13.40	12.35	20.33	17.58	11.55	11.37	14.79	15.10	13.99	14.10
100	15.63	14.03	10.80	10.92	15.94	12.14	10.95	18.97	18.57	11.89	12.07	15.29	14.22	14.06	14.69
110	11.30	13.78	10.57	9.81	16.04	10.94	10.11	17.29	17.74	10.99	12.20	15.00	12.87	12.93	14.29
120	10.16	13.22	10.18	9.21	14.40	9.73	9.22	15.52	17.02	11.31	12.44	14.78	13.22	11.64	13.92
135	10.50	10.90	9.24	8.40	13.08	8.95	8.26	14.29	15.86	9.98	11.15	14.05	11.66	11.24	13.06
150	8.22	9.08	8.72	7.64	12.25	7.58	7.59	*	14.34	8.89	10.07	13.29	10.81	10.39	12.32
165	4.27	8.51	7.53	6.76	11.49	6.59	7.06	11.13	12.88	7.31	9.14	10.44	9.57	9.94	11.42
180	5.71	7.64	7.00	5.73	11.02	5.80	6.48	10.65	11.61	7.42	8.24	10.09	8.68	9.03	10.74
195	6.29	7.21	6.43	5.33	10.29	5.04	5.27	9.78	10.68	7.05	7.27	9.67	7.80	8.14	10.11
210	1.98	6.43	5.77	4.41	9.33	4.54	4.70	8.90	9.77	6.52	6.33	8.96	7.10	7.32	10.49
225	1.95	6.26	6.04	4.14	8.59	3.92	4.35	8.13	9.02	5.76	5.50	8.73	6.33	6.58	9.82
240	0.37	5.53	6.50	3.52	7.50	4.18	3.50	7.81	8.17	5.37	4.65	9.00	5.65	6.16	8.94

* No reading from ratio isotope mass spectrometer and therefore unable to calculate percentage dose recovered.

Appendix B. Individual Percentage Dose Recovered per Hour Data for Goat's milk infant formula

	Subject														
Time	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
0	0.00	0.00	0.00	0.00	ND	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	3.74	3.90	2.60	3.67	ND	5.42	3.96	5.11	6.14	3.77	3.72	3.35	4.57	5.47	7.32
20	8.19	7.33	7.99	7.91	ND	12.42	9.99	12.49	10.97	5.07	5.31	6.76	9.93	9.97	14.03
30	10.74	12.87	9.23	*	ND	12.51	12.92	17.05	12.81	6.15	5.65	10.44	10.74	12.11	14.28
40	11.75	12.03	9.29	12.84	ND	12.27	14.28	17.93	11.81	9.79	5.61	10.06	10.66	12.73	13.72
50	12.39	14.37	8.72	14.18	ND	12.73	13.09	17.29	13.53	10.77	5.72	10.93	10.88	*	14.80
60	12.35	12.40	10.65	15.66	ND	12.90	13.38	19.34	13.75	11.52	6.20	10.76	11.15	12.26	15.11
70	12.27	11.63	9.92	15.75	ND	13.83	13.40	18.67	15.64	10.51	6.64	10.50	11.46	11.89	14.49
80	11.65	11.23	9.98	15.83	ND	13.65	12.90	18.32	15.16	9.66	7.05	9.37	11.57	11.79	14.06
90	12.12	9.25	9.61	14.20	ND	13.32	12.64	17.41	14.00	9.27	7.80	9.81	10.98	11.76	13.08
100	*	9.48	11.36	12.66	ND	11.69	11.42	15.96	13.47	9.24	8.35	9.22	10.83	12.11	12.47
110	12.52	9.95	12.28	11.54	ND	10.43	11.16	15.48	15.45	8.96	8.73	9.01	10.73	12.02	12.49
120	15.38	10.34	11.94	10.75	ND	10.20	10.24	15.51	16.79	9.03	8.66	9.09	10.51	11.56	12.10
135	15.73	10.98	11.69	9.38	ND	8.79	9.75	14.81	17.79	8.87	8.19	9.47	10.08	10.99	11.38
150	13.38	10.61	10.80	8.51	ND	7.90	9.00	13.94	16.34	7.90	7.03	10.06	9.74	10.25	11.43
165	13.11	10.59	11.12	7.60	ND	6.93	7.93	12.74	15.70	7.23	6.84	9.86	9.21	9.91	11.08
180	11.71	10.94	9.76	6.76	ND	6.14	7.66	11.22	12.75	6.66	6.35	9.75	8.63	10.18	10.17
195	10.34	11.23	9.22	5.99	ND	5.34	6.76	9.89	12.24	5.29	5.51	9.45	8.08	9.54	8.92
210	9.19	12.34	7.37	5.64	ND	4.82	5.74	9.10	11.70	6.55	5.12	9.37	7.87	8.70	7.63
225	8.31	9.90	6.89	5.05	ND	4.16	4.85	8.29	10.97	6.91	4.65	9.09	7.26	8.69	6.62
240	7.48	6.20	5.39	4.27	ND	3.93	4.20	7.72	8.00	5.86	3.91	8.06	6.85	8.64	5.83

* = No reading from ratio isotope mass spectrometer and therefore unable to calculate percentage dose recovered.

ND = No Data available as a result of exeperimental error.

Appendix C. Individual Percentage Dose Recovered per Hour Data for Whole Cow's Milk (WC)

	Subject														
Time	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
0	0.00	0.00	0.00	ND	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	6.00	2.05	2.37	ND	1.11	6.94	2.45	6.16	4.97	2.99	4.53	2.91	5.37	5.02	9.47
20	11.46	6.66	7.75	ND	3.24	9.96	7.71	14.63	12.13	7.22	8.23	6.20	12.52	8.56	11.83
30	13.34	8.92	10.15	ND	4.50	13.63	13.02	*	14.09	10.23	*	7.44	12.78	10.47	11.86
40	13.34	11.21	11.83	ND	5.28	11.26	13.00	17.69	15.35	11.38	8.81	7.64	13.09	10.68	13.10
50	13.14	11.41	12.68	ND	6.10	11.07	13.02	17.67	15.36	11.09	9.86	8.28	13.71	11.43	14.40
60	12.70	12.26	13.94	ND	6.95	13.31	12.30	18.19	14.72	10.33	10.54	8.63	13.29	12.05	14.64
70	13.68	12.54	15.28	ND	7.97	13.31	12.44	12.90	13.65	10.48	11.57	8.60	12.58	12.13	14.61
80	14.38	12.73	15.45	ND	8.64	13.30	12.73	13.30	16.10	10.96	11.66	8.80	12.75	12.20	14.39
90	13.50	*	15.74	ND	9.55	12.11	12.90	13.20	16.64	11.08	11.43	8.90	12.50	12.64	15.00
100	13.60	11.75	15.43	ND	10.11	12.69	12.41	12.92	16.82	12.01	10.70	7.33	11.59	12.35	15.89
110	13.42	12.24	14.55	ND	10.71	*	11.36	11.97	16.82	11.74	11.25	9.81	10.89	11.82	15.63
120	11.25	12.88	13.37	ND	11.57	15.01	10.49	11.81	16.80	11.66	11.27	9.96	10.99	11.69	15.05
135	9.76	12.12	12.19	ND	*	10.74	10.03	10.57	16.17	11.24	10.06	10.13	11.21	11.02	13.25
150	7.43	11.98	11.00	ND	12.43	11.55	8.72	9.76	15.52	11.85	8.88	10.71	10.24	10.54	11.88
165	7.06	11.45	10.17	ND	12.04	9.44	7.84	8.98	14.11	10.60	8.06	10.87	9.26	10.58	10.91
180	5.24	10.69	9.33	ND	11.41	*	6.68	7.32	12.48	8.85	6.22	10.29	8.81	10.47	9.58
195	5.35	9.32	9.18	ND	10.46	8.38	5.97	7.07	11.57	7.66	6.04	9.89	8.43	10.69	8.90
210	3.53	8.03	7.89	ND	9.61	*	5.15	7.28	10.80	6.96	5.65	9.40	8.23	10.60	7.93
225	3.33	7.24	6.97	ND	8.77	8.89	4.19	5.64	9.77	6.33	4.85	9.18	7.49	9.68	7.26
240	1.48	5.40	6.44	ND	7.92	2.93	3.73	5.54	9.21	5.57	*	8.54	6.92	8.49	6.63

* = No reading from ratio isotope mass spectrometer and therefore unable to calculate percentage dose recovered.

ND = No Data available as a result of exeperimental error.

Appendix D. Individual Percentage Dose Recovered per Hour Data for Whole Goat's Milk (WG)

	Subject														
Time	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	4.47	1.45	2.92	4.99	*	3.88	3.80	2.70	6.75	1.96	2.07	3.02	6.94	4.29	4.91
20	7.11	5.69	7.89	10.59	5.85	8.03	12.28	6.59	10.14	4.84	4.60	7.50	11.71	7.56	6.93
30	8.32	8.28	10.71	12.27	8.08	10.87	12.57	9.70	11.65	4.87	5.44	8.79	12.81	11.91	6.95
40	10.60	10.91	11.87	10.22	9.37	11.25	12.74	12.09	14.52	8.40	5.94	9.50	14.53	11.88	6.94
50	11.45	9.57	12.88	13.45	10.10	11.30	13.02	*	14.25	9.22	6.45	10.28	14.69	13.30	7.71
60	12.12	9.58	13.75	12.88	10.60	11.74	11.37	15.91	14.03	9.63	6.40	10.83	14.27	14.36	8.35
70	12.51	13.12	14.33	*	10.85	12.13	10.86	17.12	13.48	7.96	6.23	10.81	13.48	14.90	9.05
80	12.20	*	14.56	12.40	11.02	11.86	12.17		13.98	9.73	6.47	10.53	12.64	14.69	9.07
90	11.74	9.61	14.39	11.90	11.64	11.42	9.53	16.88	13.83	6.96	7.53	11.73	12.12	14.87	9.03
100	11.57	11.43	14.26	11.97	12.01	12.24	10.52	15.91	16.84	7.23	8.39	10.95	11.60	14.89	9.57
110	11.60	11.52	13.89	11.73	12.21	11.43	10.54	15.16	14.91	9.24	9.05	11.77	11.32	14.46	9.70
120	13.05	10.47	13.77	11.13	11.91	10.74	9.67	*	14.37	8.44	9.86	11.68	11.17	14.44	9.56
135	12.38	10.49	13.05	10.67	*	9.92	10.50	13.54	13.21	8.65	10.49	11.16	10.28	13.58	10.42
150	12.09	*	12.25	9.76	11.17	9.00	10.38	13.23	11.62	10.95	10.43	10.83	9.34	12.94	9.17
165	11.22	9.47	11.02	8.30	10.69	8.59	7.42	12.03	10.65	8.34	9.86	10.05	8.52	11.77	8.57
180	9.92	11.54	10.07	7.97	10.46	7.51	7.12	11.23	9.22	9.64	8.99	9.92	7.71	10.93	8.33
195	9.51	8.50	9.02	7.57	9.90	6.55	6.17	10.40	8.99	6.29	8.47	9.70	7.03	9.90	8.35
210	9.39	7.94	7.22	7.15	9.28	5.88	6.04	9.78	7.72	12.86	7.24	9.23	6.52	9.03	7.93
225	8.72	8.05	7.32	6.49	8.27	5.39	5.83	8.61	6.55	8.81	6.68	9.17	5.98	8.33	7.68
240	7.64	5.60	6.32	5.71	7.62	4.13	3.02	8.36	4.87	6.61	5.04	8.58	5.37	7.44	7.22

* No reading from ratio isotope mass spectrometer and therefore unable to calculate percentage dose recovered.

Appendix E. Gastric emptying parameters for individual subjects (plus mean, std deviation and std error of the means) following the consumption of four test milks

	Cow's milk infant formula (CIF)		Goat's milk infant formula (GIF)		Whole cow's milk (WC)		Whole goat's milk (WG)	
Subject	tmax	t1/2	tmax	t1/3	tmax	t1/4	tmax	t1/5
A	70	95	95	176	58	100	95	181
B	77	125	88	232	91	152	95	166
C	90	143	94	175	83	132	84	139
D	66	112	66	108	ND	ND	67	138
E	90	142	ND	ND	141	204	103	189
F	61	98	56	107	69	146	71	128
G	58	100	62	115	67	112	58	124
H	69	115	69	128	53	110	90	142
I	97	143	95	191	84	166	72	134
J	76	130	79	151	84	154	133	280
K	90	140	93	176	74.3	133.1	127	225
L	102	151	99	253	148	345	102	208
M	75	147	50	131	52	114	55	151
N	71	126	74	172	63	154	56	123
O	67	123	76	192	92	213	86	150
P	90	171	58	140	68	144	107	256
Mean	78	129	77	163	82	159	88	171
Std Deviation	13.38	21.36	16.28	43.56	28.50	60.65	23.74	48.45
SEM	3.35	5.34	4.20	11.25	7.36	15.66	5.93	12.11
N	16	16	15	15	15	15	16	16

ND= No Data available for this treatment

Appendix F. Individual subject data for plasma glucose concentration (mmol/l) following the ingestion of cow's milk infant formula (CIF)over time.

	Subject														
Time	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
0	4.65	4.42	4.52	4.36	4.68	4.66	4.38	4.39	4.82	5.68	4.58	4.99	4.67	4.73	4.10
10	4.73	4.54	4.45	4.37	4.72	4.91	4.75	4.33	4.81	5.78	4.65	4.77	4.71	5.27	4.08
20	4.76	4.73	4.82	4.53	4.86	5.66	5.36	4.68	5.00	6.13	4.77	4.76	4.70	6.31	4.82
30	4.91	4.55	5.26	4.98	4.78	6.07	5.44	4.62	4.93	6.26	5.25	5.20	4.73	6.58	4.61
40	4.87	4.61	5.18	4.54	4.79	5.57	4.98	4.64	5.01	6.54	4.91	5.62	4.68	5.43	3.91
50	4.75	4.69	5.03	4.34	4.42	4.51	4.48	4.62	5.06	6.27	4.88	5.20	4.53	4.63	3.81
60	4.70	4.58	5.04	4.30	4.24	4.21	4.42	4.38	5.05	6.21	4.66	4.65	4.44	4.46	3.47
70	4.73	4.46	4.91	4.17	4.57	4.19	4.21	4.38	5.01	6.05	4.66	4.39	4.41	3.71	3.91
80	4.94	4.37	4.37	4.22	4.70	4.06	4.38	4.10	4.86	5.75	4.50	4.35	4.42	4.15	3.93
90	5.00	4.25	4.37	4.07	4.59	4.00	4.55	4.30	4.76	5.60	4.39	4.39	4.51	4.62	3.86
100	4.93	4.40	4.83	4.02	4.58	4.15	4.60	4.63	4.95	5.80	4.52	4.55	4.61	4.50	4.02
110	5.01	4.43	4.80	4.02	4.55	4.21	4.26	4.06	4.88	5.52	4.52	4.53	0.00	4.36	3.75
120	5.00	4.36	4.97	4.11	4.56	4.46	4.11	4.10	4.77	5.53	4.18	4.67	4.22	4.68	4.01
135	5.00	4.24	4.68	4.08	4.28	4.50	*	4.40	4.50	5.39	4.82	4.67	4.27	4.62	3.80
150	5.08	4.27	4.56	4.30	4.52	4.37	4.62	0.00	4.43	5.16	4.82	4.50	4.40	4.48	4.26
165	5.02	4.20	4.53	4.08	4.39	4.29	4.38	4.30	4.37	5.06	4.48	4.68	4.26	4.62	4.26
180	5.16	4.13	4.62	4.20	4.41	4.31	4.24	4.27	4.34	4.83	4.40	4.69	4.47	4.67	4.13
195	4.99	4.13	4.46	4.13	4.34	*	4.37	4.19	4.18	4.88	4.38	4.64	4.25	4.74	4.17
210	4.78	4.02	4.63	4.17	4.43	4.10	4.33	4.24	4.29	4.72	4.23	4.56	4.37	4.60	4.11
225	4.87	4.28	4.64	4.04	4.75	4.24	4.43	4.03	4.38	4.72	4.35	4.67	5.21	4.68	3.98
240	4.58	4.23	4.39	4.16	4.26	4.16	4.00	4.10	4.38	4.68	4.37	4.57	4.89	4.82	3.96

* = No sample

Appendix G. Individual subject data for plasma glucose concentration (mmol/l) following the ingestion of goat's milk infant formula (GIF)over time.

	Subject														
Time	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
0	4.67	4.28	4.01	4.19	4.59	4.51	4.74	6.62	5.04	ND	4.37	4.49	4.62	4.31	4.61
10	4.67	4.36	4.42	4.30	4.61	5.11	4.59	5.94	5.58	ND	4.56	4.78	4.39	5.02	5.38
20	4.69	4.14	5.06	4.63	5.47	6.02	5.52	6.42	*	ND	4.79	5.57	4.47	5.23	6.46
30	4.72	4.24	5.42	4.23	5.39	5.50	6.70	6.13	5.27	ND	4.98	5.74	4.34	4.84	6.01
40	4.79	4.00	5.16	3.85	4.80	4.68	6.02	5.29	5.69	ND	4.93	5.17	4.40	4.87	4.91
50	4.70	3.98	4.83	3.72	4.75	4.00	5.04	4.96	5.34	ND	4.78	4.58	4.46	4.71	4.09
60	4.56	3.89	4.84	4.34	4.93	3.55	4.15	4.94	5.27	ND	4.56	4.40	4.44	4.13	3.98
70	4.49	4.07	4.53	3.72	4.68	3.87	3.77	5.30	5.34	ND	4.63	4.37	4.34	3.75	4.19
80	4.58	3.93	4.30	3.71	4.99	3.84	3.76	5.56	5.39	ND	4.47	4.30	4.22	3.91	4.02
90	4.58	4.32	4.09	4.25	4.62	4.44	3.81	5.87	5.24	ND	4.52	4.24	4.26	3.99	4.02
100	4.53	4.32	4.05	3.91	4.83	4.29	4.01	5.96	5.22	ND	4.59	4.26	4.44	4.66	3.88
110	4.31	4.10	4.21	3.86	4.59	4.24	4.13	6.02	5.17	ND	4.81	4.33	3.93	4.65	4.19
120	4.46	4.24	*	3.90	4.04	4.35	4.12	5.47	5.12	ND	4.77	4.33	4.52	4.56	4.15
135	4.52	4.43	4.18	4.12	5.28	4.23	4.31	5.12	5.03	ND	4.75	4.29	4.25	4.43	4.16
150	4.41	4.32	4.13	3.98	4.21	4.39	4.43	5.05	4.91	ND	4.46	4.23	4.05	4.56	4.26
165	4.55	4.29	4.11	3.93	4.47	4.50	4.19	5.18	4.67	ND	4.64	4.24	4.76	4.52	4.20
180	4.79	4.41	4.15	3.85	4.64	4.39	4.09	5.08	4.69	ND	4.66	4.31	4.93	4.36	4.10
195	4.63	4.18	4.23	3.92	4.42	4.31	4.28	5.07	4.58	ND	4.57	4.20	4.71	4.44	3.94
210	4.56	4.19	4.21	3.48	4.58	4.32	4.33	4.94	4.65	ND	4.69	4.10	4.76	4.35	4.10
225	4.67	4.26	4.19	4.30	4.51	4.28	4.76	4.92	4.58	ND	4.58	4.11	3.61	4.37	4.10
240	4.49	4.35	4.13	3.94	4.52	4.40	4.53	4.80	4.56	ND	4.47	4.00	4.72	4.44	4.12

* = No sample

ND = No Data - unable to insert indwelling cannula and draw blood samples

Appendix H. Individual subject data for plasma glucose concentration (mmol/l) following the ingestion of whole cow's milk (WC) over time.

	Subject														
Time	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
0	5.34	4.56	4.11	4.21	3.98	4.53	4.44	4.54	4.64	5.44	5.72	4.66	4.73	4.71	4.27
10	5.76	4.41	4.54	4.00	3.95	4.52	4.50	4.46	4.91	5.46	6.10	4.70	4.86	4.84	4.82
20	5.75	4.54	5.29	4.26	4.02	5.60	5.32	4.51	5.14	5.69	6.65	4.84	5.07	4.83	5.01
30	5.54	4.55	5.05	4.27	3.92	5.36	5.56	4.38	5.01	5.72	5.76	4.82	4.96	4.41	4.44
40	5.24	4.50	4.90	4.43	3.76	4.94	4.89	4.25	5.05	5.76	4.98	4.66	4.83	4.32	3.86
50	4.91	4.38	4.73	5.47	3.67	4.13	3.96	4.01	4.78	5.64	4.58	4.64	4.76	4.14	3.73
60	4.71	4.58	4.36	4.44	3.77	3.98	3.60	3.95	4.55	5.53	5.14	4.54	4.66	4.17	3.67
70	4.70	4.44	4.05	4.08	3.59	4.13	3.94	3.90	4.17	5.31	5.02	4.47	4.55	4.47	3.73
80	4.90	4.33	3.94	4.16	*	4.10	4.01	3.80	4.06	5.25	4.81	4.48	4.52	4.26	3.97
90	4.85	4.34	4.03	4.13	3.73	4.23	3.75	4.13	4.11	5.05	4.90	4.48	4.42	4.40	3.81
100	4.91	4.36	4.37	4.12	3.71	4.17	3.98	4.35	4.11	4.92	5.18	4.47	4.50	4.60	4.03
110	4.96	4.51	4.40	4.06	3.60	4.26	3.94	4.49	4.12	4.92	5.14	4.40	4.32	4.62	4.24
120	4.95	4.41	4.16	4.18	3.60	4.38	4.01	4.35	4.14	4.87	5.17	4.36	4.27	4.60	3.83
135	4.87	4.43	4.31	4.21	3.68	4.33	4.05	4.31	4.14	4.83	4.93	4.23	4.63	4.20	4.15
150	4.78	4.39	4.28	4.30	3.76	4.36	3.96	4.27	4.19	4.79	5.30	4.28	4.51	4.58	4.17
165	4.81	4.38	4.33	4.13	*	4.20	3.93	4.16	4.26	4.63	5.00	4.32	4.45	4.62	4.00
180	4.73	4.30	4.17	4.24	3.81	4.21	3.86	4.08	4.36	4.66	5.08	4.26	4.42	4.57	4.12
195	4.88	4.24	4.09	4.59	3.75	4.06	3.75	4.15	4.40	4.68	4.98	4.34	4.37	4.64	4.05
210	4.75	4.26	4.07	4.54	*	3.97	3.75	4.17	4.22	4.70	4.91	4.44	4.35	4.77	4.37
225	4.89	4.34	4.00	4.23	3.71	4.39	3.75	4.17	4.36	4.68	4.87	4.44	4.33	4.72	4.03
240	4.75	4.31	3.89	4.25	3.69	3.93	3.83	3.99	4.18	4.56	4.78	4.39	4.36	4.43	4.18

* = No sample

Appendix I. Individual subject data for plasma glucose concentration (mmol/l) following the ingestion of whole goat's milk (WG) over time.

	Subject														
Time	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
0	4.45	4.03	4.17	4.08	4.74	4.62	5.03	4.39	5.48	ND	5.17	4.80	4.71	4.41	5.62
10	4.76	4.01	4.13	4.19	5.32	4.74	5.14	4.38	5.83	ND	5.89	4.82	4.84	4.64	6.10
20	4.65	4.12	4.23	4.42	5.32	5.14	5.55	4.54	5.95	ND	5.84	5.16	4.83	5.05	6.50
30	4.56	4.22	4.34	4.40	*	5.65	6.14	4.81	5.94	ND	5.52	5.14	4.41	5.13	5.84
40	4.68	4.28	4.85	3.98	5.09	5.30	5.64	5.00	5.80	ND	5.17	4.88	4.32	4.36	5.17
50	4.73	4.31	4.91	3.87	4.88	4.79	4.79	4.78	5.70	ND	5.14	4.79	4.14	4.16	5.06
60	4.74	4.37	4.88	3.66	*	4.41	4.59	4.65	5.41	ND	5.05	4.62	4.17	4.43	4.97
70	4.67	4.13	4.66	3.45	4.47	4.20	4.29	4.63	5.35	ND	4.79	4.50	4.47	4.43	4.87
80	4.49	4.14	4.39	3.43	4.44	4.10	4.79	4.34	5.14	ND	4.81	4.46	4.26	4.33	4.94
90	4.52	4.14	4.38	3.41	4.43	3.97	4.86	4.40	4.98	ND	4.70	4.39	4.40	4.60	5.00
100	4.61	4.22	4.29	3.62	4.52	4.07	4.59	4.57	4.17	ND	4.80	4.31	4.60	4.63	5.09
110	4.49	4.15	4.19	3.56	4.54	4.11	4.55	4.52	4.73	ND	4.55	4.35	4.62	4.49	5.10
120	4.60	4.14	4.31	3.72	4.40	4.21	4.75	4.48	4.64	ND	4.82	4.31	4.60	4.52	5.13
135	4.67	4.00	4.36	3.63	4.60	4.09	4.98	4.46	4.66	ND	4.87	4.44	4.20	4.41	4.96
150	4.61	4.26	4.38	3.66	4.52	4.09	4.86	4.56	4.52	ND	4.65	4.44	4.58	4.27	5.16
165	4.80	4.41	4.32	3.71	4.66	4.23	4.54	4.59	4.52	ND	4.53	4.40	4.62	4.09	5.07
180	4.61	4.27	4.28	3.76	4.63	4.27	4.87	4.58	4.40	ND	4.50	4.47	4.57	4.65	5.23
195	4.53	4.27	4.23	3.83	4.66	4.36	4.72	4.59	4.47	ND	4.50	4.43	4.64	4.42	5.14
210	4.62	4.16	4.25	3.94	4.59	4.40	5.05	4.49	4.51	ND	4.51	4.38	4.77	4.35	5.35
225	4.69	4.18	4.25	3.85	4.54	4.23	4.83	4.38	4.39	ND	4.91	4.42	4.72	4.37	5.30
240	4.53	4.15	4.27	3.74	4.63	4.30	4.67	4.45	4.29	ND	4.62	4.42	4.43	4.40	5.12

* = No sample

ND = No Data - unable to insert indwelling cannula and draw blood samples