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DEVELOPMENT OF NUCLEAR MEDICINE METHODS FOR GASTRIC AND SMALL BOWEL MOTILITY

EFFECTS OF GLP-1 ON GASTRIC EMPTYING

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ABSTRACT

The diagnosis of functional disorders of the gastrointestinal (GI) tract requires techniques being both sufficiently sensitive to detect disturbances of the normal physiology and low-invasive enough not to interfere with the properties being studied. The purpose was to set up nuclear medicine methods for assessment of upper GI-tract motility and study the effect of glucagon-like peptide-1 (GLP-1) on gastric emptying.

Study I: In order to establish a national standard for solid gastric emptying with reference values, eight Swedish centres were included in a multicentre project, each providing 20 healthy subjects. A standardised meal consisting of a ^{99m}Tc-labelled omelet and an unlabelled soft drink was defined and reference values were established. Sub-group analysis showed that premenopausal women had a slower gastric emptying than postmenopausal women, why separate reference values should be used. Menstrual cycle, smoking habits or body mass index (BMI) within the normal range did not influence gastric emptying.

Study II: Previous studies have shown incongruent results with regard to the influence of obesity on gastric emptying. This was studied in 9 obese subjects (BMI>35 kg/m^2) and in 21 normal weight controls, using the method developed in *study I*. An association between obesity and increased gastric emptying was found.

Study III: GLP-1 is secreted from the intestinal mucosa in the presence of nutrients and acts as an incretin. An inhibitory effect of GLP-1 on upper GI motility has been shown using non-imaging techniques. The effect of iv. administered GLP-1 on gastric solid emptying was studied in 8 healthy subjects. GLP-1 had an inhibitory effect on all phases of solid gastric emptying. Analysis of pancreatic and intestinal hormones showed that peptide YY (PYY) levels decreased during GLP-1 infusion which may be due to a negative feedback mechanism. Comparison with the scintigraphic images revealed that GLP-1 release started when the ‘head of the meal’ was in the jejunum.

Study IV: The effect of GLP-1 on non-nutrient gastric emptying is unclear. The effect of iv. administered GLP-1 on gastric liquid emptying was studied in 7 healthy subjects. GLP-1 had a powerful inhibitory effect on liquid gastric emptying and also influenced the gastric distribution of liquids. No effect on water homeostasis was observed.

Study V: There is no available technique for small bowel transit examination without taking the stomach emptying into account. For this purpose, a technique based on biliary scintigraphy with ^{99m}Tc-HIDA was developed and evaluated against simultaneously performed hydrogen breath test in 30 healthy subjects. Radiotracer appearance in the duodenum and caecum, respectively, were taken for start- and endpoints of transit. There was a good correlation between transit times assessed by ^{99m}Tc-HIDA scintigraphy and hydrogen breath test, but the latter showed significant longer transit times due to the passage through the stomach. The technique may easily be set up at any nuclear medicine department where it can serve as a primary step in the evaluation of a suspected GI motor disorder.

Nuclear medicine techniques represent a feasible tool for upper GI motility examinations both in clinical and research settings. The radiation dose is low and commonly available gamma cameras can be used. In addition to reported data, such techniques are used at present for several other studies of the effect of various GI peptides.

Keywords: gastric emptying, ghrelin, glucagon-like peptide-1, ^{99m}Tc-HIDA, obesity, radionuclide investigation, small bowel transit.

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CONTENTS

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
ANOVA	Analysis of variance
BMI	Body mass index
CNS	Central nervous system
DPP-IV	Dipeptidyl peptidase IV
EGG	Electrogastrography
Gd-DOTA	Gadolinium tetraazacyclododecane tetraacetic acid
GH	Growth hormone
GHS-R	Growth-hormone secretagogues receptor
GI	Gastrointestinal
GIP	Glucose dependent insulinotropic peptide
GLP-1	Glucagone-like peptide-1
HSA	Human serum albumin
^{99m}Tc-HIDA	^{99m}Tc-N-(2,6-diethylphenylcarbomoylmethyl) iminodiacetic acid (etifenin)
iv	Intravenous
kcal	kilocalorie
keV	kiloelectron volt
LAO	Left anterior oblique
MAA	Macro aggregated albumin
MBq	megabecquerel
MMC	Migrating motor complex
MRI	Magnetic resonance imaging
mSv	millisievert
NIDDM	Non-insulin-dependent diabetes mellitus
PP	Pancreatic polypeptide
ppm	Parts per million
PYY	Peptide YY
RIA	Radioimmunoassay
ROI	Region-of-interest
T_{50}	Half-emptying time

1 INTRODUCTION

The diagnosis of different functional disorders of the upper gastrointestinal (GI) tract requires methods sensitive enough to detect disturbances of the normal physiology, rather than to diagnose diseases with histopathological morphology. Diagnoses included under this entity comprise disorders affecting motility, secretion or blood flow of the GI organs.

Investigations of gastric emptying require the use of precise, well-defined methods where the choice of technique is determined by a number of factors. A variety of techniques are available to study gastric emptying non-invasively, all of them having specific advantages and disadvantages.

For the measurement of small bowel motility, non-invasive techniques are also preferable. Among those, the vast majority measures the oro-caecal transit time. Inherent with this method is the deposition of the marker in the stomach. This means that oro-caecal transit time will regularly measure both the transit of the marker through the stomach, as well as its transit through the small bowel. By using a tracer excreted by the bile directly into the duodenum, the influence of gastric emptying on small bowel transit time can be avoided.

The purpose of this project was to set up a strictly standardised protocol for scintigraphic gastric emptying of solids possible to use in a multicentre setting. The purpose was also to within this method establish general reference values for healthy subjects of various characteristics, as well as to use GI peptide hormones as a tool to effect solid and liquid gastric emptying. Furthermore, development of a non-invasive ‘easy-to-use’ scintigraphic technique for evaluation of small bowel transit was also within the frame of this project. Taken together, this implies that a complete non-invasive methodology for the diagnosis of functional disorders of the upper GI tract would be at hand.

2 BACKGROUND

The knowledge of gastric digestion and motility made a considerable step forward by the work of Dr William Beaumont in the beginning of the 19th century. In 1822 Dr Beaumont treated the young French-Canadian voyageur, Alexis St. Martin, who had been severely injured by an accidental gun blast. The injury healed with a persistent fistula into the stomach with a six cm wide opening to the body surface. A long series of experiments took place between 1825 and 1833 where the physiology of digestion and gastric juice actions was studied [1]. Beaumont's groundbreaking experiments still form the basis of the knowledge of the digestive system.

The discovery of the Röntgen rays in 1895 opened completely new non-invasive ways to study the motility of the GI tract. A pioneer work was presented by Cannon 1898, in a study of gastric motility in cat. Using bismuth contrast medium mixed with the food, he described the influence of food intake on gastric motility [2].

Gastric emptying studies using radionuclides began in the mid 60s. The far most known report came from Griffith et al who used a ^{51}Cr-labelled porridge meal and a scintiscanner for gamma ray detection [3].

Since the introduction of radionuclide gastric emptying tests considerable improvements have been made in both methodology and operational equipment, and scintigraphy has become the 'golden standard' for measurements of gastric emptying both in research and in the clinical setting. Other methods that have evolved over the years to become increasingly used for studies of gastric emptying in the clinic are ultrasonography and, more recently, magnetic resonance imaging.

GASTRIC AND SMALL BOWEL MOTOR CONTROL

The main function of the stomach is to act as a reservoir of ingested foodstuffs, and to perform mechanical and chemical breakdown of the contents to a fluid chyme that is delivered to the duodenum at a controlled rate. In the small bowel, the nutrient chyme is further degraded to molecular components by digestive enzymes to be absorbed through the gut wall, a process facilitated by the fed motility pattern.

The stomach can be divided in two different functional regions. After ingestion of a solid meal, the proximal and distal parts of the stomach are separated by a 'midgastric transverse band' [4,5] suggested to represent a physiological division important for the regulation of intragastric distribution of solid contents [6]. A gastric pacemaker area located at the upper part of the great curvature generates a slow-wave basal rhythm (pacesetter potential) with a frequency of three waves per minute. This also sets the maximum frequency of gastric contractions [7,8]. The gastric slow-waves are propagated in aboral direction, while the proximal gastric region remains silent without any spontaneous depolarisations.

The small intestine measures approximately 4-6 m in length depending on muscle tone and is divided into duodenum, jejunum and ileum. The sharp bend at the ligament of Treitz represents the anatomical division between the duodenum and the jejunum while the junction of jejunum and ileum is indistinct, but the aboral three fifths are generally referred to as the ileum.

A pacemaker area is located just distal of the pylorus and generates a slow-wave with a frequency of 10-12 waves per minute, which is higher than the gastric basal rhythm [9]. The initiation of a muscle contraction needs a depolarisation superimposed on the slow-wave rhythm, a phenomenon modulated by neurohormonal inputs and locally co-ordinated by the enteric nervous system [10].

Fed motility

After food intake, the gastric fundus and the upper part of the corpus function as a reservoir for the contents. The adaptive relaxatory reflex, described by Cannon and Lieb 1911 [11], successively activates the proximal stomach to accommodate an increasing volume of contents with little change of luminal pressure. This is followed by a tonic contraction propelling gastric liquids and redistributing solids to the distal stomach. From their origin in the mid-corpus, motor waves travel distally to the antrum and ends with closure of the pylorus and a powerful terminal contraction of the antrum. This motoric pattern grinds the solid contents against the closed pylorus in a repetitive process that facilitates the breakdown of solids and mixing with gastric juice [12].

There is a striking difference between the emptying of liquids and solids from the stomach. Ingested liquids are rapidly distributed throughout the entire stomach. The emptying of non-caloric liquids begins immediately and is directly proportional to the volume present in the stomach in a first order exponential process with a half-emptying time of 15-20 min, where the gastro-duodenal pressure gradient is the driving force [13-17]. The gastric emptying of solids has a biphasic pattern. During the first phase, the *lag-phase*, the solids are redistributed from the gastric fundus and broken down to smaller particles (1-2 mm) which can then pass through the pylorus during the *linear emptying phase* [18,19] (Fig. 1). Caloric contents are delivered more slowly to the duodenum than non-nutrient liquids due to a negative feedback mechanism mediated by duodenal receptors, thereby maintaining a constant delivery rate of nutrients into the bowel [14,20,21]. A negative feedback mechanism on the upper gut motility has also been seen during nutrient infusion to the ileum [22]. The gut-derived peptide hormones glucagone-like peptide-1 (GLP-1) and peptide YY (PYY) have been proposed to exert this ‘ileal brake’ mechanism, and a recent study has shown the presence of GLP-1 containing L-cells in the duodenum [23]. Immediately after food intake, the small bowel motility is increased to reach a maximum at about 30 min when the stimulation of motility GI peptide hormones and neurones are considered to be most prominent. This fed motility takes place all over the small bowel, but most markedly at the angle of Treitz. In spite of its predictability and duration of about four hours after a regular 600-kcal meal, this physiological motor pattern has not been mathematically characterised. This means, that we cannot yet use this motor response to food intake for diagnostic purposes [24].

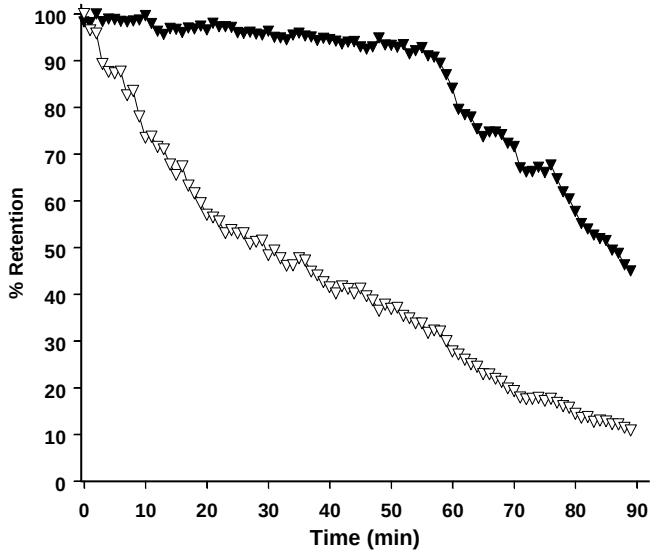

Figure 1: Gastric emptying curves for a solid (^{99m}Tc-labelled omelet, q) and liquid (^{111}In-labelled soft drink, ∇) meal in a healthy volunteer. Liquid emptying begins instantly in an exponential fashion while the linear solid emptying begins after the lag-phase.

Fasting motility

After cessation of the digestive processes a cyclic pattern of GI motor activity, secretion [25] and blood flow occurs [26,27], which migrate from the distal stomach towards the ileum. This pattern, named the migrating motor complex (MMC), was first described in dogs by Szurzewski 1969 and later in several species including man [28-32]. In man, the MMC is mainly featured by 5-15 min periods of pressure waves that recur at highly variable intervals, but usually repeated every 80-120 min. The MMC cycle is divided into three phases where phase I is characterised by quiescence. In phase II random, irregular contractions are present, while phase III consists of the characteristic continuous phasic contractions lasting for up to 15 min. Not all MMCs propagate along the entire small bowel, why the MMC cycle length increases in distal direction [33]. The propagation velocity of the MMC is also higher in the proximal part of the small bowel and slows down in distal direction [33].

The neurohormonal control of the MMC is complex and not fully understood. The enteric nervous system plays a major role in this control, as shown in experiments in dogs were local blockade of the enteric nervous system (i.e. atropine, hexamethonium, tetrodotoxin) blocked the MMC propagation [34]. Of physiological interest are luminal bile acids, which seem to be of importance for the initiation of fasting motility as shown in animal and human studies [35-37]. This is also in agreement with findings in man where pancreatico-biliary secretions in the gut lumen seem to be a prerequisite for the initiation of phase III of the MMC [38].

The gut peptide motilin has a cyclic plasma level pattern in synchrony with the MMC.

The plasma motilin peak precedes the MMC phase III in the stomach, suggesting that motilin is involved in the formation of the MMC activity front [39,40], but the mechanism of cyclic motilin release is still unclear, even if the bile has been suggested to be a main releaser of motilin [37,41]. Later research has shown that a novel GI peptide hormone, ghrelin, [42], which has structural similarities to motilin [43] and is present in the mucosa of the stomach, exerts a prominent stimulatory action on the MMC [44].

The secretory and motor pattern of the MMC has been ascribed as a 'house-keeper' function rinsing the fasting gut from unabsorbed digestibles and accumulated debris in preparation for the next meal [45-47] and thereby most likely acting as a protective mechanism for bacterial overgrowth of the small intestine [31].

Since the MMC pattern is clearly defined, attempts have been made to use manometry recordings of this recurring motor pattern for diagnostic purposes. This has been successful only to a limited extent as only some extreme patterns of motility disturbances have been agreed upon to be of pathological significance [24]. A major drawback of these attempts is inherent with its physiological control showing a rare occurrence and irregularity of motor activity, which will require prolonged invasive manometry in order to capture a motility disorder.

GASTROINTESTINAL PEPTIDES

Glucagon-like peptide-1

Glucagon-like peptide-1 (7–36) amide (GLP-1) is a 30 amino acid peptide produced by posttranslational processing of proglucagon by the 'L-cells' in the intestinal mucosa [48]. The secretion of GLP-1 into the circulation is stimulated by nutrients in the gut. GLP-1 is together with GIP (glucose dependent insulinotropic peptide) a potent incretin [49,50], —'meal related gut hormone with insulinotropic effect' [51]. In addition to the powerful insulin releasing effect, GLP-1 also inhibits glucagon release [52]. GLP-1 has a major inhibitory effect on gastric emptying, thereby acting as an 'ileal brake'. This mechanism is mainly mediated via the vagus nerve [53-57], but endocrine mechanism should also be considered [58].
Another candidate for the inhibition of upper gut motility by nutrients in the ileum is PYY. Like GLP-1, PYY is found in the 'L-cells' of the intestinal mucosa and has also an inhibitory effect on gastric secretion and upper gut motility [59,60].
Gastric acid and pancreatic exocrine secretion is reduced by GLP-1, mediated via afferent vagal pathways [57,61-63].
Receptors for GLP-1 are expressed at different site in the CNS, especially in the hypothalamic paraventricular nucleus and central nucleus of the amygdala [64], this prospecting a role of GLP-1 in the regulation of food intake and appetite. In animals intraventricular injection of GLP-1 reduces the intake of food and water [64,65], while injection of the GLP-1 antagonist exendin has been shown to increase food intake [64]. In humans, infusion of GLP-1 reduces appetite and food intake not only in healthy subjects, but also in obese individuals and diabetic patients [66-68].
Taken together, the physiological effects of GLP-1 make it an interesting drug for

diabetes treatment, especially of non-insulin-dependent diabetes mellitus (NIDDM) [52]. Since the insulinotropic effect of GLP-1 is glucose-dependent there is little risk of therapy-induced hypoglycemia [69,70]. The efforts of GLP-1 treatment is, however, limited by the rapid inactivation of circulating GLP-1 mediated by dipeptidyl peptidase IV (DPP-IV) resulting in a $t_{1/2}$ of only 1-2 min [71]. Current developing pathways are based on either long-lived GLP-1 analogues as exendin-4 or DPP-IV inhibitors [72].

Ghrelin

Ghrelin, a 28-amino-acid peptide was discovered in 1999 [42] as the natural ligand of the growth hormone secretagogues receptor (GHS-R), which is mainly found in the pituitary and hypothalamus but also in peripheral organs as myocardium and adipose tissue [73,74]. The stomach, especially the fundus, is the major origin for circulating ghrelin, and up to 70% decrease in plasma ghrelin levels has been seen in patients who have undergone total gastrectomy [75]. Ghrelin is a strong stimulator of growth hormone (GH) release and to a lesser extent it also stimulates the release of prolactin, ACTH and cortisol [76]. The orexigenic (appetite-stimulating) effect has been shown both in animal and human experiments where iv. administered ghrelin increased appetite and food intake [77-79], probably by activation of neurons in the hypothalamic nucleus arcuate which are involved in the regulation of feeding behaviour [80]. Gastric motility is modulated by ghrelin. Animal studies have shown increased gastric emptying after ghrelin administration [81], why the use of ghrelin for treatment of postoperative ileus has been proposed [82]. In the hitherto only published human study by Wren et al [78] no effect was seen on gastric emptying, as measured by paracetamol absorption test.
Plasma levels of ghrelin are strongly correlated with feeding status although the underlying mechanism is not clear. Before a meal the ghrelin levels are increased, and after ingestion the levels decrease, the latter probably in relation to blood glucose levels [83]. Increased plasma ghrelin levels are seen in catabolic conditions as in anorexia nervosa and cancer cachexia but also after dietary weight reduction [84], while obesity is correlated with low ghrelin levels [85,86]. Another interesting finding is the high ghrelin levels seen in obese patients with the Prader-Willi syndrome [87].
The evolved data makes ghrelin to an important link for the understanding of the brain-gut axis in the control of nutritional and metabolic homeostasis. This may lead to new therapeutic approaches for treatment of obesity and cachectic disorders.

SCINTIGRAPHIC TECHNIQUES FOR ASSESSING UPPER GASTROINTESTINAL MOTILITY

Gastric emptying

The use of radionuclides for gastric emptying studies began in the mid 60s as an effort to overcome the disadvantages of the existing methods (i.e. intubation and radiological techniques), especially in clinically practice. The first publication came from Griffith et al 1966 presenting a method utilising a ^{51}Cr-labelled egg and porridge meal and a scintiscanner for gamma ray detection [3].

Another, but more complicated method using a stationary scintillation detector was developed by Brömster et al [88,89] as a modification of a method utilised for absorption studies [90]. Using this methodology, the test meal consisted of a nutrient liquid formula tagged with ^{131}I-human serum albumin (HSA). The radiotracer was rapidly absorbed in the duodenum, which diminished the acquisition disturbances from the radioactivity in gut segments near the stomach, but correction was needed due to re-secretion of ^{131}I into the stomach.

The early methods for assessing gastric emptying by radionuclide techniques were hampered by several factors caused by equipment and available radionuclides. Up to 10 min were required for the scintiscanner to accomplish a scan, which made the method inaccurate, especially in subjects with rapid gastric emptying. Data were graphically collected as marks on a recording paper showing the stomach distribution, which had to be manually counted. The early gastric emptying radionuclides were far from optimal for radiation safety reasons. They had long physical half lives (^{51}Cr 27.7 days, ^{131}I 8 days) and high-energy emissions (^{51}Cr 320 keV, ^{131}I 364 keV and also a high amount of beta-particles). As radioiodine also accumulates in the thyroid, the gland had to be blocked before the examination.

The introduction of gamma cameras for gastric emptying tests [91] made many subsequent acquisitions possible which improved the accuracy in patients with rapid gastric emptying or dumping. An important step forward came with the availability of ^{99m}Tc, which both reduced the radiation doses and increased the imaging quality [92].

Methodological development for simultaneous testing of both liquid and solid gastric emptying began with the work of Heading et al [93,94] by labelling the liquid and solid components of the meal with radioisotopes emitting different photon-energies which could be separately detected. To overcome the problems with dissociation of the solid phase marker, Meyer et al developed a technique employing *in vivo* ^{99m}Tc-sulphur colloid labelled chicken liver [95]. The *in vivo* labelling of the solid phase is, for natural reasons, not a practical method and *in vitro* labelling of solids with ^{99m}Tc-sulphur colloid or ^{99m}Tc-albumin colloid have been shown to be reliable alternatives [96-98].

General considerations of methodology

Test meal

The composition of the test meal should include both a liquid and a solid component. If only one component can be radio-labelled, the solid phase is considered to be more sensitive and thus preferable. The caloric load should exceed 300 kcal [99,100].

Data acquisition and corrections

Gastric emptying is affected by body posture. It is slower in supine compared with sitting or standing, why the latter alternatives are preferable [101]. Infrequent data sampling can affect the estimation of the initial lag phase [102,103].
When utilising 'low-energy' radioisotopes such as ^{99m}Tc, depth correction for the anterior movement of the ingesta from fundus to antrum has to be undertaken since gamma ray attenuation will lead to an underestimation of the emptying rate, if only anterior acquisitions are made [104,105] (Fig. 2).

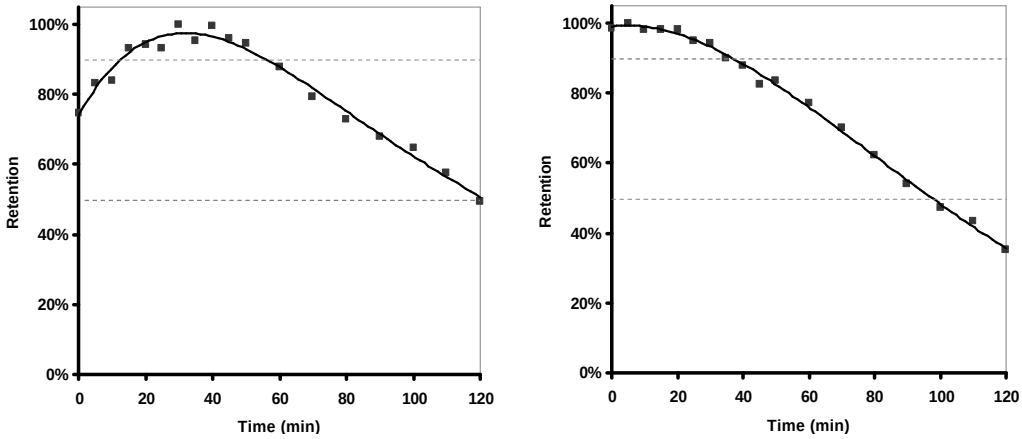

Figure 2: Effect of tissue attenuation. Solid gastric emptying study (^{99m}Tc-labelled omelet) in a healthy volunteer.
Left image- Anterior acquisitions. Note the increased retention, due to anterior movement of the gastric contents, during the lag-phase.
Right image- Geometric mean corrected data from anterior and posterior acquisitions.
Dotted lines are at 90% and 50% retention respectively.

The geometric mean correction method ($\sqrt{anterior\ x\ posterior}$) is well accepted and facilitated by the use of modern dual head gamma cameras for simultaneous anterior and posterior acquisitions. In single head systems the acquisitions have to be performed with alternating positioning of the subject. The left anterior oblique method (LAO) is a less common alternative where the position of a single head camera is parallel with the fundus-antrum axis and thereby reducing the differences in attenuation during the examination [106]. With the 'lateral image' method, a complementary lateral view is used to determine the stomach depth for attenuation correction [107]. Mathematical methods based on the scatter ratio have never gained any widespread application [108].
Physical decay of short-lived radioisotopes (i.e. ^{99m}Tc with the physical half live of 6 hours) would give rise to significant errors if not corrected for.
Dual isotope studies performed to simultaneously evaluate both liquid and solid gastric emptying require a special data correction in order to compensate for 'down-scatter' into the lower energy window, or direct interference if the radioisotopes have similar emitting gamma energies [14,109].

Data analysis

The completely different gastric emptying pattern of liquids and solids requires separated data analysis. Gastric liquid content empties in a mono-exponential manner and data is often presented only as T_{50} (half-emptying time).
The biphasic solid emptying data are usually presented as lag-phase, linear emptying rate, T_{50} (half-emptying time) and retention values at certain times. The most controversial parameter has been the lag-phase, and several definitions for its estimation have been proposed. Visualisation of the radiotracer appearance in duodenum [102] is probably the most physiological estimation but seldom used and requires frequent imaging. More common methods comprise analysis of the time activity curve (percentage drop from maximum) [107,110] or pure mathematical analysis utilising *modified power exponential curve fitting* ($y(t)=1-(1-e^{-kt})^{\beta}$) [111]. Comparative studies have shown roughly similar results for all the methods except for the *modified power exponential curve fitting*, which overestimates the lag period [112,113].

Small bowel transit

Scintigraphic methods for small bowel transit studies have not gained the same widespread use as scintigraphy for gastric emptying, neither for clinical nor for research purposes. In the vast majority of reports, the radiotracer is given orally, thus taking both gastric emptying and small bowel motility in account. The test meal can consist of either liquid, solid or liquid-solid with digestible or non-digestible contents [114-118]. A direct delivery of the radiotracer to duodenum overcomes the influence of gastric emptying rate. Intestinal intubation, on the other hand, can inflict the motility and thereby also transit times [119].
The use of a radiopharmaceutical for hepatobiliary scintigraphy (^{99m}Tc-HIDA) gives access to a physiological pathway to the duodenum through the biliary tree. This approach has been used in animal studies [120-122], but only in one single report in humans without any reference values [123].
There is no ‘golden standard’ for how to analyse and present scintigraphic small bowel transit data. A calculation of the ‘head of the meal’ transit is easy to perform by subtracting the time of first duodenal arrival from first caecal arrival time [115]. Several quantitative or semi-quantitative methods for the estimations of the ‘bulk of the meal’ transit have been proposed. Ten percent colonic filling with or without subtracting the time for 10% gastric emptying is a feasible method, but takes only the leading column of contents in account [124]. Subtracting the gastric half-emptying time from half colonic filling time is another approach [125]. Deconvolution techniques [116] measure the transit spectrum of contents through the small bowel but require very long examination times and are also hampered by the complex mathematical processing of data [126].

NON-SCINTIGRAPHIC TECHNIQUES FOR ASSESSING UPPER GASTROINTESTINAL MOTILITY

Gastric emptying

Radiology

Radiological examinations for evaluation of upper GI motility with barium meals are simple to perform at any radiology department. Most of the techniques are non-invasive and well tolerated by the patients. The volume of the residual barium in the stomach can only be assessed in qualitative terms and the method is therefore considered insensitive for any but the most severe gastric motility disorders.

Ultrasonography

Gastric emptying, wall motion, transpyloric flow and gastric accommodation have been studied by different ultrasonography techniques [127-130]. Assessment of gastric emptying can be performed by measurements of the changes in antral cross-sectional area [131]. The few studies performed by parallel measurements of gastric emptying with ultrasonography and scintigraphy have demonstrated good agreement between the methods, both for liquid and solid meals [132,133]. Ultrasonographic measurements of gastric emptying are, however, time-consuming and dependent on a skilled operator.

MRI

Magnetic resonance imaging (MRI) can be used for gastric motility examinations, permitting the study of both liquid and solid emptying [134-136], as well as gastric contractions and accommodation [137-139]. The first reports described liquid gastric emptying using Gd-DOTA (gadolinium tetraazacyclododecane tetraacetic acid) as an MRI marker [134,135,140] while evaluation of solid gastric emptying with MRI technique can be performed without the use of contrast agents [136].

Intubation

The simplest intubative gastric emptying test is the saline load test. This, however, has the disadvantage of not taking the gastric secretion into account. Utilising this test, 750 ml of physiological saline solution is given through the gastric tube and if >200 ml can be recovered after 30 min it indicates abnormal gastric retention [13,141,142].
The use of a non-absorbable marker in the test volume, as polyethylene glycol or phenol red, makes it possible to estimate the amount emptied and the contribution of gastric secretion.
Intubation techniques have previously provided information of both liquid and solid gastric emptying [13] while the complexity and subject inconvenience has limited their use.

Breath test

Both stable (^{13}C) and radioactive (^{14}C) carbon isotopes, bound to various substrates, can be used for measurement of liquid or solid gastric emptying [143]. Octanoic acid (a

medium chain triglyceride) is the mostly used substrate for labelling of solid meals, and acetate can be used as a substrate to label liquid meals. When the digested meal is emptied into the duodenum, the carbon-labelled substrate is rapidly absorbed and metabolised in the liver to be exhaled as CO_2. By applying mathematical calculations on the exhaled carbon isotope levels, gastric emptying parameters are indirectly assessed [143].

Drug absorption

Liquid gastric emptying can be estimated by the paracetamol absorption test. Paracetamol is rapidly absorbed from the small intestine and gastric emptying is the rate-limiting step since no absorption takes place in the stomach [144,145].
Drug absorption tests require repeat blood samples to capture the rise of the plasma drug concentration. Parameters expressing gastric emptying rate can then be derived from the generated time-concentration curves.

Small bowel transit

Radiology

Small bowel transit evaluated by a barium meal has proven to be inaccurate [146]. Methods with orally administered radio-opaque markers can be used for assessment of colonic or total gut transit, but give little information on gastric emptying or small bowel transit [147,148].

Intubation

With intubation, transit in short small bowel segments can be studied [149], but its use is limited by the complexity of the small bowel intubation process.

Breath test

Breath hydrogen test utilising a non-absorbable carbohydrate marker was introduced by Bond and Levitt 1975 [150]. The carbohydrate, usually lactulose, passes unabsorbed through the stomach and the small intestine to the caecum. In this, the lactulose is degraded by the microflora resulting in a high hydrogen production, which is exhaled and detected in the breath. The time between ingestion of the carbohydrate and the rise in breath H_2, thus reflects the oro-caecal transit time. Bacterial overgrowth in the small intestine may cause early hydrogen peaks which are interpreted as a rapid transit.

Drug absorption

Orally administered salicylazosulfapyridine passes unabsorbed to the caecum where colonic bacteria split the drug. Its metabolite sulfapyridine is rapidly absorbed from the caecum and can be measured in the blood. By this, the oro-caecal transit time can be assessed [151,152].

Gastric and small bowel motor activity

Manometry

Both for antro-pyloro-duodenal and for small bowel manometry, multiple recording points are necessary to evaluate the propagation of motor events. The recordings can be undertaken stationary with a perfused multichannel open tip system, or ambulatory with micro-tip transducers connected to a portable recording system. Antro-pyloro-duodenal manometry requires that the recording points are placed both in the aboral part of antrum and proximal duodenum. In small bowel manometry, a recording point at the ligament of Treitz is crucial since the highest MMC frequency usually is seen there [33,153,154].

Electrogastrography (EGG)

EGG and small bowel electromyography are today purely experimental techniques, which have not yet made their way into the experimental motility lab, even less for clinical purposes.

3 AIMS

The aims of this thesis were to develop and improve nuclear medicine methods for gastroenterologic diagnostics of upper GI tract motility. The developed techniques have been utilised for following purposes:

1) Establish a valid diagnostic test for solid gastric emptying in order to achieve a reliable standard for gastric emptying studies.

2) Evaluate the influence of obesity and gender on solid gastric emptying.

3) Study the effect of GI peptide hormones on the different phases of solid and liquid gastric emptying with special reference to *GLP-1* and *ghrelin*.

4) Evaluate the feasibility of scintigraphic transit investigations of the small intestine as a diagnostic tool for the detection of motility disorders.

4 MATERIAL AND METHODS

SUBJECTS

Paper I

In a multicentre study setting participated eight Swedish centres, each providing 20 healthy subjects, of different age and gender. Totally 160 subjects were recruited (69 men, 91 women, mean age 42.3 years, range 17-80). In order to evaluate the intra-individual variation in emptying kinetics, a repeated examination was done in altogether 12 subjects at five different centres. Both examinations for each individual were made at the same centre within a week.

Paper II

Nine heavily obese (BMI > 35 kg/m^2) patients (3 men, 6 women, mean age 38.8 years, range 25-63) and 21 normal weight volunteers (11 men, 10 women, mean age 47.9 years, range 30-79) were recruited for the study. All included subjects were healthy.

Paper III

Eight healthy men were recruited for this study (mean age 33.6 years, range 24-43). Each subject was studied with GLP-1 and saline infusion in a randomised cross-over fashion at two occasions, 1 week apart.

Paper IV

Seven healthy men were recruited for this study (mean age 27.0 years, range 20-43). Each subject was studied with GLP-1 and saline infusion in one-way blinded fashion.

Paper V

Healthy subjects: Results were based on 30 healthy subjects (14 men, 16 women, mean age 32.3 years, range 19-77). One male was a hydrogen non-producer; hence calculations of lactulose transit were based on 29 hydrogen producers.
Patients: Twenty-three patients (8 men, 15 women, mean age 38.2 years, range 19-68) who had undergone routine work-up including small bowel manometry for a motility disorder with symptoms of abdominal pain or diarrhoea.

Thesis - ghrelin

In a separate study the effect of ghrelin on gastric emptying was studied. This was carried out in 8 healthy volunteers (5 men, 3 women, mean age 26.5 years, range 20-35). Each subject was studied with ghrelin and saline infusion in a randomised cross-over fashion at two occasions, 1 week apart.

METHODS

Scintigraphy

All scintigraphic studies started in the morning after an overnight fast.

Solid gastric emptying

Papers I-II

All examinations were undertaken using the same strictly standardised study protocol according to meal, examination technique and data evaluation.

The meal consisted of a ^{99m}Tc-labelled omelet and a non-labelled soft drink. The omelet was made of two medium size eggs (57-65 g), 15 g wheat flour and 100 ml milk (3% fat), 5 g margarine and salt and basil for flavour. Total energy content of the omelet was 1300 kJ (310 kcal), composed of 19 g fat (57 energy %), 18 g protein (22 energy %) and 16g carbohydrate (21 energy %). ^{99m}Tc-labelled macroaggregates (12-15 MBq) of human serum albumin (TechneScan LyoMAA, Mallinckrodt Medical, Petten, The Netherlands, or Pulmonate, Amersham International plc, Little Chalfont, UK) were added to the batter. The stability of the labelling of the meal was tested *in vitro.* Mixing the chopped omelet with either 100 ml 0.1 M HCl (pH 1.2) or gastric juice (pH 2.3), 1.5% or 2.3% of the radioactive label respectively was recovered in the supernatant after centrifugation.
A non-labelled soft drink of 150 ml (energy content 250-290 kJ, 60-70 kcal) was served as the liquid component of the meal. The non-labelled liquid component can serve as reference in future examinations of simultaneous liquid and solid gastric emptying.

Imaging began direct after the meal was finished (T_0) with 1-min frontal and dorsal acquisitions in sitting position. In *paper I* the subjects at one centre were investigated in standing position due to camera technical reasons. Acquisitions were made at 5-min intervals for the first 50 min followed by 10-min intervals up to a total examination time of 120 min.

In *paper I* the different local imaging equipment were used at the participating centres. In *paper II* imaging was performed using a 400T Maxicamera II (General Electric, Milwaukee, WI, USA) equipped with a general purpose collimator, acquisition matrix 128 x 128 operating a PDP 11/73 computer (Digital Eq. Corp., Maryland, MA, USA).

Examinations were evaluated by manually outlining a ROI in each digitised image. Geometric mean was used for attenuation correction. Correction for physical decay was done using an Excel (Microsoft Corp., Redmond, WA, USA) software macro converting the given count values to percent of maximum plotted against time. A linear fit operation by least-square regression was performed by the Excel macro and applied to the linear part of the curve which was visually identified. The following parameters were calculated from the emptying curve (Fig. 3):

- Lag-phase: Time between T_0 and the intercept of the regression line at the 90% level.
- Half-emptying time (T_{50}): Time between T_0 and the intercept of the regression line at the 50% level.
- Emptying rate (%/min): Defined as the slope of the linear emptying curve.
- Retention 60, 90, 120 min: Defined as percentage gastric retention of the meal by using the values of the regression line at these time points.

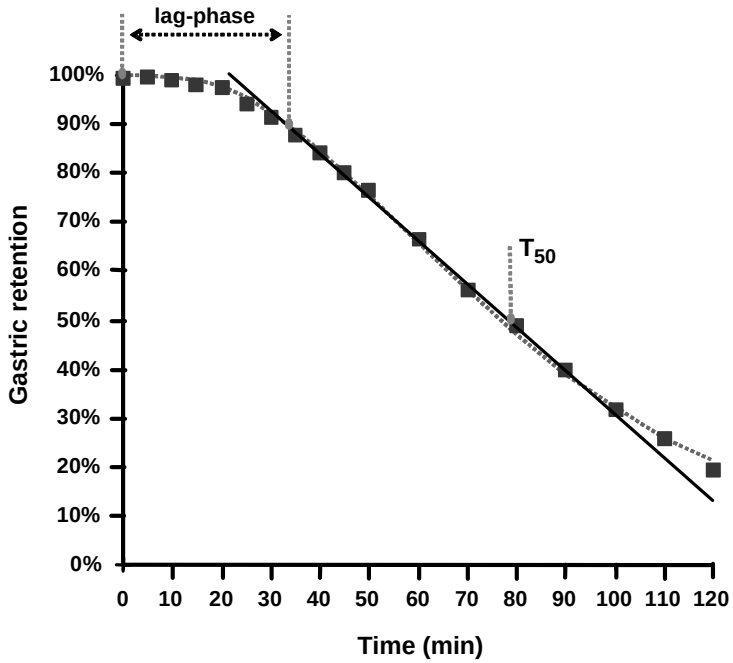

Figure 3: Typical gastric emptying curve for a solid meal (omelet). The initial lag-phase is followed by linear emptying. The slope of the solid line represents the linear emptying rate.

Evaluation of the agreement between repeated examinations was made according to Bland and Altman [155]. For each subject the mean value of the two examinations was plotted against the difference of the first and second examination. This was then evaluated against the difference between the first and second examination for all subjects (mean ± 2 SD).

Paper III

Together with the onset of the meal the intravenous infusion was started with either 0.9% saline or GLP-1 (0.75 pmol x kg^{-1} x min^{-1} Bachem AG, Bubendorf, Switzerland) dissolved in 0.9% saline containing 1% albumin (Albumin Kabi, 200 mg/ml). The infusion continued for 180 min.
The technique for scintigraphic solid gastric emptying differed slightly from the method described in *paper I - II*. Water, instead of a caloric soft drink, was served to the meal. All studies were performed on a dual head gamma camera (Biad XLT, Trionix Inc., Twinsburg, OH, USA). A late acquisition (180 min) was added to the protocol.

Liquid gastric emptying Paper IV

Thirty min before the intake of radio-labelled water, an intravenous infusion of either 0.9% saline or GLP-1 (0.75 pmol x kg^{-1} x min^{-1}, Bachem AG, Bubendorf, Switzerland) dissolved in 0.9% saline containing 1% albumin (Albumin Kabi 200 mg/ml) was started and continued for 45 min after intake of water. Ten MBq ^{99m}Tc-DTPA mixed with 330 ml tap water at room temperature was consumed at T_0. Thereafter, simultaneous dynamic anterior and posterior 1-min acquisitions were collected for 45 min. All examinations were performed using a dual head gamma camera (Biad XLT, Trionix Inc., Twinsburg, OH, USA) and with the subject in sitting position. Geometric mean was calculated for each corresponding image set. The stomach was manually outlined by a ROI on the digitised images to obtain the gastric radioactivity. After correction for physical decay, time-activity curves were generated. In order to evaluate the relative distribution of the radioactive marker between the proximal and distal stomach during the emptying process, the ROI was divided into a proximal and distal part which in most cases were clear using the mid-gastric notch as reference point [156].

^{99m}Tc-HIDA small bowel transit Paper V

The subjects laid comfortably on the couch with the gamma camera in position over the abdomen. Imaging began immediately after iv. administration of 120 MBq ^{99m}Tc-HIDA, with dynamic 1-min anterior acquisitions. The studies were performed on a Maxicamera 400T (General Electric, Milwaukee, WI, USA) equipped with a low-energy general-purpose collimator, acquisition matrix 64x64 and operating on a PDP 11/73 computer (Digital Eq. Corp., Maynard, MA, USA) or a Hermes computer system (Nuclear Diagnostics, Hägersten, Sweden). In the normal subjects each examination was evaluated in two different ways. First, duodenum and proximal caecum were outlined by separate ROIs and the count rate was evaluated against the background activity within each ROI. Doubling the background counts was defined as the time of appearance in the ROI of question, which were taken as start and end-points of transit. In the second evaluation, start and end-points were defined by the clearly visible appearance of radioactivity in the duodenum and caecum, respectively.
In the patient group only visual determination was made. Due to limitations of time allotted for examinations, scintigraphy had to be terminated when the normal transit range, by broad marginal, had been surpassed.

Together with onset of the meal intravenous infusion of either 0.9% saline or ghrelin (10 pmol x kg^{-1} x min^{-1} Neo System, Strasbourg, France) dissolved in 0.9% saline containing 1% albumin (Albumin Kabi, 200 mg/ml) was started. The infusion continued for 180 min.
The technique for scintigraphic solid gastric emptying differed slightly from the method described in *paper I - II*. Instead of a caloric soft drink, 330 ml tap water was served to the meal and 15 ml lactulose (Laktulos 633 mg/ml, Pharmacia, Stockholm, Sweden) was given with the meal in order to perform hydrogen breath test for later validation studies. All studies were performed on a dual head gamma camera (Biad XLT, Trionix Inc., Twinsburg, OH, USA). A late acquisition (180 min) was added to the protocol.
Blood samples for later analysis of ghrelin were taken at T = -20, -10, 0, 10, 20, 30, 40, 50, 60, 90, 120 and 120 min.

Hydrogen breath test *(Paper V)*

In the normal subjects, the hydrogen breath test and HIDA small bowel transit were performed at the same session. None of the patients underwent hydrogen breath test. Duplicate breath samples for hydrogen evaluation were taken with 10 min-intervals (Exhaled hydrogen monitor, GMI Medical Ltd, Renfrew, Scotland). Hydrogen concentration were determined by the mean of each duplicate sample within a range of <50%. If the measurement fell out of this range, a triple measurement was obtained and the mean of the two most similar measurements was used. Two basal breath samples were taken before iv. administration of ^{99m}Tc-HIDA. The liver uptake and bile excretion of the radioactive marker was continuously monitored on the computer screen. When the radionuclide was accumulated in the gallbladder and central bile ducts, 15 ml lactulose (Laktulos 633 mg/ml, Pharmacia, Stockholm, Sweden) was given orally.
The reason for giving lactulose first when ^{99m}Tc-HIDA had accumulated in the bile was to establish as much synchrony in emptying to the small bowel as possible of the two different markers. The hydrogen breath test transit time was calculated as the period from the time of intake of lactulose to the end-point when the breath hydrogen concentration reached values ≥5 ppm above basal level.

Intestinal manometry *(Paper V)*

Gastrointestinal manometry was carried out in the patient group as part of the clinical follow up. Each patient underwent either stationary 8 h- or ambulatory 24 h-manometry recordings.

Stationary 8 h gastrointestinal manometry: Studies were performed with a multichannel catheter (William Cook, Bjaeverskov, Denmark) perfusion technique (Arndorfer Medical Specialities, Greendale, WI, USA) with six recording points located in the antro-duodeno-jejunal region. Each channel was continuously perfused with degassed water and connected to an external pressure transducer. Digital

recordings at 4 Hz were obtained by connecting the respective transducers via a PC Polygraph (Synectics, Stockholm, Sweden) to a personal computer. Evaluation of the motility recordings was performed with Polygram Lower GI version 6.40 (Synectics).

Ambulatory 24 h gastrointestinal manometry: Studies were performed using a tip transducer technique (Synectics) with three recording points located in the duodeno-jejunal region. The recording was stored in a 2 Mb data logger and thereafter transferred to a personal computer. Evaluation of the motility recordings at 4 Hz was performed with Multigram version 6.31 (Synectics).

With both types of motility recordings, the patients were intubated with a recording tube placed in the GI tract with one recording point located at the angle of Treitz for reference [24,157]. Gastrointestinal motility disorders were diagnosed using criteria for specific manometric abnormalities [24,157]:

- *Fasting state:* (a) decreased amplitude of pressure waves, (b) marked increase in baseline pressure during phase III activity, (c) aberrant propagation or configuration of the interdigestive MMC, (d) bursts of non-propagated phasic pressure activity and (e) sustained uncoordinated phasic pressure activity.
- *Fed state:* (a) paucity of fed motility response, (b) early interdigestive MMC, (c) bursts of non-propagated phasic pressure activity or (d) minute clusters of motor activity.

Blood samples and radioimmunoassays *(Papers III–IV)*

Blood samples: All samples were collected in pre-chilled heparinized tubes and centrifuged at 4°C for 10 min at 2000 g. Plasma was collected and stored at -20°C for analysis in one series for each project.
Paper III: Venous blood samples were taken 20 and 10 min before intake of the radio-labelled omelet, and then at the same time points as the scintigraphic acquisitions. GLP-1 (C- and N terminal), PYY, glucose, insulin, glucagon and C-peptide were analysed.
Paper IV: The time of intake of the radio-labelled water was set as T_0. Blood samples were taken at T = -40, -30, -20, -10, 0, 5, 10, 15, 20, 30 and 40 min. GLP-1 (C- and N-terminal), glucose, insulin, vasopressin, sodium and osmolality were analysed.

RIA for GLP-1 (N- and C-terminal) [158-160], PYY [161,162], C-peptide (Euro-Diagnostica, Malmö, Sweden), insulin (DAKO Insulin Kit K6219, Copenhagen, Denmark), pancreatic glucagon [163] and vasopressin (RB 319, Euro-Diagnostica) were done.
Glucose was analysed by an enzyme assay (mutarotase and glucose dehydrogenase; Boeringer-Mannheim, Mannheim, Germany) with a Hitachi 917 automatic analyser (Kyoto, Japan). Plasma sodium was measured potentiometrically using an automated method (Vitros Instruments, Johnson & Johnson Clinical Diagnostics Inc., Rochester, NY, USA). Plasma osmolality was measured using a freezing point depression osmometer (Roebling, Germany).

Data analysis

All values are means ± SD, SEM or median (range), as appropriate, and $P < 0.05$ was considered statistically significant.

Paper I Statistical evaluation was performed using Student's *t*-test and ANOVA with the Tukey-Kramer post test as appropriate. Regression analysis was carried out to evaluate correlations.
Paper II The lag-phase and T_{50} were illustrated using Kaplan-Meier plots and differences were analysed using the log-rank test and Student's *t*-test for unpaired variables. The gastric emptying rate and retention at 60, 90 and 120 min were analysed using Student's *t*-test for unpaired variables.
Paper III The gastric lag phase, linear emptying rate, and T_{50} were statistically evaluated by means of Wilcoxon signed-rank test for matched pairs. The gastric emptying curve and plasma concentrations of C- and N-terminal GLP-1 were analysed by employing an ANOVA for repeated, paired measures, with time and treatment as factors. For glucose, insulin, C-peptide, PYY, and glucagon the changes from baseline (calculated by using the mean fasting value at-20, -10, and 0 min) were compared with the results employing ANOVA for repeated, paired measures, with time and treatment as factors.
Paper IV Gastric retention at 45 min and mean plasma concentration of peptides, glucose, sodium and osmolality during fasting and preload were evaluated using Wilcoxon signed-rank test for matched pairs. The gastric emptying curve and plasma concentrations of peptides, glucose, sodium and osmolality were evaluated by ANOVA for repeated measures with time and treatment as dependent factors.
Paper V Non-parametric Friedman test in combination with Dunn's multiple comparisons post-test was used for comparison between scintigraphic and lactulose transit times. Linear correlation was calculated and presented as r and P-values.

Thesis – ghrelin Lag phase, linear emptying rate, T_{50} and retention at 60, 90 and 120 min were statistically evaluated by means of Wilcoxon signed-rank test for matched pairs.

Ethics

All studies were approved by the Northern Research Ethics Committee and the Radiation Safety Committee at the Karolinska Hospital.

5 RESULTS

Gastric emptying in healthy and obese subjects *(Papers I-II)*

The gastric emptying curves of the healthy volunteers showed a biphasic emptying pattern with a lag-phase followed by a linear emptying phase.
There was a significant difference between men and women for all the computed emptying parameters. Women had a longer lag-phase and a slower linear emptying rate than men resulting in a later T_{50} and higher retention values at 60, 90 and 120 min (Table 1).
Dividing the female group according to menopausal status revealed a significant difference in all emptying parameters except linear emptying rate (Table 1).

Table 1: Gastric emptying results in the multicentre study *(paper I)*.

	Males (n=69)	Females (n=91)	Premenopausal women (n=73)	Postmenopausal women (n=18)
Lag phase (min)	28.3 ± 11.2	34.2 ± 11.7**	35.5 ± 11.7	28.8 ± 10.2$^{\#}$
Emptying rate (%/min)	0.76 ± 0.15	0.66 ± 0.15***	0.65 ± 0.15	0.73 ± 0.15
T50 (min)	83.4 ± 16.3	97.5 ± 21.4***	100.4 ± 21.4	85.6 ± 17.3$^{\#\#}$
Retention 60 min (%)	65.3 ± 10.6	72.4 ± 9.6***	73.7 ± 9.4	66.9 ± 8.8$^{\#\#}$
Retention 90 min (%)	42.8 ± 13.8	52.5 ± 12.8***	54.3 ± 12.5	45.0 ± 11.9$^{\#\#}$
Retention 120 min (%)	21.8 ± 14.6	32.9 ± 16.1***	35.3 ± 15.4	23.1 ± 15.7$^{\#\#}$

Values are means ± SD. Student's t-test showed: **P<0.01 and ***P<0.001 in comparisons between males and females, and $^{\#}$P<0.05 and $^{\#\#}$P<0.01 in comparisons between premenopausal and postmenopausal women.

Separation by age gave similar results if the cut-off was set to 50 years. For men, no differences were seen after separation in corresponding age groups. Regression analysis showed no age-dependent differences in the male group. In the female group the lag-phase was shortened by age while the linear emptying rate increased, together resulting in a decrease of T_{50}. Both the lag-phase and T_{50} decreased by age in the female subgroup below 50 years, while no such correlation were seen in the corresponding male subgroup.
No significant differences on gastric emptying parameters were seen when pre-menopausal women were divided according to menstrual cycle phase. Neither BMI within the normal range nor smoking habits or investigation centre showed any significant impact on the emptying parameters.
Repeated gastric emptying examinations showed a high intra-individual reproducibility with individual differences within ±2SD of overall mean difference for each of the computed gastric emptying parameters.
T_{50} (P=0.03) but not the lag-phase turned out to be significantly shorter in the obese group.
Both the lag-phase (P=0.01) and T_{50} (P<0.01) were shorter in obese females than normal-weight females.

Gastric emptying and gastrointestinal peptide hormones

Glucagon-like peptide-1 *(Papers III-IV)*

Solids

Infusion of GLP-1 at doses reaching high physiological levels caused a delay of gastric emptying, as evidenced by a prolonged lag phase (P=0.01), T_{50} (P=0.01) as well as a slower linear emptying rate (P=0.01) (Fig. 4). These results were also reflected at measurements of gastric retention at 60, 90, 120 and 180 min.

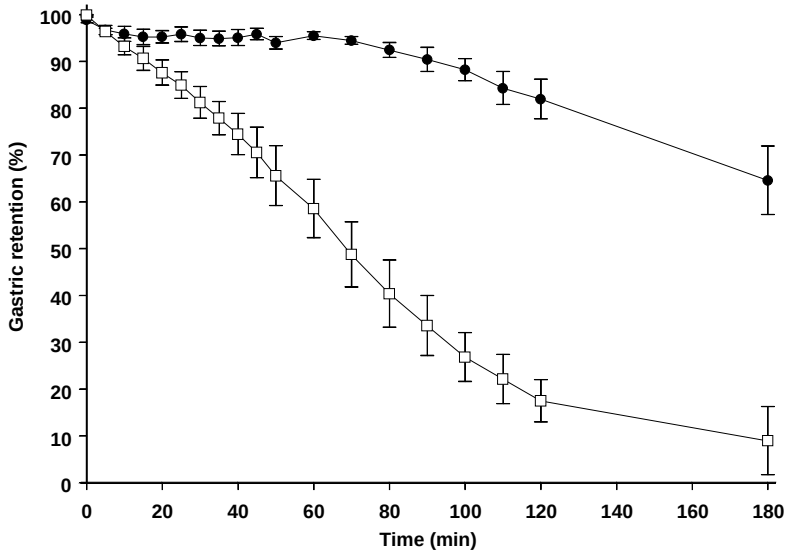

Figure 4: Means ± SEM of solid gastric emptying measured by scintigraphy in 8 healthy male volunteers. Intravenous infusion of GLP-1 (0.75 pmol x kg $^{-1}$ x min $^{-1}$, ˜) or saline (£) for 180 min.

Significantly elevated plasma concentrations of both C- and N-terminal GLP-1 were seen during infusion of 0.75 pmol x kg^{-1} x min^{-1} GLP-1. During saline infusion there was a postprandial bimodal peak of GLP-1 C-terminal secretion at 15-40 and at 50-100 min. Comparison with the scintigraphic examinations revealed the location for the 'leading edge' of the radio-labelled meal at proximal jejunum and ileum, respectively. Baseline plasma concentrations of glucose, insulin, C-peptide, glucagon and PYY did not significantly differ before saline and GLP-1 infusion. Postprandial glucose, insulin, C-peptide and PYY concentrations were significantly lower during GLP-1 infusion compared to saline infusion (Table 2). Both glucagon and PYY concentrations fell below baseline during GLP-1 infusion.

Table 2: Baseline glucose and hormone levels in 8 healthy male volunteers. Effect of GLP-1 infusion (0.75 pmol x kg $^{-1}$ x min $^{-1}$) compared with saline after a 300 kcal omelet meal.

		Baseline		Infusion	
		NaCl	GLP-1	Effect during GLP-1 infusion	P time x treatment interaction effect
GLP-1	pmol/l	-	-	á	P < 0.003
Glucose	mmol/l	5.2 ± 1.5	5.2 ± 1.0	â	P < 0.001
Insulin	mU/l	7.3 ± 1.3	7.2 ± 1.8	â	P < 0.004
C-peptide	nmol/l	0.76 ± 0.13	0.74 ± 0.14	â	P < 0.001
Glucagon	pmol/l	26.0 ± 1.5	27.7 ± 1.0	â â*	P < 0.001
PYY	pmol/l	4.8 ± 1.9	7.3 ± 1.9	â â*	P < 0.04 #

Values are means ± SEM
*below baseline during GLP-1 infusion
ANOVA for repeated, paired measures with time and treatment as factors during infusion. #P for treatment effect.

Liquids

Similar findings as in solid emptying were obtained with non-caloric liquids. A marked reduced emptying, with an increased gastric retention at 45 min (P=0.02), was seen during GLP-1 infusion (Fig. 5). Further analysis of the intragastric distribution of contents revealed an increased accumulation of the fluids in the fundus (P<0.001).

Significantly elevated plasma concentrations of both C- and N-terminal GLP-1 were seen during infusion of 0.75 pmol x kg^{-1} x min^{-1} GLP-1, while saline infusion had no effect on the plasma concentrations. Baseline plasma osmolality and concentrations of glucose, insulin, vasopressin and sodium did not differ significantly before saline and GLP-1 infusion. During GLP-1 infusion the glucose concentrations were lower compared to saline infusion, both during the first 30 min and after liquid ingestion. No significant difference of insulin concentrations was seen between saline and GLP-1 infusions. For the time period after liquid ingestion, there was a tendency towards higher plasma insulin concentrations during GLP-1 infusion, however, not reaching statistical significance (P=0.06).
Plasma osmolality and concentrations of vasopressin and sodium were not significantly different during GLP-1 infusion compared to saline during the first 30 min or after liquid ingestion (Table 3).

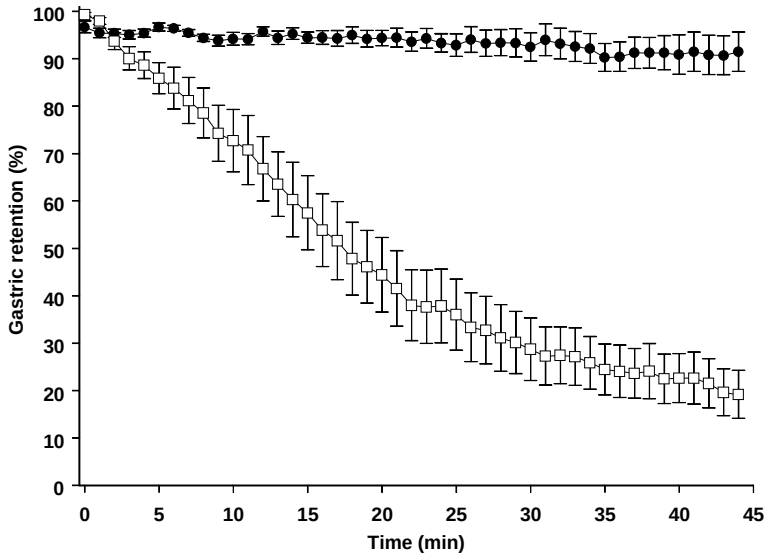

Figure 5: Means ± SEM of liquid gastric emptying measured by scintigraphy in 7 healthy male volunteers.
Intravenous infusion of GLP-1 (0.75 pmol x kg $^{-1}$ x min $^{-1}$, ˜) or saline (£).
Infusions started 30 min before the intake of water and continued for 45 min.

Table 3: Baseline plasma glucose, sodium, osmolality and hormone levels in 7 healthy male volunteers. Effect of GLP-1 infusion (0.75 pmol x kg $^{-1}$ x min $^{-1}$) compared with saline after ingestion of 330 ml tap water.

		Baseline		Infusion	
		NaCl	GLP-1	Effect during GLP-1 infusion	P time x treatment interaction effect
GLP-1	pmol/l	-	-	á	P < 0.002
Glucose	mmol/l	4.8 (4.3–5.1)	4.8 (4.5–5.5)	â	P < 0.001
Insulin	mU/l	6.0 (4.5–16.0)	7.5 (2.0–16.0)	(á)	P = 0.06
Vasopressin	pmol/l	0.65 (0.2–1.65)	0.55 (0.1–1.0)	—	P = 0.19
Sodium	mmol/l	151.7 (146.4–162.1)	151.5 (143.3–165.3)	—	P = 0.20
Osmolality	pmol/l	286.2 (284.0–287.5)	284.1 (278.5–290.0)	—	P = 0.74

Values are medians (range)
ANOVA for repeated, paired measures with time and treatment as factors during infusion.

Thesis – ghrelin

Infusion of ghrelin at doses giving a physiological level hastened gastric emptying as verified by a shortened gastric lag phase, T_{50}, linear emptying rate and reduced retention at 60, 90 and 120 min (Table 4, Fig. 6).

Blood samples are under analysis and the results are yet not available.

Table 4: Results of solid gastric emptying measured by scintigraphy in 8 healthy volunteers. Intravenous infusion of ghrelin (10 pmol x kg $^{-1}$ x min $^{-1}$) or saline for 180 min.

		NaCl	Ghrelin
Lag-phase	(min)	26.5 ± 3.8	16.2 ± 2.2*
Emptying rate	(%/min)	0.82 ± 0.04	1.25 ± 0.10*
T50	(min)	75.6 ± 4.9	49.4 ± 3.9*
Retention 60 min	(%)	62.0 ± 3.8	34.3 ± 6.4*
Retention 90 min	(%)	37.3 ± 4.6	8.4 ± 3.2*
Retention 120 min	(%)	14.8 ± 3.9	0 ± 0$^{\#}$

Values are means ± SEM

*P=0.01 and $^{\#}$P=0.02 Wilcoxon signed rank test for matched pairs.

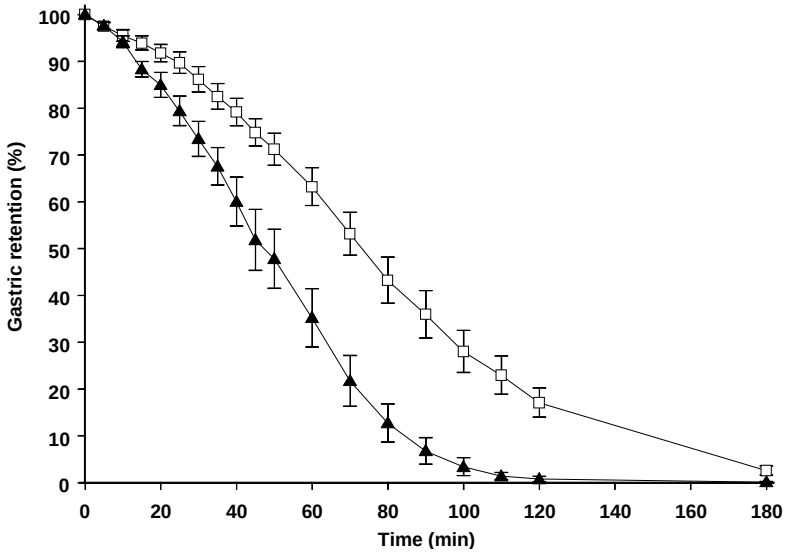

Figure 6: Means ± SEM of solid gastric emptying measured by scintigraphy in 8 healthy volunteers. Intravenous infusion of ghrelin (10 pmol x kg $^{-1}$ x min $^{-1}$, p) or saline (£) for 180 min.

^{99m}Tc-HIDA small bowel transit *(Paper V)*

There was no difference in HIDA transit times either by visual or ROI-based evaluation of the tracer arrival to duodenum or caecum respectively. Lactulose transit was, on the other hand, significant longer compared with the HIDA transit evaluations. A high correlation was seen between the different assessments of transit time (Table 5).
In the group of patients with GI motility disorders, shown as a clinical reference, 17 of 23 had a pathological motility pattern verified by manometry. In 14 of these, HIDA transit was prolonged with abdominal pain as the main symptom in 10 of the patients. Five patients had a normal manometry, but prolonged HIDA transit, in four of them abdominal pain was the main symptom.

Table 5: Transit times and correlations for assessments by ^{99m}Tc-HIDA small bowel scintigraphy and lactulose breath test in 30 volunteers.

	HIDA		Lactulose
	Visual	Calculated	
Transit times (min)	79.3 ± 30.9	77.9 ± 31.1	100.1 ± 43.4*
Correlation	r=0.99	r=0.72	
	P<0.0001	P<0.001	

Values are means ± SD
Non-parametric Friedman test in combination with Dunn's multiple comparisons post-test showed: *P<0.05 for lactulose vs HIDA visual and calculated.

6 DISCUSSION

As a result of the increasing technical development a numerous non-invasive methods for GI motility studies have evolved over the last decades, thereby replacing techniques with lower accuracy or being more invasive [164].
Scintigraphy is considered to be the 'golden standard' for assessing gastric emptying, both in research and in clinical praxis [165]. Since the first reports nearly 40 years ago, the method has undergone major improvements, but so far there has been no standardization or consensus on how to perform the examination, not even between nearby centres [166]. This has been a considerable problem because many methodological parameters can affect the results, and thereby complicate comparisons between different study cohorts. The need of a strictly standardized method based on large number of normal controls has been asked for by authors in the field [166-168].

One goal of this project was to establish a nationwide 'easy-to-use' standard for scintigraphic gastric emptying studies with reference values based on an adequate number of healthy subjects in a multi-centre setting.

In a majority of patients, emptying of solids better distinguish pathology than emptying of liquids [99]. ^{99m}Tc-labelled MAA is easy to handle, and the binding to egg-protein is almost as strong as in chicken liver labelled *in vivo*, why a ^{99m}Tc-labelled omelet meal was chosen [97,98]. Labelling the liquid component with another radioisotope (i.e. ^{111}In) would in the majority of patients give little extra information, but increase the complexity, costs and radiation doses to the subjects. A dual isotope examination of the liquid and solid phases (10 MBq ^{111}In, 20 MBq ^{99m}Tc) corresponds to an effective dose equivalent of approximately 3.5 mSv while our solid phase examination with 15 MBq ^{99m}Tc only gives 0.3 mSv. This is an important factor to take in account if repeated examinations are needed to monitor disease development or in research settings.

To avoid the influence of gravity [101] examinations were undertaken in the upright position.
Depth-correction was done with the geometric mean method, which has proven to be most accurate, and also fits 'modern' dual-head camera systems [169]. Imaging was undertaken more frequently for the first 50 min in order to distinguish the lag-phase. Emptying data were calculated from a linear-fit operation by least-square regression, applied to the linear part of the curve. Evaluation according to the 'modified power exponential' equation was not performed, since this method is purely mathematical and does not take the lag-phase into account. The problem of how to properly define the lag phase still remains an open question [112,113]. The present definition, as a 10% drop from maximum counts, was made from our initial data, suggesting that the emptying from the stomach into the bowel had a lengthy onset. Thereby, we computed the time until the linear emptying began, rather than the first portion emptied to the duodenum. A variant of this lag-phase definition is to adopt linear curve fits for the first and second phase of the emptying curve, setting the intersection as the end of the lag-phase [170].

The influence of gender, age, menstruation cycle and BMI has been unclear and reports with divergent results, usually based on a relatively small number of subjects, have not solved these issues [171-182]. The large number of subjects in the multi-centre study offered an opportunity for sub-group analyses.

Gender had a significant impact on the gastric emptying parameters. Premenopausal women had a slower gastric emptying compared to postmenopausal women as well as compared to men of any age. This was due to both a prolonged lag-phase and a slower linear emptying rate and gave rise to an increased T_{50} and higher late retention values. Splitting the females by age gave almost the same result as splitting by menopausal status if the cut-off was set to 50 years. Consequently this could act as an alternative grouping parameter since the menopause is a transitional process over several years. For men there were no significant differences in gastric emptying by age.
The observations that gastric emptying differed between pre- and postmenopausal women, but no difference was seen according to phase in the menstrual cycle might reflect that a prolonged change in sex hormones as seen after menopause [183], rather than the cyclic pattern seen during the menstrual cycle, is required to change the gastric emptying pattern. Oestrogens mediated increase in nitric oxide synthase activity [184] or decrease in vagal activity [185], may explain the difference in gastric emptying rate between pre- and postmenopausal women.

The influence of BMI on gastric emptying has in earlier reports shown contradictory results, ranging from delayed to rapid gastric emptying [171,172,176,178,179]. Among the healthy subjects in the multi-centre study there was no correlation between BMI and gastric emptying rate. This indicates that a BMI within a ‘normal’ range does not affect gastric emptying. The severely obese women in *paper II* had, on the other hand, a faster gastric emptying than normal weight women in the control group.

Acute cigarette smoking has been shown to delay gastric emptying [186]. In the present study, the subjects were instructed to refrain from smoking at least 8 hours before the examination and no differences between smokers and non-smokers were seen, which indicates that smoking influence gastric emptying in a reversible way.

GASTRIC EMPTYING AND GASTROINTESTINAL PEPTIDES

The GI canal is the largest endocrine organ in the body, affecting not only GI motility but also exerting an important role in metabolic control and feeding behaviour [64,84,187]. Among the 'novel' GI peptide hormones, GLP-1 and ghrelin have gained a special interest for their role in GI motility, energy homeostasis and influence on feeding behaviour [85,188].

GLP-1

The presence of nutrients in the distal small bowel stimulates the secretion of GLP-1, which in turn has a powerful insulin releasing effect but also inhibits glucagon release, thereby exhibiting incretin effects [49,50]. Moreover, the release of GLP-1, stimulated by the bowel nutrients, inhibits upper GI tract motility thereby acting as an ileal brake [22,58]. As a result the nutrients are emptied to the bowel at a slow rate, which helps to blunt postprandial blood glucose levels [189]. This may be the most important physiological role of GLP-1 [56].
An inhibitory effect of GLP-1 on gastric emptying has been shown in earlier studies, performed with 'non-imaging' techniques [53,54,56,189,190]. These reports, however, gave no information about the distribution of gastric or gut contents in relation to blood glucose and peptide hormone levels. In the present studies of either solids or non-caloric liquids, an almost complete inhibition of gastric emptying was seen.

GLP-1 AND SOLID EMPTYING

The bimodal peak of plasma GLP-1 seen during saline infusion corresponded, at comparison with the scintigraphic images, anatomical to the appearance of the 'head of the meal' in the proximal part of jejunum and ileum, respectively. These findings indicate a continuous recruitment of GLP-1 through the small intestine and not only from distal ileum and beyond, where the GLP-1 releasing 'L-cell-population' is denser [23,191], a finding that been seen also in other studies [192-194]. The drop of PYY concentrations below baseline during GLP-1 infusion, indicate a negative feedback mechanism of GLP-1 on PYY secretion. In experiments with isolated perfused porcine ileum, infusion of GLP-1 has been shown to inhibit PYY secretion, this probably through somatostatin release from intestinal D-cells [195].
The lower postprandial insulin response during GLP-1 infusion can be explained by the early reduction in glucose and glucagon levels, in combination with the markedly reduced emptying of nutrients to the bowel. This is according with other studies, which have shown that the insulinotropic effect of GLP-1 is glucose-dependent [69,70].

GLP-1 AND LIQUID EMPTYING

The gastric emptying mechanisms and kinetics are different for liquids and solids [6]. Emptying of non-caloric liquids follows an exponential pattern, thought to be driven by the gastro-duodenal pressure gradient generated by the muscular tonus in fundus [13]. The liquid content is normally immediately spread in the entire stomach. In *paper IV* there was a striking difference in gastric handling of the liquids between the examinations with saline and GLP-1 infusion. GLP-1 almost completely arrested the liquid emptying, and trapped the liquid contents in the proximal stomach.

In a recent human study by Schirra et al [196], infusions of GLP-1 at physiological and supra-physiological levels, had a relaxatory effect on the fundus accomplished by a reduction in the frequency of phasic contractions. The fundus relaxation can explain the increased amount of radioactivity retained in the proximal stomach during GLP-1 infusion. Since the scintigraphic examinations were undertaken in the upright position, a higher degree of emptying through a relaxed pylorus during GLP-1 infusion would be expected due to gravity force. This finding could possibly be explained by a direct contractile effect of GLP-1 on the pylorus. GLP-1 infusion at physiological and supra-physiological levels has been shown to inhibit antro-duodenal motility while pyloric contractions increase, both in the fasting and postprandial state [197].
Plasma glucose levels were, as expected, reduced during GLP-1 infusion. The only insignificant rise in plasma insulin was probably due to the combination of the lowered glucose levels and the lack of absorbable nutrients in the bowel. No effect of GLP-1 on plasma vasopressin, sodium, or plasma osmolality was seen in the present study. In rodents, peripheral administered GLP-1 has been shown to decrease plasma levels of vasopressin [198]. The reason for this disparity is not clear, but maybe the study period was not long enough to show changes in salt and water balance.

GHRELIN

Ghrelin has in animal studies been shown to increase food intake and accelerate gastric emptying [77,79,81,82]. The orexigenic effect of ghrelin has also been shown in human studies while the effect on gastric emptying has been unclear [78,84]. The only published report, done with the paracetamol absorption test for the assessment of gastric emptying, showed no effect during ghrelin infusions at 5 pmol x kg^{-1} x min^{-1} [78]. In the present study, there was a significantly increased gastric emptying during iv. ghrelin infusion at 10 pmol x kg^{-1} x min^{-1}. This effect was seen on both the lag-phase and linear emptying rate and, consequently, also affected the half-emptying time and late retention values. The disparity between the studies may, at least at some extent, be due to the higher infusion rate. The different methodology in the assessment of gastric emptying is another possible explanation thus the paracetamol absorption test measures emptying of the liquid phase while in the present study the emptying of solids was examined, which in general, is more sensitive to detect emptying disturbances in the clinical setting [99].

^{99m}Tc-HIDA small bowel transit

Small bowel motor activity is readily measured by manometric recordings, but the method is complex and not employed at all gastroenterology centres, and can only examine the upper part of the small bowel. Small bowel transit is preferably studied in a non-invasive manner [119]. At various scintigraphic methods for assessment of small bowel transit, the radiotracer has usually been administered orally, why the gastric emptying rate has to be taken in account. As an alternative route, we have used iv. ^{99m}Tc-HIDA, a well-established radiopharmaceutical for hepatobiliary examinations. This method gives a direct access to the duodenum via the biliary tree. Moreover, as no nutrients are given, the method measures the small bowel motility in the fasting state, which makes this technique unique. This is otherwise important at manometry examinations [154]. In order to develop an 'easy-to use' method, the appearance of the radiotracer in duodenum and caecum, respectively, were taken as the start- and endpoints. Thereby, complicated deconvolution calculations demanding long camera times were avoided. There was no difference in HIDA transit times by calculation of count-rate rise (at start- and end-points) or visual determination, why the latter is preferred due to its ease. Lactulose transit times were on the other hand longer, probably influenced of gastric emptying rate and of the time required to metabolise lactulose to hydrogen [199].
^{99m}Tc-HIDA small bowel scintigraphy can be suitable as a primary step in the investigation of suspected GI motor disorders, especially in hospitals without manometry facilities.

SUMMARY AND CONCLUSIONS

Nuclear medicine methods provide functional information in addition to radiological examinations in many fields of medicine. By using ^{99m}Tc the radiation dose can be held to a minimum. The large number of available radiopharmaceuticals opens up possibilities to examine almost any organ system or physiological process. The radionuclide technique is almost non-invasive and permits the use of pharmacological agents in negligible amounts. In practice, this excludes the risk of interference with normal physiologic processes which is fundamental in the study of GI motility. The sensitive and precise registration technique together with digital sampling allows also the detection of small variations in the normal physiology.

Taken together, nuclear medicine methods have qualities of great importance for the study of GI motility, which have been shown in the clinical setting as well as in the daily routine.

The present thesis has illustrated the fundamentals and available assessment methods in the upper GI motility field, with emphasis on gastric emptying and scintigraphy. Moreover, the impact of GI peptides GLP-1 and ghrelin on gastric emptying has been presented:

- A national standard for solid gastric emptying with validated reference values has been established.
- Gender differences in gastric emptying advocate for separate reference values for premenopausal women.
  Severe obesity is associated with increased gastric emptying rate.
- GI peptides GLP-1 and ghrelin influence the gastric emptying in opposite directions.
- ^{99m}Tc-HIDA is a feasible tool for primary investigation of suspected small bowel motor disorders.

The developed gastric emptying method has been used in several other GI peptide studies. Reports have been published regarding the effects of GLP-2 and orexin, and in ongoing projects, the effect of GIP, PP and PYY are studied.

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