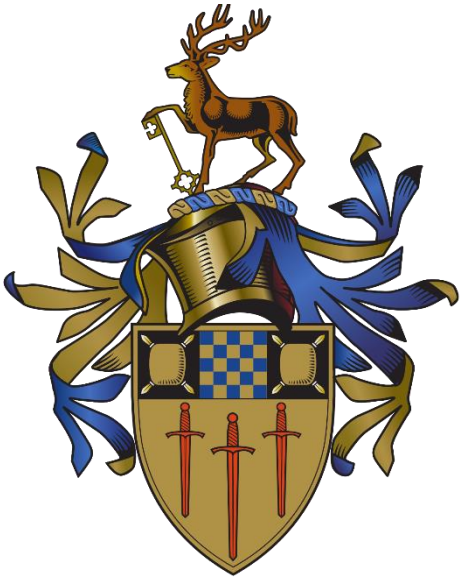
Literature Review of Gastric Emptying Methodology and Mathematical Modelling using the ^{13}C-Octanoic Acid Breath Test

Marwan Elnesr 6408066
Joseph Prollins 6421457
Organisation – University of Surrey
Department of Chemical and Process Engineering
Supervisor – Dr. Michael Short



Declaration

The following literature review is a combined result of writing undergone by both individuals carrying out the aforementioned research project. Shared contributions have been made to significant sections of the report by both individuals with no sections being written by one individual solely.

Contents

List of Figures

List of Tables

Symbols

Symbol	Definition
β	Mathematical constant
γ	Gamma
^{13}C	Carbon-13 isotope
^{14}C	Carbon-14
^{51}Cr	Chromium-51 isotope of chromium
^{99m}-Tc	Technetium-99m isotope of technetium-99
a	Mathematical constant
A_B	Amount of ^{13}C-octanoic acid within breath compartment
A_C	Amount of ^{13}C-octanoic acid within central body compartment
AC_{Plasma}	Amount of acetaminophen in plasma
AC_S	Amount of acetaminophen in stomach
AC_{SI}	Amount of acetaminophen in small intestine
A_I	Amount of ^{13}C-octanoic acid within intestine compartment
A_P	Amount of ^{13}C-octanoic acid within peripheral body compartment
A_S	Amount of ^{13}C-octanoic acid within stomach compartment
b	Mathematical constant
c	Mathematical constant
C_1	Central body compartment
C_2	Peripheral body compartment
C_I	Small intestine compartment
CO_2	Carbon Dioxide
D_G	Gastrointestinal tract depot compartment
D_S	Stomach depot compartment
FP_G	Proportionality factor representing first-pass effect into small intestine
G_D	Amount of glucose in duodenum (first section of small intestine)
G_I	Amount of glucose in ileum (final section of small intestine connecting to large intestine)
G_J	Amount of glucose in jejunum (middle section of small intestine)
G_S	Amount of glucose in stomach
G_{Plasma}	Amount of glucose in plasma
k	Mathematical constant
k_{12}	Transfer rate constant from central body to peripheral body (s^{-1})
k_{21}	Transfer rate constant from peripheral body to central body (s^{-1})
k_a	Rate constant for absorption (s^{-1})
k_{DJ}	Transfer rate constant from duodenum to jejunum
k_g	Rate constant for gastric emptying (s^{-1})
k_{JI}	Transfer rate constant from jejunum to ileum
K_{mAC}	Acetaminophen amount in small intestine giving 50% of maximum first-pass effect
K_{mG}	Glucose amount which results in 50% of maximum absorption rate
k_{nres}	Rate for constant for elimination via non-breath routes (s^{-1})
k_{res}	Rate constant for elimination via breath (s^{-1})
k_{SD}	GE rate constant (same as k_g used in other literature)
m	Total cumulative *C recovery as t → ∞
r	Linear correlation coefficient
r^2	Least squares regression coefficient
RA_{maxX}	Maximum rate of absorption from each segment of small intestine
t	Time (s)

$t_{1/2}$	Half-emptying time (s)
$t_{1/2,\ b}$	Half-emptying time for breath test (s)
$t_{1/2,\ corr}$	Corrected half-emptying time (s)
$t_{1/2,\ s}$	Half-emptying time for scintigraphy test (s)
t_{asc}	Ascension time (s)
t_{lag}	Lag time (s)
$t_{lag,\ b}$	Lag time for breath test (s)
$t_{lag,\ corr}$	Corrected lag time (s)
$t_{lag,\ s}$	Lag time for scintigraphy test (s)
t_{lat}	Latency time (s)
V_1	Volume of central compartment
V_2	Volume of peripheral compartment
$\dot{V}CO_2$	Rate of CO_2 production
$\dot{V}CO_{2DM}$	Directly measured rate of CO_2 production
$\dot{V}CO_{2PR}$	Predicted rate of CO_2 production from resting
$\dot{V}CO_{2BSA}$	Predicted rate of CO_2 from body surface area
V_{MaxAC}	Maximum rate of first-pass metabolism

Acronyms, Initialisms and Abbreviations

Acronym, Initialism or Abbreviation	Definition
CL	Clearance
CLD	Intercompartmental clearance
DAISY	Differential Algebra for Identifiability of Systems
EN	Enteral Nutrition
GE	Gastric Emptying
GEC	Gastric Emptying Coefficient
GES	Gastric Emptying Scintigraphy
GEVMC	Gastric Excitatory Vagal Motor Circuits
GIVMC	Gastric Inhibitory Vagal Motor Circuits
GVMC	Gastric Vagal Motor Circuits
IGGET	Italian Group for Gastric Emptying Test
IGI	Integrated Glucose-Insulin
IIV	Interindividual Variability
JSSMR	Japan Society of Smooth Muscle Research
LRA	Linear Regression Analysis
MDZ	Midazolam
MDZ CL	Midazolam Clearance Modelling
MEPE	Mixed Effects Parameter Estimation
MRI	Magnetic Resonance Imaging
NLRA	Non-Linear Regression Analysis
OBT	Octanoic Acid Breath Test
ODE	Ordinary Differential Equation
PK	Pharmacokinetic
PK PD	Pharmacokinetic – Pharmacodynamic
SIA	Structural Identifiability Analysis

1. Introduction

1.1. Preamble/ Project Outline Description

Gastric Emptying (GE) is a biological process by which the contents of the stomach are emptied into and absorbed by the small intestine (Jacoby, 2017). This occurs through a series of controlled mechanisms which will be discussed in greater detail later in the theoretical background section (Section 2 of the review. GE is essential to the functionality of the human body and digesting system as the absorption of digested food into the small intestine is what allows the nutrients in the food to be distributed in the blood to areas in the body allowing for growth, energy and cell repair (NDDIC, 2017). Due to the nature of GE process, it is especially difficult to accurately determine the rate of GE and gastric emptying time in an individual's body without the use of tracer molecules and specialist equipment. Measuring GE is important as it allows the diagnosis of particular gastric conditions in individuals induced by undesirable symptoms e.g. vomiting, nausea, stomach pains etc. Different gastric conditions can have adverse effects on the GE process where some conditions accelerate GE rate e.g. hypoglycaemia and others slow down the GE rate e.g. hyperglycaemia (O'Donovan, et al., 2004). As such, precise diagnosis of these gastric conditions is crucial in order to prescribe the correct GE-modifying drugs and dosages for patients.

There are different methods to estimate the GE rate in individuals. Based on published literature in the field, main acknowledged techniques and methods in the context of GE are:

1. Radioscintigraphy
2. Stable Isotope (Carbon-labelled) Octanoic Acid Breath Test
3. Semi-mechanistic pharmacokinetic (PK) modelling using ^{13}C-OBT test results

Radioscintigraphy has been widely acknowledged as the most accurate 'gold standard' technique for determining GE rate in individuals. However, the reliance of the technique on radioactive tracers and gamma rays limits its application on pregnant women and children (Ghoos, et al., 1993). Stable isotope octanoic acid breath tests (OBT) were proposed as new novel techniques to estimate GE by equating the rate of Carbon-labelled octanoic acid molecules being digested and oxidised to carbon dioxide (CO_2) which is eventually exhaled in the breath. However, there was a need to further develop the OBT method as there were still discrepancies between results for OBT and radioscintigraphy. Furthermore, although these techniques were able to make progress in estimating GE rate, they still were not able to explain the physiological background for variables used in equations to determine GE rate. As a result, semi-mechanistic PK models were developed to analyse OBT results which were able to obtain results similar to those by radioscintigraphy and explain the physiological meaning behind the mathematical variables used in determining GE rate.

This literature review intends to focus on semi-mechanistic pharmacokinetic models in the context of accurately determining GE rates from experimental data using the ^{13}C-octanoic acid breath test. Therefore, the literature that will be focused on ideally will include: background theory of GE process, semi-mechanistic models about GE, GE using the ^{13}C-octanoic acid breath test and modelling of OBT results using semi-mechanistic, neural networks and non-linear mixed effects techniques. As there is no published literature on modelling of GE test results using neural networks, papers reviewing will cover application of neural networking in areas outside GE. Whilst some papers may not feature modelling of gastric emptying, they do help provide theoretical background that is vital to understand about the topic before, understanding and application of modelling in GE can take place. Review will focus on modelling of GE systems but will also extensively cover previously administered techniques for determining GE and how effective they were in comparison to semi-mechanistic modelling. Although these previous methods are more widely recognised and validated as GE estimation models, semi-mechanistic modelling is able to combine the non-invasive and zero radiation burden features of OBT with the accurate GE rate estimation obtained by radioscintigraphy.

1.2. Aims and Objectives

The aims and objectives of this literature review report will be to carry out critical analysis of papers relevant to the GE and OBT processes and modelling of GE rate based on OBT results using semi-mechanistic PK models, non-linear mixed effects models and neural networks. With reference to and critique of published literature, the literature review report aims to cover the following:

1. Basic biological background of GE process based on key steps involved
2. Importance of understanding how GE process functions in the body
3. History of tests used to determine GE rate and methodology for these tests, namely radioscintigraphy and OBT. This will analyse the following to determine the practicality of both tests:
    - Background for test and key features
    - Method for testing
    - Test meal
    - Measuring techniques
    - Sampling
    - Results interpretation
4. Statistical interpretation techniques for analysing GE test results
5. Mathematical analysis of OBT data curves using defined variables
6. Clinical applications of OBT and diagnosing gastric conditions
7. Application of semi-mechanistic modelling using pharmacokinetic models in OBT to determine GE rate
8. Application of semi-mechanistic modelling using pharmacokinetic models in other applications
9. Application of other modelling techniques to further improve GE models e.g. Mixed Effects Parameter Estimation (MEPE) Non-linear Modelling

1.3. Hypothesis

The main hypothesis for this research topic is that the *'semi-mechanistic pharmacokinetic modelling is the most effective method of determining GE rates from experimental data'*. Theoretical Background

1.4. Purpose of the Field of Research

As mentioned in the preamble/ project outline description section, research into the field of GE rate estimation is necessary to allow diagnosis of different gastric conditions in the human body. Gastric conditions are, by nature, difficult to diagnose as their symptoms are very similar depending on the impact of the condition on the GE rate. Some conditions (e.g. gastroparesis) limit/ slow down the GE rate resulting in the following symptoms:

- Nausea
- Vomiting
- Abdominal pain etc.

The most common causes of delayed GE rate are: 1) an obstruction in the pylorus (gastric outlet channel connecting the stomach to the small intestine which food passes through) and 2) gastroparesis where delayed GE is due to abnormal behaviour of the stomach muscles which causes food to remain in the stomach. Delayed GE rate is also prevalent in individuals who have recently had gastric/ stomach surgery as this can often lead to gastroparesis.

Other conditions (e.g. hypoglycaemia for individuals with diabetes mellitus) have an adverse effect in accelerating the rate of GE resulting in the following symptoms:

- Diarrhoea
- Weakness or feeling light-headed after eating etc.

Understanding GE is important to be able to interpret GE test results for diagnosis of gastric conditions or screening for patients about to undergo gastric surgery (Szarka & Camilleri, 2009). Furthermore, accurate GE tests allow for the diagnosis of particular GE rate modifying drugs which adjust the GE rate of the individual affected based on the gastric condition. Due to the nature of gastric conditions, correct diagnosis can only be done based on accurate sampling and analysis of results from the GE test.

2. Theoretical Modelling

2.1. Gastric Emptying and its Importance

2.1.1. What is Gastric Emptying

Gastric Emptying (GE) is the process by which the food contents of the stomach are emptied into the duodenum (first section) of the small intestine. Figure 1 shows a basic level diagram of the human digestive system with labels to the key organs involved. These organs are also integral to the GE process:

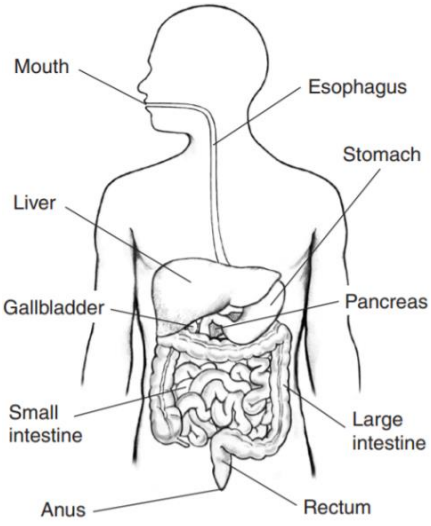


Figure 1 – Labelled diagram of key organs in human digestive system (NDDIC, 2017)

- Mouth – ingestion of food and initial digestion using saliva and teeth
- Oesophagus – delivery of food to stomach
- Stomach – breakdown of large food particles into chyme using gastric juice, peptide acid and churning/ contraction
- Pancreas – providing pancreatic juice containing digestive enzymes
- Liver – providing bile juice containing digestive enzymes
- Small intestine – absorption of nutrients through small intestine walls into bloodstream to be delivered to areas around the body
- Rectum, anus, gallbladder, mouth etc. – elimination of indigestible and waste material from the body

Figure 2 is a diagram at a basic level of detail of the digestive system showing the physiological processes involved in the gastrointestinal system.

The diagram highlights GE as being primarily based in the gastrointestinal transit stage which means transportation (transit) of material from the stomach (gastro) to the small intestine (intestinal). Typical material involved in this process is liquids, digestible solids and indigestible food residues (Goyal, et al., 2019). Water (major liquid involved in GE) usually leaves the stomach immediately whereas digestible solids are first compressed and broken down into chyme, containing particles less than 2-3 mm in size (Goyal, et al., 2019). This often lasts 2-3 hours after a meal has been consumed and is known as the Digestive Period. Any remaining large/ indigestible solid food particles after the digestive period are emptied into the small intestine and this begins the next stage known as the Inter-Digestive Period.

The GE process involves complex biomechanical activity regulated by neuromuscular activity and migrating motor complex (MMC) which utilises electrical waves of varying frequencies to vary the intensity of contractions in the stomach (Goyal, et al., 2019). Figure 3 shows a detailed anatomy of the human stomach and channels connecting the stomach to the small intestine which control the GE process in the gastrointestinal system.

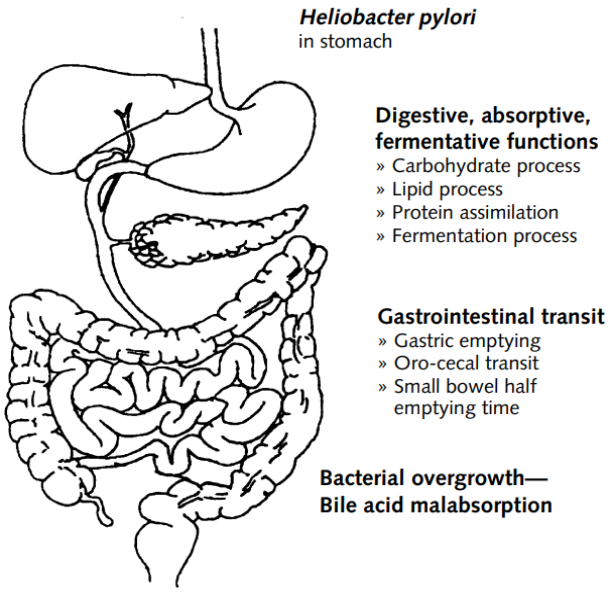


Figure 2 – Key physiological gastrointestinal functions involved in gastric emptying process (Ghoos, et al., 2002)

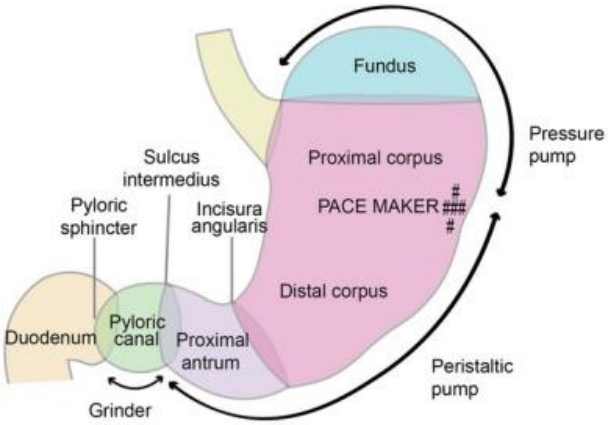


Figure 3 – Anatomy of the human stomach, gastric tunnel (Magenstrasse) and pylorus before duodenum (Goyal, et al., 2019)

It also shows the three key interconnected compartments in the stomach chamber:

1. Pressure Pump
2. Peristaltic Pump
3. Grinder

These structures constitute individual features of the stomach involved in the digestion stage and GE into which allows for liquids to bypass the solid food in the stomach into the duodenum resulting in rapid GE rate. The pylorus allows for grinding of food into chyme using acid-pepsin enzyme (Goyal, et al., 2019). Figure 4 focuses on the interconnections between the stomach and duodenum which food is transported through before absorption of material in the small intestine.

The gastrointestinal system is very complex and consists of various detailed processes and systems beyond the scope of this literature review. However, it is important to understand basic details of the individual processes involved to analyse which areas have the largest influence on the GE rate.

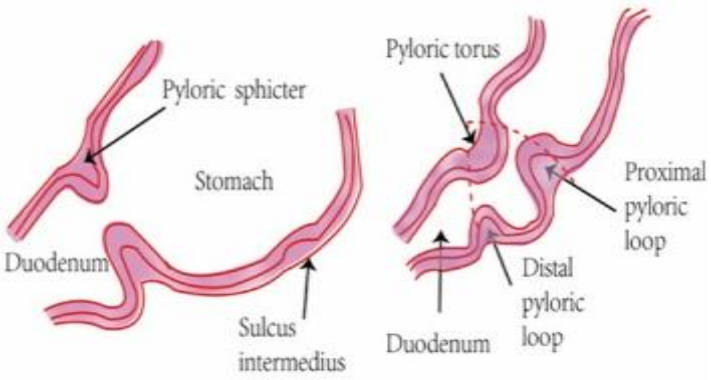


Figure 4 – Anatomy of pylorus responsible for grinding of material into chyme and transferring it into the duodenum (Goyal, et al., 2019)

2.1.2. Key processes and features

The gastrointestinal system/ digestive process can be summarised in Table 1:

Table 1 – Summarised digestive and gastric emptying processes in gastrointestinal system (Goyal, et al., 2019)

Process/ Stage	Key features
Digestive Period	• Filling Phase - Food ingested and fills stomach. Fundic compliance (relaxation of fundic section in stomach) so stomach can accommodate more food. No contractions occur at this point • Pumping Phase – Slow contractions begin in stomach allowing food to be mixed with gastric acid and pepsin to form chyme. This is then transferred to the pylorus. The antrum (wide section in pylorus) must fill to a certain level before food is moved to the duodenum – defined as lag-phase of GE process and is associated with a lag-time (t_{lag}). • Time taken for 50% of the ingested food to be emptied into the duodenum is defined as the gastric half-emptying time ($t_{1/2}$). • Pyloric contractions and relaxations further grind up the food before it is projected into the duodenum. Pressure Waves in the pylorus are used to control contraction and relaxation properties. • Relaxation of the pressure pumps in the stomach controls rate of GE of material into the duodenum. Weaker or disorganised contractions by the peristaltic pump can result in poorer mixing and overall reduced GE rate as there is more indigestible material and vice versa for strong contractions and faster GE rate. • Pylorus also regulates GE rate as it connects the stomach to the duodenum. The pylorus and duodenum must coordinate with each other through contractional activity to control transfer of material out of the stomach. GE rate may be slowed down with increased duodenal contractions and decreased pyloric contractions.

Inter-Digestive Period	• Gastric motility (movement of food through digestive system from ingestion at mouth to elimination from the body) and GE used to empty stomach of indigestible solids. • Controlled by neuro-hormonal activity in a Migrating Motor Complex (MMC) used to regulate movement of food through digestive system • MMC consists of four unique phases (Phases I to IV) • Gastric vagal motor circuits (GVMC) regulate GE process through stimulation (GEVMC – Gastric excitatory vagal motor circuits) and inhibition (GIVMC – Gastric inhibitory vagal motor circuits)
Small Intestine Mixing	• Muscles in small intestine wall mix food from stomach with juices from other organs (liver, pancreas etc.)

Absorption, Metabolism, Distribution and Elimination	• Key processes in small intestine for digested material:
	  ○ Absorption of digested material into the small intestine
	  ○ Metabolism of digested material to yield key components required by body e.g. amino acids, glucose, cholesterol etc.
	  ○ Distribution and Transport of material through blood stream to lungs via pulmonary circulation or to body tissue via systemic circulation (Sanaka & Nakada, 2010)
	  ○ Elimination of material from the body via sweat, urine, excretion, exhalation etc.

2.1.3. Factors affecting rate of GE

There are a variety of factors contributing to a change in the GE rate. These could vary from diet and lifestyle of individual to conditions affecting the gastric system. Table 2 summarises the key factors that should be considered when evaluating test results that suggest an accelerated/ reduced GE rate in an individual. This is important in prescribing gastric medication or deciding if gastric surgery is necessary.

Table 2 – Factors affecting rate of GE in the human body

Factor	How does it affect GE rate
Diet	• Different types of food may take longer/ less time to digest thus, resulting in a different GE rate e.g. protein-based food would take more time to digest thus, longer GE rate compared to vegetable-based food which would have lower GE rate • Drinking lots of fluids could accelerate GE rate allowing for softening and ease of digestion of bulk solid material • This is a particularly important factor to consider for GE rate tests where individuals would eat a test meal containing a tracer component to monitor GE rate in the body e.g. OBT (see section 2.2.4). Composition of this test meal is important in determining the GE rate.
Meal	• Composition (see points made above for diet factor) • Meal volume – Increased volume (i.e. consuming a lot of food) means longer digestion time in stomach thus, slower GE rate • Meal temperature – Increased temperature means food is easier to break down thus, faster GE rate

	• Particle size – Large particle size of food components would increase digestion time to form chyme thus, reducing GE rate
Exercise	• Exercise after consuming a test meal improves body thermoregulation and carbohydrate digestion where carbohydrates are known for reducing GE rate thus, increased carbohydrate digestion accelerates the GE rate (Foster, 1994) • Exercise may also improve mixing in the stomach and allow for faster digestion (care should be ensured as exercising immediately after consuming food can case abdominal pain) • Standing up or sitting down while consuming food and after eating can also affect GE rate. Laying down can reduce GE rate by > 50% after one hour (Szarka & Camilleri, 2009)

Gastric conditions	• Cause upsets in digestion thus, affecting the GE rate
	• There are diagnosed based on common symptoms (see section 2.1)
	• Some examples cases are:
	  ○ Obstruction in pylorus channel connecting stomach and duodenum
	  ○ Gastroparesis – nerves and muscles controlling flow of material in stomach do not function properly
	  ○ Genetic/ lifestyle conditions e.g. diabetes types I and II
	• There are many other conditions affecting different aspects of the gastrointestinal and digestive systems

2.2. Testing Methods for Determining Gastric Emptying Rate

2.2.1. Introduction

There are various testing methods for determining GE rate to diagnose delayed GE across published literature. The main incentive for GE testing is to be able to diagnose particular gastric conditions or screen patients who are about to undergo gastric surgery to prepare them for it (Szarka & Camilleri, 2009). A list of the principal tests and their main methodologies can be summarised in Table 3 developed by (Bruno, et al., 2013):

Table 3 – Principle diagnostic tests for determining delay in gastric emptying rate (Bruno, et al., 2013)

Test	Method
Gastric Scintigraphy	99-m technetium sulfur-colloid labeled low fat, egg-white meal. Scinti-scanning at a minimum of 1, 2 and 4 h after test meal ingestion in the upright position.
Stable isotope breath tests	The non-invasive 13-C-labeled octanoate breath test is an indirect means of measuring gastric emptying. It is a medium chain triglyceride which is bound to a solid meal such as a muffin. After ingestion and stomach emptying, 13-C octanoate is rapidly absorbed in the small intestine and metabolized to 13 CO2 which is expelled from the lungs during expiration. The rate limiting step for the signal appearing in the breath is the rate of gastric emptying.
Radiopaque markers	Indigestible markers, i.e. 10 small pieces of nasogastric tubing, none of the markers should remain in the stomach on an X-ray taken 6 h after ingestion with a meal.
Ultrasonography	Emptying measurement of a liquid meal by serially evaluating cross-sectional changes in the volume remaining in the gastric antrum over time.
Magnetic resonance imaging	Using gadolinium to measure semi-solid gastric emptying and accommodation using sequential transaxial abdominal scans.
Single-photon emission CT	99-Tc pertechnetate that accumulates within the gastric wall rather than the lumen and provides a three-dimensional outline of the stomach. Measurement of regional gastric volumes in real-time to assess fundic accommodation and intragastric distribution can be made.
Swallowed capsule telemetry	The ingestible "SmartPill®" (VA Boston Healthcare System, MA, USA), or telemetry capsule measures pH, pressure and temperature using miniaturized wireless sensor technology. The time taken for the pill to be expelled from the stomach into the duodenum is measured by monitoring the time point at which the acid readings of the stomach are replaced by the dramatic increase in pH as the capsule enters the duodenum.
Antroduodenal manometry	A water-perfused or solid- state manometric catheter is passed from the nares or mouth and placed fluoroscopically into the stomach and small bowel to measure actual gastroduodenal contractile activity. The frequency and amplitude of fasting, interdigestive and post-prandial contractions can be recorded, and the response to prokinetic agents can be assessed.
Electrogastrography (EGG)	Measurement of gastric slow-wave myoelectrical activity *via* serosal, mucosal or cutaneous electrodes. It is most conveniently recorded with cutaneous electrodes positioned along the long axis of the stomach.

The focus of this literature review will be primarily based on the gastric scintigraphy and stable isotope breath tests. However, it is important to be aware of other tests that could be utilised to determine GE rate. Most of these tests are overlooked as they are only able to estimate GE rate for liquid/ semi-solid meals where this research focuses on solid-based meals. Furthermore, some tests involve administration of foreign devices into the patient's body to estimate GE e.g. nasogastric tubing for radiopaque markers test and manometric catheter for antroduodenal manometry test etc. (Bruno, et al., 2013) which is not comfortable for patients. Furthermore, historical published research justifies radioscintigraphy as the 'gold standard' technique for directly estimating of GE rate due to the precision of the results obtained from it. On the other hand, OBT is considered as being a less accurate (with respect to accuracy of results) but more readily available, non-invasive and significantly cheaper test compared to scintigraphy.

2.2.2. Radioscintigraphy

Historically, the process of estimating GE rate has been difficult to predict due to the vast number of processes that contribute to it. In the 1930s, scintigraphy first came about utilising X-rays to better understand the human body performance; however, there was still limited understanding in predicting the rate of GE. This was later developed in 1966 into radioscintigraphy, which used Gamma (γ) rays and a radioactive tracer molecule ^{99m}Tc-albumin colloid embedded into a test meal to monitor gastric movement in the body (Ghoos, et al., 1993).

Conventionally, scintigraphy was based on the time taken for a radiopaque meal (i.e. opaque to X-rays so they don't pass through it) to leave the stomach; however, it was later found out that this was not accurate as it only gave an indication of the time taken for a meal to leave the stomach which does not actually represent the GE rate (Griffith, et al., 1968). Thus, a new method was developed where a test meal was labelled with chromium-51 (^{51}Cr), which is a radioactive tracer molecule, and the rate at which ^{51}Cr leaves the stomach is measured using a scintiscanner (external counting device) (Owen, et al., 1966).

The methodology for the radioscintigraphy test (sometimes referred to as GES or Gastric Emptying Scintigraphy in literature) was as follows (Owen, et al., 1966):

1. Patients must fast overnight before conducting the test
2. Radioactive sodium chromate (Na_2CrO_4) tracer molecule containing ^{51}Cr is prepared at a concentration of 200 μC (unit of electric charge) in a 5 ml. saline solution
3. Breakfast meal prepared consisting of the following food (amounts will vary depending on nature of test):
    - Porridge
    - Scrambled eggs
    - Bread
    - Milk
    - Butter

    First, the bread, milk and butter ingredients are prepared separately with no tracer component added.
4. Porridge and scrambled eggs are prepared with sodium chromate solution incorporated into them
5. Patient consumes meal and the scintiscanner is used to detect rays emitted by the tracer molecule once the meal is finished

GE was estimated by measuring the gamma rays emitted by ^{51}Cr molecule in the stomach using the scintiscanner. (Owen, et al., 1966) decided to use a scanning mechanism rather than a conventional stationary counter device; this is due to the variation in the shape and asymmetry of the stomach and the overall proximity of the small bowel thus, allowing for accurate GE measurement ensuring that the ^{51}Cr molecule was in fact leaving the stomach and not at a different stage of the GE process which further validated the testing technique developed by the authors. Patients had to lie down between two detectors fitted to a scintiscanner device (Owen, et al., 1966). Standardised patient positioning was important as a patient changing from lying down to sitting/ standing could slow down GE by more than 50% in 1 hour (Szarka & Camilleri, 2009). Gamma rays emitted by ^{51}Cr in the test meal are detected by the scanner device and the patient is moved longitudinally by shifting the table-top (adjustable table) 1cm continuously until the entire patient's stomach had been scanned from the outside by the device (Owen, et al., 1966). A printer which is synchronised to move in time with the table-top adjustments is used to record the pulses from the detectors and recording paper is marked based on the number of pulses. A sample of recording paper (termed as a 'scintiscan) is shown on Figure 5 which represents the distribution of the ^{51}Cr tracer molecule in the stomach (in the test meal) and the overall outline of the stomach (Owen, et al., 1966):

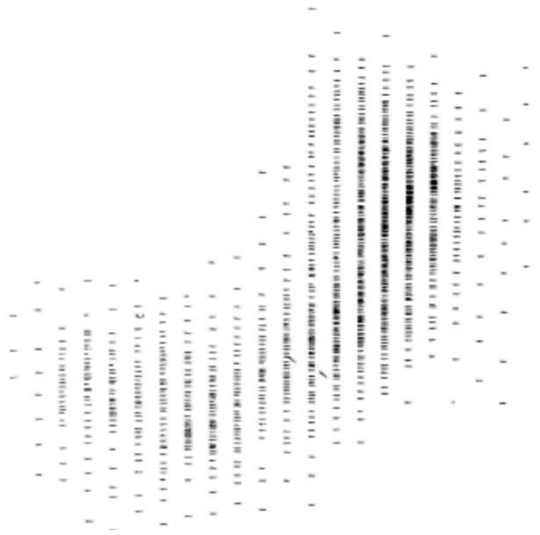


Figure 5 – Scintiscan of upper abdomen representing distribution of ^{51}Cr tracer molecule in the stomach during GES test

Scanning commences once the meal is finished and is repeated every 30 minutes until the stomach is completely empty with each scan taking approximately 6-10 minutes to complete (Owen, et al., 1966). The stomach outlined was deduced from the first scan as shown in Figure 5; this was used a reference point for future scans as well as two fixed points on the skin. The total number of marks in the outlined section was counted and this was approximated to the radioactive content of the stomach at the time of scanning (Owen, et al., 1966).

It was ensured that the GES test technique was validated by a variety of protocols throughout the testing process (Owen, et al., 1966):

1. Detectors fitted in scintiscanner used directional collimators (devices used to align particles/ waves in a particular direction) in order to differentiate the stomach from the lower abdomen so correct analysis of gastric emptying of the stomach is carried out
2. Two detectors were positioned coaxially above and below the table-top to allow the external counting rate to be independent of the depth of radioactivity within the patient's abdominal cavity
3. Verification of the counting rate obtained was carried out by separately scanning specific ^{51}Cr solutions in a pseudo-dummy rubber stomach container immersed in a water tank and the results obtained were directly proportional to the count rate
4. Different factors that could affect the count rate in the stomach were investigated:
    - Different concentrations of ^{51}Cr solution with a fixed amount of ^{51}Cr tracer
    - Addition of other fluids e.g. gastric juice and saliva

    It was observed that these factors did not affect the total count rate in the dummy stomach system.

Overall, the technique was deemed accurate enough such that, by continuous scanning of the test meal labelled with ^{51}Cr at constant speed, radioactivity in the stomach and a correlated gastric emptying pattern could be quantified (Owen, et al., 1966). Furthermore, analysis of patient results using GES led to the development of a new variable which was integral to future research in understanding gastric emptying and this variable is known as gastric half-emptying time or $t_{1/2}$; this was correlated with the exponential drop in the external count which is equivalent to the half-life curve for a radioactive tracer molecule. In this paper, $t_{1/2}$ was defined as the half-life of the test meal in the stomach and used to represent the rate of emptying of food from the stomach into the small intestine due to its exponential nature. The least squares statistical method was used to determine $t_{1/2}$ for each patient and the patient sample size was based on 26 chosen patients both with and without gastro-duodenal disease (condition which causes inflammation to oesophagus, stomach and duodenum resulting in formation of gastric ulcers). This allowed for a wide range of results to validate the test and investigate the impact of gastric conditions on GE and $t_{1/2}$. Figure 6 shows the test results of emptying of the stomach contents against the half-emptying time for both healthy and affected patient samples (Owen, et al., 1966):

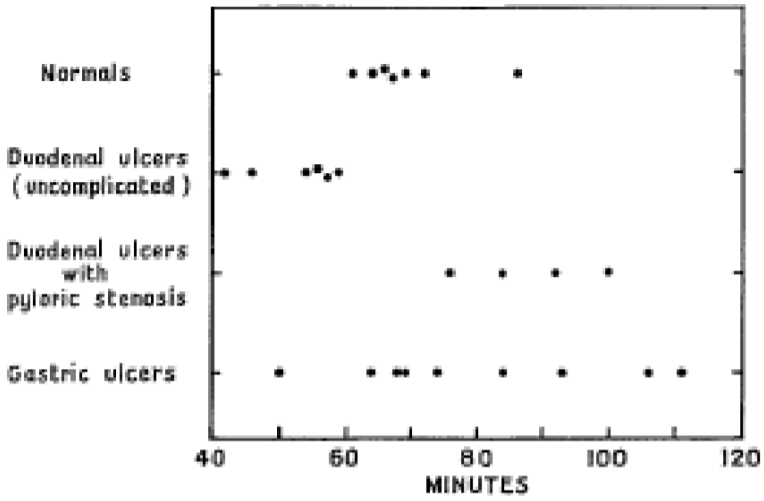


Figure 6 – Results for GES test based on gastric half-emptying time ($t_{1/2}$) for patients with/ without gastro-duodenal disease (Owen, et al., 1966)

It is clear that the GE rate is not the same when comparing healthy patients to those affected by gastro-duodenal disease. Patients with duodenal ulcers are seen to have an accelerated GE rate due to lower $t_{1/2}$ values across patients whereas some patients without duodenal ulcers are seen to have their GE rate slowed down. It should be noted that some patients with gastric conditions still reflected 'normal' GE rates which emphasises the need for repeated testing and a larger patient sample size to reduce deviation in test results.

This paper and GES made a breakthrough in the field of GE rate testing with solid foods and developing physiological factors controlling the GE rate and process. Although GES proved to be very effective at estimating GE rate in patients and assessing impact of gastric conditions on the GE rate, there were some key issues with the work carried out by (Owen, et al., 1966) related to technical aspects and the testing process itself that limited its use. Most of these have been brought up by future published literature analysing the feasibility and application of GES in GE rate testing; these are summarised as follows:

- Future published literature introduced an integral stage in the GE process known as lag phase. This was based on a delay in the GE process before the stomach contents began to empty and was associated with a delay variable (t_{lag}). There is no mention of this stage or variable at all in the work published by (Owen, et al., 1966) which would greatly influence their results and conclusions made as GE rate would be affected by t_{lag}.
- It was later discovered that ^{51}Cr was not a suitable tracer molecule as it is not able to be fully retained in the test meal during GES test and did not emit strong enough radiation (compared to other tracers e.g. ^{113m}In, ^{99m}Tc etc.) to be picked up by the detectors
- This technique was based on rough adjustments of the detector probes to develop an overall outline for the stomach. Not only is this a tedious process (especially due to how long the scanning process would take and having to manually count and note the dots), but the detectors may pick up undesired radioactivity from surrounding areas (e.g. lower abdomen, colon etc.), thus yielding false count data (Chaudhuri, 1973).
- ^{51}Cr has a sufficiently long half-life such that it limits the possibility of repeated GES testing on the same patient over the allocated testing period (Heading, et al., 1971).

Other more generalised limitations were based on the lack of standardisation involved in (Szarka & Camilleri, 2009):

- Testing meals containing tracer molecules
- Patient positioning during scanning
- Timing around acquiring imaging of stomach and gastric system areas

One of the key limitations with GES is that there are too many variations to carry out the test and it is important to develop a normal standardised range for completing GES tests (Seok, 2011). This led to the American Neurogastroenterology and Motility Society and the Society of

Nuclear Medicine to publish a standardised protocol for an 'Egg Beaters' meal which is a standard manual for GES tests to follow when preparing test meals for the patients which was used as a reference for future published literature analysing GES (Tougas, et al., 2000).

A new paper was published (Griffith, et al., 1968) which addressed the limitations of the GES test itself from (Owen, et al., 1966). (Griffith, et al., 1968) were also able to go further by carrying out detailed statistical analysis of the results between healthy patients and patients with gastric ulcers, duodenal ulcers and stomach neoplasms. Results shown were similar in that there a delay in the GE rate for patients with malignant stomach conditions and pyloric stenosis (Griffith, et al., 1968).

GES was further optimised in future published literature using a new tracer molecule known as Technetium-99m-sulphur colloid (^{99m}Tc-SC) and this became the tracer molecule chosen for GES tests nowadays. Later research showed that retention of the tracer within the test meal was essential to ensure the tracer does not dissociate from the solids into the liquid phase which has significantly faster GE rate as liquids require no trituration (i.e. homogeneous mixing of solids to obtain a uniform digestible chyme mixture) (Szarka & Camilleri, 2009). This involved in-vitro simulation testing of retention of ^{99m}Tc-SC tracer in test meal under pseudo-gastric conditions described in (Szarka & Camilleri, 2009).

Other issues brought up by most recently published literature addressing the application and social implications of GES were (Ghoos, et al., 1993):

- Requires prolonged availability and operation of complex and expensive equipment
- Competent operation by trained personnel
- Uses penetrating γ ray emitters which prevent repeated use on one subject
- Radiation exposure make it unsuitable for pregnant women and children. This also makes it uncomfortable for patients particularly, as the radiation dose in GES exceeded that using conventional X-ray techniques when obtaining a single radiograph of the abdomen (Owen, et al., 1966)

Overall, GES provided an opportunity for determining GE rate accurately and precisely to allow diagnosis of gastric conditions for individuals. Although there were some key limitations with the test, it explained key GE processes and variables necessary for the method to be further developed which ultimately, led to the development of the isotopic breath tests (OBT).

2.2.3. Magnetic Resonance Imaging (MRI)

(Szarka & Camilleri, 2009) went on to investigate the application of Magnetic Resonance Imaging (MRI) technique instead of the conventional automatic scintiscanner. Results showed that MRI was able to successfully distinguish between healthy patients and those suffering from gastroparesis through measured gastric motility index based on monitoring antral contractions (area where mixing and digestion of food occurs in the stomach using physical contractions). However, users were not able to directly determine the rate at which the contents of the stomach were emptied. As a result, estimates were made based on the intensity of the relative signals in ex vivo imaging of the test meal in a container with 0.01 M hydrochloric acid solution added to simulate the gastric system conditions (Szarka & Camilleri, 2009). Scanning was done as soon as the meal was ingested of the gastric area and the results were shown in Figure 7 (Szarka & Camilleri, 2009):

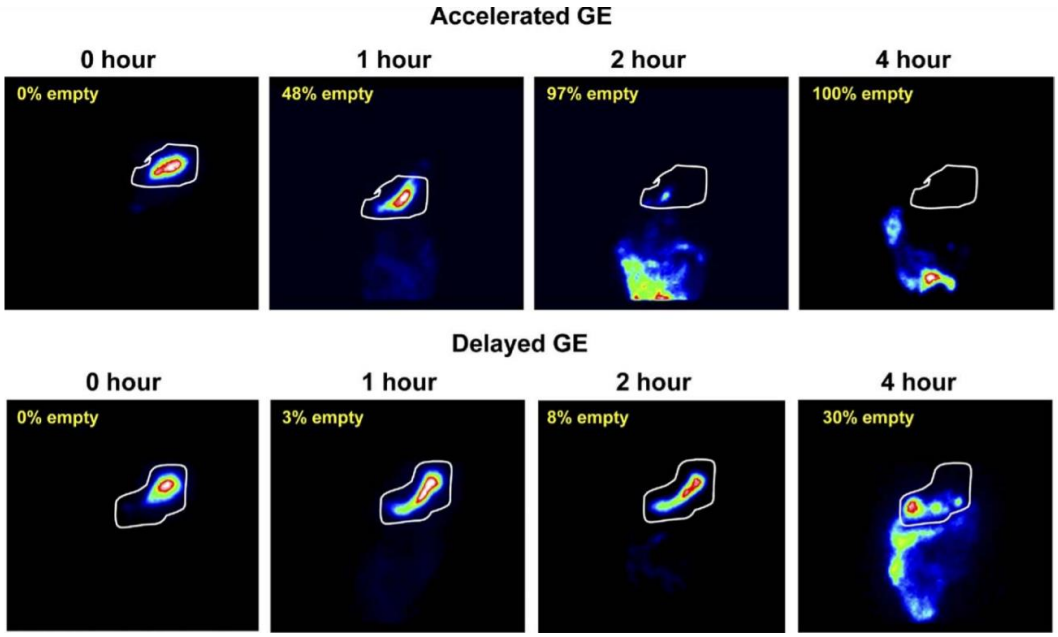


Figure 7 – MRI scan results of gastric system in a GES test immediately after test meal ingestion for patients with gastric conditions

Overall, Szarka and Camilleri, 2009 advised against further pursuit of MRI application as a method for GE rate testing due to it being significantly more expensive than conventional GES. Furthermore, MRI requires further trialling before it could be validated as a GE testing procedure as the test results were not better than those obtained by GES (Szarka & Camilleri, 2009).

2.2.4. Octanoic Acid Breath Test

This section covers the detail of the OBT methodology from test meal preparation to results and sampling and analysis. Although the method by Ghoos et al., 1993 will be the primary source for developing this section, references will be made to other sources including adaptations to the OBT method to optimise the test. Further analysis and critique of the method by Ghoos et al., 1993 and other published literature will be carried out in section 4.0.

2.2.4.1. Background of OBT

To allow a more readily available and practical test to be used which presented minimal radiation burden, the Octanoic acid Breath Test (OBT; sometimes, referred to as isotopic breath test in literature) was developed. This is based on measuring GE rate of solids by means of retention of ^{14}C- or ^{13}C- octanoic acid molecule as a solid in a test meal and monitoring the absorption of this into the body (GE) based on its oxidation to carbon dioxide (CO_2) exhaled in the breath. OBT was able to target the limitations of GES where (Ghoos, et al., 1993):

- It can be readily field tested and does not require specialist analytical equipment
- It is easy to perform
- It is non-invasive and can be repeated in short-term periods
- Significantly reduced radiation burden for ^{14}C-OBT (50-100 times less than GES) and zero radiation burden for ^{13}C-OBT (Siegel, et al., 1983) thus, allowing GE testing on pregnant women and children
- Minimal radiation burden also allows safe repeated/ simultaneous testing on patient without having to worry about radiation overexposure with GES
- It provides excellent sensitivity, specificity and both positive and negative predictive values that are comparable to GES
- It can be done overnight and does not require so much time investment as GES

- It generates reproducible test results

2.2.4.2. Testing methodology

As described by (Ghoos, et al., 1993) and (Perri, et al., 2005), the methodology and biological steps for the OBT are as follows:

1. A test meal is prepared – typically, an egg sandwich – with ^{14}C- or ^{13}C- octanoic acid label being added into the egg yolk of the meal. After homogenising the yolk, the egg white is prepared with carbon-label with either ^{14}C- or ^{13}C-ocatnoic acid.
2. Upon ingestion of the test meal, $^{14}C/^{13}C$- octanoic acid molecule is retained in the solid phase of the meal as it passes through the gastric system
3. Upon reaching the duodenal lumen, the test meal is rapidly disintegrated and broken down from the solid phase
4. $^{14}C/^{13}C$- octanoic acid is rapidly absorbed through intestinal mucosa and oxidised to CO_2 in the liver
5. Rate of CO_2 exhalation in the breath after consumption of the test meal is used to determine the GE rate

Based on the work carried out by Ghoos et al., 1993, there were no distinct differences between using ^{14}C compared to ^{13}C-octanoic acid. Furthermore, as there is still a minute radiation burden with ^{14}C-OBT, it can be said that ^{13}C-octanoic acid is the most suitable tracer molecule for OBT. ^{14}C-OBT involves a combination of both OBT and scintigraphy techniques where the GE rate results are analysed based on CO_2 exhalation rate from the breath but using beta scintillation counting to measure the CO_2 concentration over a specified time period (Ghoos, et al., 1993).

For OBT, egg is the most commonly chosen test meal as “octanoic acid is readily solubilised in egg yolk, which is lipophilic in nature” (Ghoos, et al., 1993). As mentioned above, octanoic acid is suitable for this application as it is firmly retained in the solid phase of the test meal as it passes through the gastric system so this ensures that minimal C-octanoic acid is lost in other areas of the body. The main factor controlling the rate of CO_2 exhalation in the breath after oral administration of $^{14}C/^{13}C$- octanoic acid is the rate of disintegration of the egg yolk test meal from the solid phase in the duodenal lumen – this is known as the *rate limiting step* (Ghoos, et al., 1993). The main drawback with OBT is that it assumes that the rate of absorption of $^{14}C/^{13}C$- octanoic acid and oxidation to CO_2 (i.e. GE) is directly proportional to the rate of CO_2 exhalation in the breath. However, there are various other processes occurring which contribute to the overall GE rate; examples of these processes are: absorption, metabolism, elimination (by means other than CO_2 exhalation) and distribution and it is commonly agreed that not considering these processes is what results in the discrepancy between OBT and radioscintigraphy results (Sanaka & Nakada, 2010). This discrepancy is accounted for by using a model correction factor which is used to correct OBT variables to those by radioscintigraphy. Such variables used in determining GE rate and analysing gastric system are gastric half-emptying time ($t_{1/2}$) and lag time (t_{lag}). Depending of the testing method, these can be further defined based on unique subscripts e.g. $t_{1/2,\,b}$ refers to half-emptying time for OBT whereas $t_{1/2,\,s}$ refers to half-emptying time for radioscintigraphy.

The ^{13}C-Octanoic Acid Breath Test (^{13}C-OBT) was a method developed (Ghoos, et al., 1993) to measure the gastric emptying rate of solids via a non-radiative breath test which can be utilised on children and pregnant women. As previously mentioned, the methodology for ^{13}C-OBT is based on equating the rate of CO_2 exhaled in the breath after ^{13}C-octanoic acid (added to egg yolk of test meal) is oxidised to CO_2 in the duodenum.

2.2.4.3. Kinetics of ^{13}C-octanoic acid in the human body

In order to develop a semi-mechanistic pharmacokinetic model for determining GE rate based on OBT results, it is important to understand the mechanisms of the OBT process and the kinetics of the ^{13}C-octanoic acid tracer molecule throughout the human body while it is retained

in the test meal. Understanding these mechanics provides insight into the processes that contribute to the GE process thus allowing a more accurate representation of GE rate that is not just based on rate of CO_2 exhalation from the breath and rate of emptying of the stomach.

Figure 8 (Perri, et al., 2005) shows the sequential metabolic steps involved in the GE process from ingestion of the test meal containing ^{13}C-octanoic acid to hepatic oxidation (oxidation in the liver) of the octanoic acid to CO_2 and elimination from the body as exhaled breath.

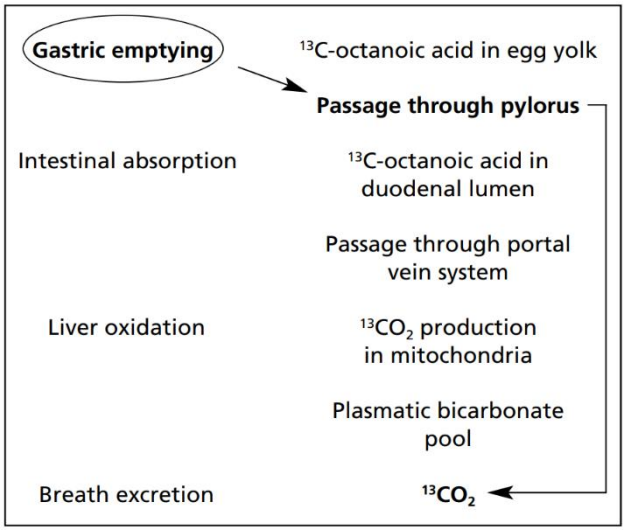


Figure 8 – Sequential metabolic steps for ingestion of egg test meal containing ^{13}C-otanoic acid (Perri, et al., 2005)

As previously mentioned, the rate limiting step is the gastric emptying of egg yolk into the duodenum. Perri et al., 2005 state that the rate of CO_2 exhalation and CO_2 appearing in the breath is unaffected by other metabolic steps (namely, absorption and oxidation) on account of studies done by Ghoos et al., 1993 and that CO_2 appears in the breath "almost immediately with little inter-subject variability" (Perri, et al., 2005). However, discrepancies between the OBT and scintigraphy experimental results invalidates this statement and highlights the key disadvantage of OBT where it does not consider other processes contributing to GE rate. Examples of such process are other metabolic absorption processes, distribution and elimination by means other than exhalation of breath (Sanaka & Nakada, 2010).

The kinetics of ^{13}C-octanoic acid during the ^{13}C-OBT are extremely complex and at a level of detail beyond the scope of this research topic. To allow a better understanding of the metabolic steps, Figure 9 below from Sanaka et al., 2010 shows a human body model explaining OBT theory and metabolic activity of ^{13}C-labelled component in the test meal:

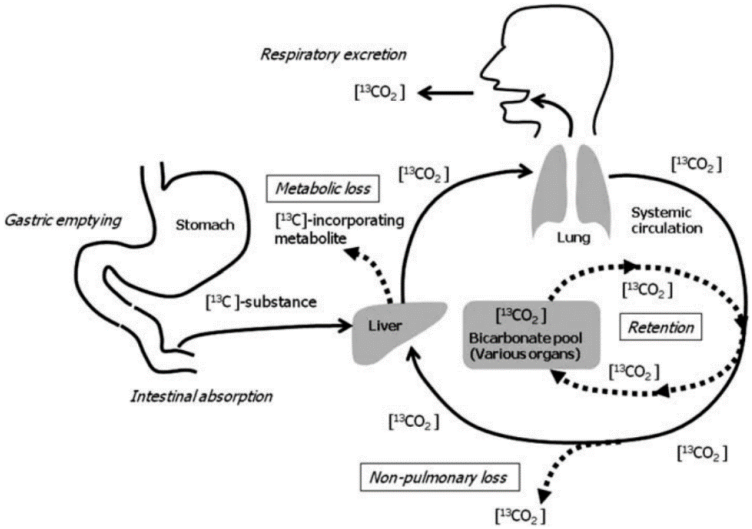


Figure 9 – Model of human body system explaining theory behind OBT (Sanaka & Nakada, 2010)

Although the details of this model are explained in Sanaka et al., 2008, a more concise overview is covered in Sanaka & Nakada, 2010 which defines the key kinetics of ^{13}C-OBT based on four key processes (Sanaka & Nakada, 2010).

First, upon ingestion of the test meal containing ^{13}C-labelled compounds, the labelled compounds are rapidly absorbed in the small intestine and not the stomach where the test meal is digested but ^{13}C label (e.g. ^{13}C-octanoic acid) is retained. Fatty acids are transported to the liver using the portal venous system (used to transport blood from the small intestines, pancreas and spleen to liver). ^{13}C label is removed from the original tracer compound and incorporated into generation of metabolic products e.g. glucose, cholesterol, CO_2, amino acids etc. Then, the ^{13}C label compound is 'fixed' in a metabolic/ bicarbonate compound pool consisting of products that cannot be exhaled (e.g. glucose, amino acids etc.) and the rest is converted to CO_2. Following hepatic (i.e. in the liver) metabolism, $^{13}CO_2$ is released into the blood through pulmonary circulation (deoxygenated blood transported from heart to lungs) and exhaled in the breath. Some of the CO_2 transported in the blood by pulmonary circulation escapes and flows into the systemic circulation which involves transport of oxygenated blood from the heart to body tissue and vice versa for deoxygenated blood. Circulating $^{13}CO_2$ in the systemic circulatory system enters bicarbonate/ metabolic product pools where it is 'fixed' or 'retained' before being eliminated (Sanaka & Nakada, 2010).

1. Fixation — Temporary storage of $^{13}CO_2$ (from hepatic oxidation of ^{13}C label in the liver) in bicarbonate/ metabolic product pools before it is eliminated from the body by pulmonary circulation (elimination by breath) or systemic circulation (elimination by other means). This is associated with 'slow turnover'.
2. Retention — Temporary storage of $^{13}CO_2$ (from hepatic oxidation of ^{13}C label in the liver) in bicarbonate/ metabolic product pools before it is eliminated from the body by pulmonary circulation (elimination by breath) or systemic circulation (elimination by other means). This is associated with 'rapid turnover'.

Various literature defines retention and fixation differently. Many authors characterise fixation and retention as the same term interchangeably (Sanaka & Nakada, 2010). Sanaka & Nakada, 2010, define fixation as having slow turnover where time taken for release of $^{13}CO_2$ from bicarbonate pools back into the circulatory system is significantly longer than retention which is defined as having rapid turnover (i.e. $^{13}CO_2$ is released quickly from bicarbonate pools) (Sanaka & Nakada, 2010). Fixation is also sometimes defined as the 'incorporation of the ^{13}C label into metabolic products' (Sanaka & Nakada, 2010). It can be said that fixation and retention result in a delay in the pulmonary $^{13}CO_2$ elimination where some of ^{13}C label is recycled back from the bicarbonate pools to pulmonary circulation where it is exhaled as $^{13}CO_2$ in the breath (Sanaka & Nakada, 2010).

As briefly mentioned above, $^{13}CO_2$ is retained/ fixed in the bicarbonate pools before it is returned to the systemic circulation where it is either redirected towards pulmonary circulation to be eliminated by breath (defined as turnover) or elimination from body by other means e.g. urine, faeces, sweat etc (defined as loss).

3. Turnover — Elimination of $^{13}CO_2$ as breath where it is recycled back into the pulmonary circulation from the bicarbonate pools in the body tissue and systemic circulation.
4. Loss — Irreversible removal of $^{13}CO_2$ from the body via a non-pulmonary route in systemic circulation e.g. excretory system as urine and faeces, sweat etc.

It can take a long time before the ^{13}C label is fully eliminated from the body (> 12 hours) (Sanaka & Nakada, 2010).

By defining these four processes, important kinetic factors which cause discrepancies between the inlet ^{13}C label contained in the test meal and the outlet $^{13}CO_2$ exhaled in the breath (Sanaka & Nakada, 2010):

1. Incorporation of ^{13}C label into metabolic products – fixation
2. Distribution of $^{13}CO_2$ into bicarbonate pools with rapid turnover – retention
3. Distribution of $^{13}CO_2$ into bicarbonate pools with slow turnover – fixation
4. Elimination of ^{13}C label via non-pulmonary methods – loss

As mentioned earlier in this section, there is a much higher level of complexity in the kinetics of ^{13}C label which will not be covered in this theoretical background. Sanaka & Nakada, 2010 cover the complex kinetics and state the key processes which contribute to ^{13}C activity in the body which affects the GE process and overall GE rate. These processes are (Sanaka & Nakada, 2010):

- Metabolism – postgastric (between small intestine and liver) and hepatic
- Absorption – in small intestine and bicarbonate pools in body tissue
- Transport – in pulmonary circulation and systemic circulation (to/ from heart to/from body tissue)
- Distribution – $^{13}CO_2$ between blood in systemic circulation and body tissue
- Elimination – pulmonary (breath) and non-pulmonary (other means)

2.2.4.4. Patients and Sampling

Patient sample sizes were chosen based on both healthy individuals and ones suffering from gastric conditions. In Ghoos et al., 1993, samples were based on choosing a wide age range between 19 and 71 years old with both male and female patients. For patients suffering from gastric conditions, 20 patients were chosen who had symptoms of epigastric distress and nausea with no history of diabetes mellitus, previous gastrointestinal surgery or use of medication affecting gastric mortality; this is important to ensure that no former treatment/ surgery had been undergone which could improve the GE process and affect validity of results carried out. However, Ghoos et al., 1993 did not investigate GE in individuals with other gastric conditions, while Perri et al., 2005 did, by discussing the clinical applications of ^{13}C-OBT in diagnosis of gastric emptying conditions in individuals based on OBT results where the test is sensitive and precise enough to detect changes to the GE process induced by gastric-enhancing drugs taken e.g. motilin-receptor agonists, cisapride, anticholinergics and octreotide (Perri, et al., 2005). Furthermore, Perri et al., 2005 went further to investigate the effect of taking these gastric drugs on OBT results and $t_{1/2}$ and t_{lag} variables (defined earlier in section 2.1.3). Further investigations were carried out to determine the effect of using different drugs and dosages on the GE rate enhancement which presents an opportunity for utilisation of OBT in clinic-pharmacological studies. Generally, published literature tended to focus on GE of individuals with specific gastric conditions and compared those results to GE of healthy individuals. Few went further to investigate the impact of certain GE modifying drugs on the GE rate of individuals with gastric conditions and the clinical applications of GE test results. For example, Schommartz et al., 2006 chose to study diabetic gastroparesis,

To evaluate intraindividual (within subjects themselves based on day-to-day variability) and interindividual (between different subjects), Ghoos et al., 1993 repeated ^{14}C- and ^{13}C-OBT studies three times over a period of 3 weeks to analyse the interchangeability between the two OBTs. This was the first major step in the development of the ^{13}C-OBT as the studies showed that there was little to no variability in the results between ^{14}C- and ^{13}C-OBT meaning that ^{13}C-OBT could now be used as an established non-invasive method for determining GE rate with zero radiation burden on the patient. This set up the foundation for future research and highlighted the need for moving away from radioscintigraphy and radiative methods. Furthermore, mathematical modelling-based methods for more accurate determination of GE rate would not have been possible without the initial research by Ghoos et al., 1993.

2.2.4.5. Testing Procedure

Different OBT measuring techniques to determine the GE rate were employed by different papers but the majority used the same principal methods. Ghoos et al., 1993 covered a very detailed method where both ^{14}C- and ^{13}C-OBTs were carried out. For ^{14}C-OBT on normal individuals not affected by any gastric conditions, the CO_2 exhalation rate is measured immediately after "intraduodenal administration of 74 kBq (Becquerel - unit for measuring radioactivity) of ^{14}C-octanoic acid" (Ghoos, et al., 1993) as follows:

- Every 3 minutes for 30 minutes then;
- Every 5 minutes for 30 minutes and finally;
- Every 15 minutes for 3 hours

For remaining patients, breath test sampling was done over 2 hours with samples taken every 15 minutes. ^{13}C-OBT involved an additional step in utilising infrared mass spectrometry (IRMS) or infrared spectrometry (IS) in order to differentiate between $^{13}CO_2$ and $^{12}CO_2$ content in the breath (Bruno, et al., 2013). Furthermore, the weight and height of each patient must be determined in order to calculate the body surface area. Ghoos et al., 1993 proposed using a height-weight formula developed by Haycock et al., 1978 to calculate surface area empirically where:

$$SA = W^{0.5378}\, H^{0.3964} \times 0.24625 \qquad \textbf{Equation 1}$$

Where, SA is the body surface area in m^2, W is weight in kg and H is height in cm. (Haycock, et al., 1978).

Patients were also subjected to the radioscintigraphy test in the method by Ghoos et al., 1993 with sampling being done over 2 hours: every 10 minutes for the first hour and every 15 minutes for the second hour (Ghoos, et al., 1993). Methodology for GES by Ghoos et al., 1993 was the same as that described in section 2.3.2.

To minimise the risk of false positive/ negative data, patients should fast at least 8 hours before the test and should drink only non-carbonated water (Bruno, et al., 2013).

Once sampling and results collection was complete, mathematical analysis was carried out.

Bruno et al., 2013 summarise the ^{13}C-OBT methodology in table:

Table 4 – Methodology for carrying out ^{13}C-OBT (Bruno, et al., 2013)

Step 1	Patients should fast at least 8 hours before conducting the OBT. Only natural/ non-carbonated water can be consumed.
Step 2	Two breath samples are collected for each patient before meal is consumed (t = 0).
Step 3	Weight and Height is determined for each patient in order to calculate body surface area using Haycock et al., 1978 formula or other method.
Step 4	^{13}C-labelled meal prepared (conventionally, egg meal but dependant on test study). 250 ml water provided with meal.
Step 5	Meal consumed by patient.
Step 6	Breath samples collected based on pre-determined test intervals. Bruno et al., 2013 proposed 15-minute intervals over 4 hours whereas Ghoos et al., 1993 proposed 15-minute intervals over 2 hours. Patient must not do the following after consuming the meal during the study:

	• Drink • Eat • Smoke • Sleep • Carry out any physical exercise
Step 7	Breath sample analysis using Infrared Mass Spectrometry (IRMS) or Infrared Spectrometry (IR) to distinguish $^{13}CO_2$ from $^{12}CO_2$ in exhaled breath.
Step 8	Results interpretation and therapy discussion with patient.
Step 9	Statistical evaluation with OBT results from other patients to investigate trends and deviation and variability in studies.

2.3. Mathematical Analysis

The following method is taken from Ghoos et al, 1993. Other published literature on the OBT use the same equations with slightly different notation. However, similar equations have been used in other studies (González, et al., 2000). Although González et al., 2000 were investigated GE of liquids, the governing equations for OBT are still applicable.

For the OBT, two equations have been derived to fit the data. Equation 2 effectively fits the measured percentage $^{13}CO_2$ recovered in breath per hour. Equation 3 is derived from the percentage cumulative ^{13}C excreted in the breath as time tends to infinity.

$$y = at^b e^{-et} \qquad \textbf{Equation 2}$$

Where, y is the percentage of *C excreted in the breath per hour, t is time in hours and a, b, and c are constants.

$$y = m\left(1 - e^{-kt}\right)^{\beta} \qquad \textbf{Equation 3}$$

Where, y is the percentage of cumulative *C in the breath, t is the time in hours, k, and β are constants and m is the total cumulative *C recovery when time is infinite.

According to Gonzalez et al., percentage of $^{13}CO_2$ excreted in the breath per hour (y) can be defined based on Equation 4 which is the differential form of Equation 3:

$$y = mk\beta e^{-kt}\left(1 - e^{-kt}\right)^{\beta-1} \qquad \textbf{Equation 4}$$

Conducting nonlinear regression analysis allows three parameters to be derived: half-emptying time, the lag phase and the gastric emptying coefficient (GEC), these parameters are represented by Equation 5, Equation 6 and Equation 7 respectively.

$$t_{(1/2,\ b)} = \left[-\frac{1}{k}\right] \times \ln[1 - 2^{-\frac{1}{\beta}}] \qquad \textbf{Equation 5}$$

$$t_{lag,\ b} = \frac{\ln(\beta)}{k} \qquad \textbf{Equation 6}$$

$$GEC = \ln(a) \qquad \textbf{Equation 7}$$

Using the GE half-emptying time and GEC, a relationship was determined with the scintigraphy half-emptying time ($t_{1/2s}$) via correlation and linear regression analysis which also applies to the relationship between the lag phases of scintigraphy and OBT ($t_{lag,\ s}$ and $t_{lag,\ b}$ respectively) (Ghoos, et al., 1993).

Correction of the OBT lag phase and half-emptying time ($t_{lag,\ b}$ and $t_{1/2,\ b}$) was required due to the discrepancy in these variables between OBT and scintigraphy. Ghoos et al., 1993 proposed a correction time of - 66 minutes which is used to validate the OBT results to they are in line with scintigraphy (Ghoos, et al., 1993). Gonzalez et al., 2000 propose a correction time of -53 minutes and define new corrected time variables ($t_{lag,\ corr}$ and $t_{1/2,\ corr}$) which allows for simpler mathematical analysis (González, et al., 2000).

2.3.1. Statistical Analysis

Statistical analysis is used to interpret the results obtained from the OBT due to the variability in data between different patients. In literature comparing the results from scintigraphy and OBT, correlation and linear regression methods are used to compare the scintigraphy half-emptying time ($t_{1/2,\ s}$) to the data obtained by OBT breath sample analysis which is breath test half-emptying time ($t_{1/2,\ b}$) and gastric emptying coefficient (GEC which is equivalent to ln (a) as per section) (Ghoos, et al., 1993). This was often carried out using programming or statistical software e.g. Matlab, PROC NLIN program (for non-linear regression models) by SAS (statistical software) (Ghoos, et al., 1993). This is significantly more efficient and less time-consuming than manual statistical analysis using Excel and is utilised by most recently published literature carrying out analysis of GE test results. Excel Solver function offers an alternative for carrying out statistical analysis with ease and could be used as a tool for verify the results of the mathematical software models.

In some literature, half-emptying times ($t_{1/2}$) between scintigraphy and OBT were related by linear regression analysis (LRA) using correlation coefficient (r) and a low level of probability significance ($P \leq 0.05$). This is also used as a measure to detect how close data is to a fitted regression line; however, it is less accurate than non-linear regression analysis (NLRA) as OBT test results do not follow a linear trend. On the other hand, LRA is simpler to carry out and can be done without relying on programming or modelling software compared to NLRA which cannot be done for studies of this complexity and magnitude without modelling software.

Linear regression analysis involves fitting experimental data to a linear line with the following equation:

$$y = a + bx \qquad \textbf{Equation 8}$$

Where y is the dependent variable, x is the independent variable, b is the gradient of the regression line between two points on the line and a is the y-intercept (x = 0).

For non-linear regression analysis, experimental data is fitted to polynomial curves of varying orders based on the desired accuracy of the regression analysis. For second order polynomial, the regression curve has the following equation:

$$y = a + bx + cx^2 \qquad \textbf{Equation 9}$$

For a third order polynomial line:

$$y = a + bx + cx^2 + dx^3 \qquad \textbf{Equation 10}$$

Increasing the order of the polynomial would result in a more accurate non-linear regression coefficient but would also increase the complexity of the problem and amount of time required for the program to solve the model. A sensible polynomial order should be considered by the program developer e.g. 2nd or 3rd order polynomial which would yield an accurate correlation result, while not being too complex in its nature.

3. Modelling

3.1. Gastric-Emptying Modelling

Literature within the field concerning semi-mechanistic modelling of GE and OBT is limited. The general aim is to develop semi-mechanistic pharmacokinetic models and evaluate their effectiveness concerning how the OBT results can be curve-fitted towards the scintigraphic results hence establishing correlations between indirect and direct measurement of GE.

Work completed by Ogungbenro & Aarons, 2011b focused on structural identifiability analysis (SIA) using the software package: Differential Algebra for Identifiability of Systems (DAISY). The aim of the paper was to develop a new semi-mechanistic mode to analyse OBT results and how effective DAISY as a simulation model. The model was designed to classify mathematical model parameters into globally identifiable, locally identifiable or non-identifiable in the context of GE, absorption, distribution, metabolism and elimination processes for ^{13}C-octanoic acid.

Within the same year, the authors release another paper building upon their previous model relating to semi-mechanistic modelling regarding GE (Ogungbenro & Aarons, 2011a). This was developed in MATLAB and went beyond the first in Ogungbenro & Aarons, 2011b by analysing the results of the semi-mechanistic PK model against results yielded by other known OBT methods: Modified exponential method, Ghoos and Wagner-Nelson.

Semi-mechanistic models for GE tend to split the body into compartments e.g. stomach, intestine, central body, peripheral body and breath (Ogungbenro & Aarons, 2011a). This is to consider processes which, when unaccounted for, result for inaccurate representation of GE by only considering rate of CO_2 exhalation in breath as a direct measurement of GE rate (Ogungbenro & Aarons, 2011a). Alongside the OBT GE model, rate constants representing kinetics of other processes in Sanaka et al., 2010, k_g, k_a, k_{res}, k_{nres}, k_{12}, k_{21}, were developed. These represented GE, absorption (with baseline GE as a constraint), expulsion via breath, expulsion via non-breath routes, central body to peripheral body and peripheral body to central body (representing distribution and metabolism). This is represented in Figure 10 where the input for the model is the stomach with the output in the breath expulsion.

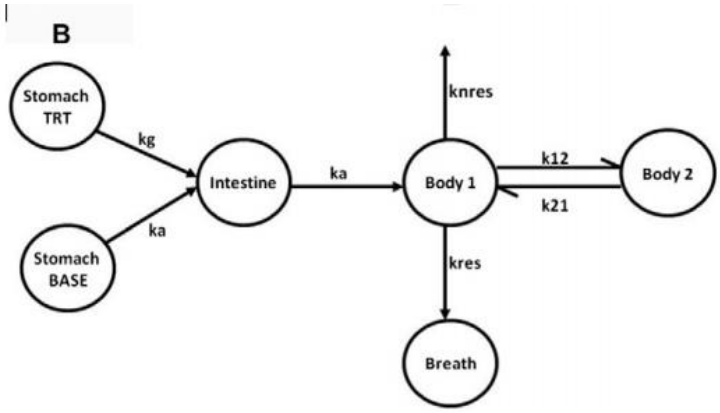


Figure 10 – Graphical representation for the semi-mechanistic model. In this case, showing a treatment and baseline approaches (Ogungbenro & Aarons, 2011a)

Approaches currently undertaken, focus on mathematical curve fitting of the models between the OBT and scintigraphy in order to generate accurate GE results.

Equation 11 through to **Equation 15** represent the mass transfer from each compartment within the model based on rate constants where A_S, A_I, A_C, A_P and A_B represent the amount of ^{13}C-octanoic acid within the stomach, intestines, central body, peripheral body and breath respectively (Ogungbenro & Aarons, 2011a). The transfer rates are based on first order ordinary differential equations (ODEs).

$$\frac{dA_s}{dt} = -kg \cdot A_s \quad \textbf{Equation 11}$$

$$\frac{dA_1}{dt} = (kg \cdot A_s) - (ka \cdot A_I) \quad \textbf{Equation 12}$$

$$\frac{dA_c}{dt} = (ka \cdot A_1) + (k21 \cdot A_P) - (k12 + kres + knres)A_c \quad \textbf{Equation 13}$$

$$\frac{dA_P}{dt} = (k12 \cdot A_C) - (k21 \cdot A_P) \quad \textbf{Equation 14}$$

$$\frac{dA_b}{dt} = kres \cdot A_C \quad \textbf{Equation 15}$$

Overall, the simulation operated against the modified experimental modal, Ghoos method and Wagner-Nelson method.

Ogungbenro et al., 2014 further developed a semi-mechanistic model focusing on the pharmacokinetic application of double-peaked profile phenomenon due to GE based on the application of a gastric-modifying drug known as Levodopa. Ogungbenro et al., 2014, explore the concept of double peaks within gastric modelling due to interruptions and how to create models to analyse such phenomena. This paper focuses on the use of levodopa (GE modifying drug) and how its use affects the pharmacokinetic profiles based on plasma drug concentration. Whilst this paper doesn't use the ^{13}C-OBT, it does develop a semi-mechanistic model regarding GE using an indirect method of measuring it. It is also more recent than the previous paper mentioned in this literature review covering semi-mechanistic modelling which was completed by Ogungbenro & Aarons, 2011b. The paper successfully develops a semi-mechanistic model to interpret the double-peak phenomenon providing a good fit in contrast to OBT data. Review of the model and its features is conducted in the discussion in section 4.

3.2. Other Modelling Approaches

It is worth investigating modelling applications outside of the context of gastric emptying and OBT, especially as knowledge of semi-mechanistic models regarding gastric emptying is not extensive. Semi-mechanistic models have been developed using a variety of approaches for numerous applications. Some useful reports can be found within this section.

te Braake, et al., 1999 wished to show how neural networks and fuzzy models can be simply incorporated into a semi-mechanistic modelling environment. The incentive was to create a semi-mechanistic (i.e. alternative) model to model a biochemical process that is cheaper and has a shorter development time than a fully mechanistic model, whilst still providing satisfactory performance. The authors focused on a semi-mechanistic model based on the enzymatic conversion of Penicillin-G. They compared two different types of black-box models (models that can be viewed by their inputs and outputs, but not their processing method): a fuzzy model and a neural network model. The neural network is used to the prediction of kinetics of Penicillin-G within the semi-mechanistic model. Both models (fuzzy and neural network) provide good predictions of the numerical conversion kinetics with combination of a white-boxed model allowed overall process behaviour to be accurately determined using fundamental analysis based on first principles.

te Braake, et al., 1998 investigated an approach based on a combination of white-box (models that can be viewed by their inputs and outputs, with their processing method understood) and black-box (defined above) techniques based on neural networks. White-box models are usually governed by fundamental first principles mass and energy balances. In the context of GE, the five-compartment model of GE process by Ogungbenro & Aarons, 2011a and the ODEs representing the mass transfer around each compartment (using rate constants) is a suitable example of a white-box model in the application of semi-mechanistic modelling in GE systems (Ogungbenro & Aarons, 2011a). In the approach by te Braake et al., 1998, neural networks were developed using both white-box and black-box techniques where the known parts of the process were based on first principles and the unknown parts as black-box models which is then incorporated into the white-box model (te Braake, et al., 1998). The application involved developing semi-mechanistic modelling of chemical processes.

Simulation results from this model by te Braake et al., 1998, show that using neural networks in the application of semi-mechanistic modelling was significantly better and more effective than non-linear black-box models. However, the main restriction of neural network semi-mechanistic models is that they require enough process data to be available for the model development to be feasible. For example, if a particular process prevents the operator from being able to make changes to the process in order to monitor various operating variables and parameters, the neural network model is subsequently invalidated. This is important in the application of developing semi-mechanistic models for GE rate as a semi-mechanistic model must first be developed on the foundation of a white-box model and first principles e.g. mass and energy balances. However, many studies would require scaling up of the results from one individual study to a whole array allowing comparisons to be made between different individuals. Furthermore, neural networks in semi-mechanistic modelling allow the interpretation of areas of uncertainty around a particular system to be made which could be particularly useful in the context of relating the impact of different digestive processes to the GE rate in an OBT.

Ogungbenro, et al., 2011c developed a population pharmacokinetic semi-mechanistic model based on the GE function for acetaminophen plasma concentration in critically ill patients (Ogungbenro, et al., 2011c). The population modelling was carried out using a non-linear mixed effects analysis software (NONMEM) with the most effective model being four-compartment (stomach, intestine, central and peripheral) semi-mechanistic model; a three-

compartment model was also trialled with the compartments being: depot (absorption rate constant) and volumes of both central and peripheral compartments as the second and third elements of the model. Diagrams of both models are shown in Figure 11A and B. Diagram notation is defined in Table 5 which follows Figure 11A and B. Both models were formulated on the basis of assuming first order rate parameters for each compartment (Ogungbenro, et al., 2011c). Relevant first order ODEs have been utilised in other literature addressing semi-mechanistic modelling of GE systems (see section 3.1) (Ogungbenro & Aarons, 2011a).

Where:

Table 5 – Definition of notation used in semi-mechanistic GE compartment models

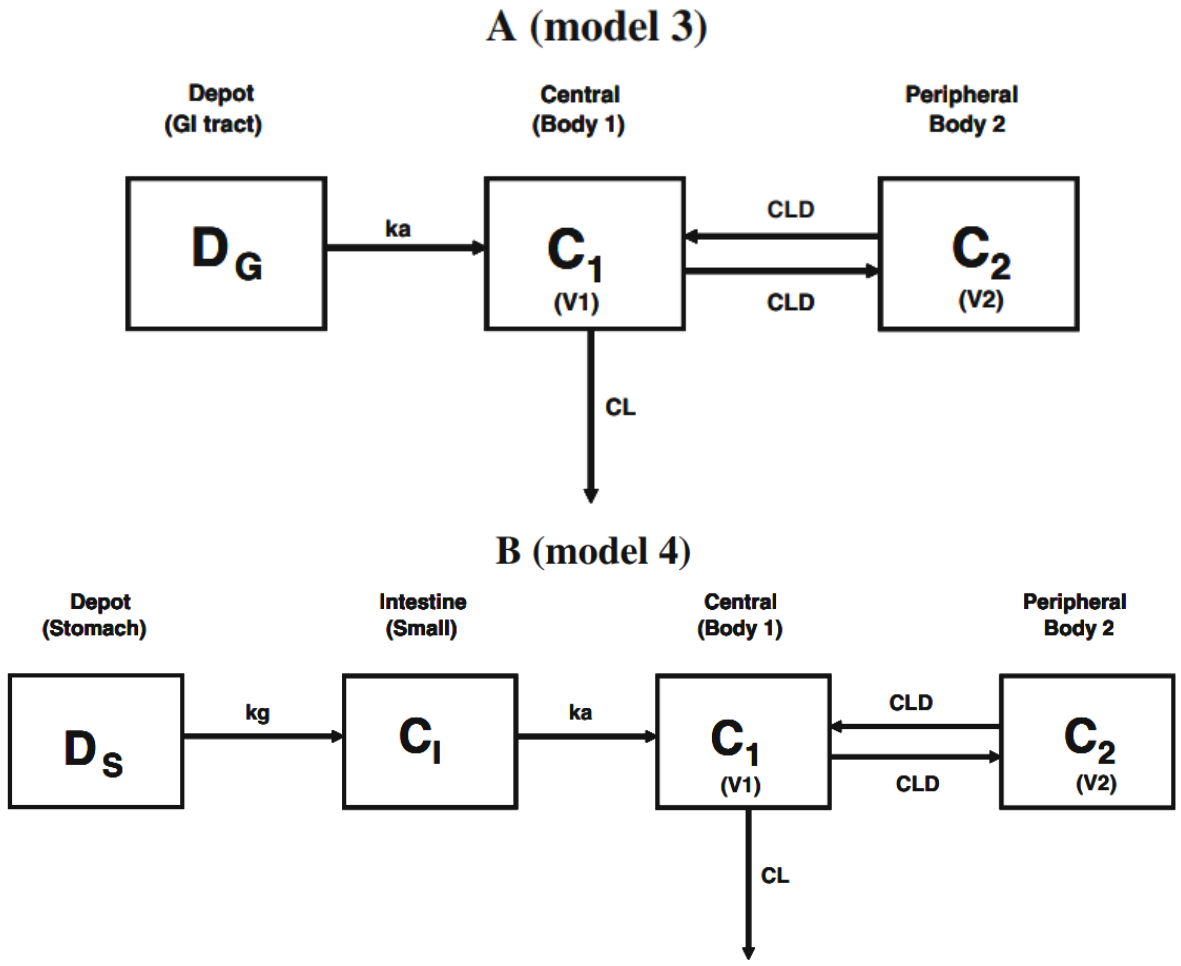


Figure 11 – Three-compartment (A) and four-compartment (B) first-order absorption models for GE analysis process (Ogungbenro, et al., 2011c)

(Ogungbenro, et al., 2011c)

Variable	Definition
D_G	Gastrointestinal tract depot compartment
D_S	Stomach depot compartment
C_1	Central body compartment
C_2	Peripheral body compartment
C_I	Small intestine compartment
V_1	Volume of central compartment
V_2	Volume of peripheral compartment
CL	Clearance

CLD	Intercompartmental clearance
k_a	Absorption rate constant
k_g	Gastric emptying rate constant

30 patients were sampled and they were further divided into two groups based on tolerance to acetaminophen and orally administered/ Enteral Nutrition (EN - patients fed internally as they are unable to orally take in food). Prokinetic agents (drugs) are used to enhance gastrointestinal motility by influencing the frequency/ intensity of contractions in the stomach wall thus, allowing for enhanced GE in patients.

Overall, the semi-mechanistic PK model was able to accurately interpret the GE rate in critically ill patients with varying tolerance to EN. Results show that the four-compartment model was superior to the three-compartment model, particularly due to the inclusion of the small intestine as a separate compartment to the stomach. This was able to provide a better fit for data overall as compared to conventional two-compartment simpler models. Analysis carried out in this paper explores the capability of application of semi-mechanistic PK modelling in prescribing drug dosages for gastric conditions to control the GE in affected individuals. This is validated by exploring both drug absorption and impaired GE (due to underlying gastric conditions) as individual factors affecting GE where Ogungbenro et al., 2011c were able to improve rate of GE in patients who are intolerant to EN after treating them with prokinetic agents to enhance gastric motility and GE (Ogungbenro, et al., 2011c).

Alskär, et al., 2015 wished to use knowledge of physiology to improve a previously published semi-mechanistic model (Integrated Glucose-Insulin model or IGI) that described plasma insulin and glucose concentrations after some induced changes to glucose concentrations. This model's performance was compared with previously published empirical models in order to further develop the IGI model with regards to explaining glucose absorption and impact of different glucose dosages on GE during testing (Alskär, et al., 2015).

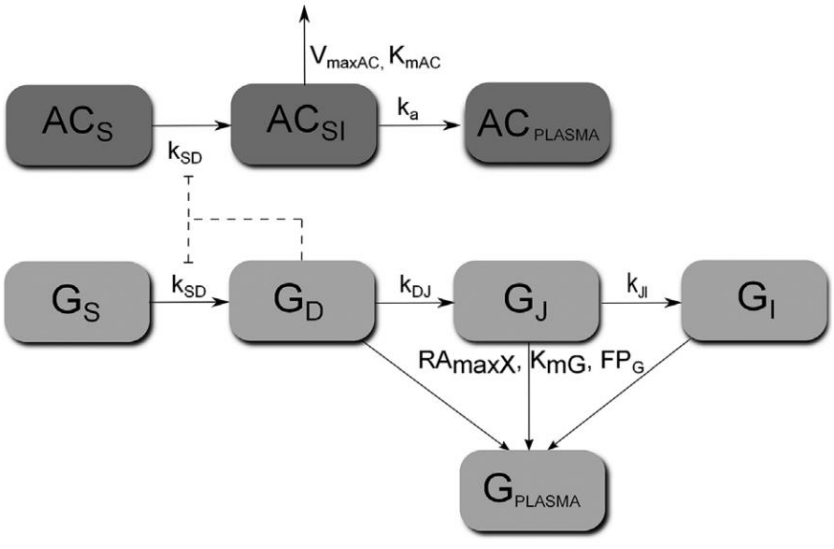


Figure 12 – Two-compartment semi-mechanistic model for glucose absorption and GE in patients (Alskär, et al., 2015)

Modelled data was obtained from patient samples consisting of healthy individuals and patients with type 2 diabetes and the model was developed using this data as well as understanding of GE process and glucose inhibitory effect on GE. The model was based on a simple two-compartment model assuming first-order kinetics; a diagram of the compartment model is shown in Figure 12. Diagram notation is defined in Table 6 which follows Figure 12.

Although three- and four-compartment models have shown better success in semi-mechanistic modelling GE applications, the complexity of glucose absorption kinetics combined with GE

makes it difficult to develop and model a three- or four-compartment model for this application. However, developing such a model would be beneficiary for the progression and validation of the model by Alskär et al. 2015.

Where:

Table 6 – Definition of notation used in semi-mechanistic GE and glucose absorption compartment models. Full lines indicate material flow and dashed lines indicate control mechanisms (Alskär, et al., 2015)

Variable	Definition
G_S	Glucose in stomach
G_D	Glucose in duodenum (first section of small intestine)
G_J	Glucose in jejunum (middle section of small intestine)
G_I	Glucose in ileum (final section of small intestine and connection to large intestine)
G_{Plasma}	Glucose in plasma
AC_S	Acetaminophen in stomach
AC_{SI}	Acetaminophen in small intestine
AC_{Plasma}	Acetaminophen in plasma
V_{MaxAC}	Maximum rate of first-pass metabolism
K_{mAC}	Acetaminophen amount in small intestine giving 50% of maximum first-pass effect
k_a	Absorption rate constant
k_{SD}	GE rate constant (same as k_g used in other literature)
k_{DJ}	Transfer rate constant from duodenum to jejunum
k_{JI}	Transfer rate constant from jejunum to ileum
RA_{maxX}	Maximum rate of absorption from each segment of small intestine
K_{mG}	Glucose amount which results in 50% of maximum absorption rate
FP_G	Proportionality factor representing first-pass effect

Although the complexity of this GE and glucose absorption two-compartment model and detailed kinetics involved in glucose absorption is beyond the scope of this research topic, it is important to still consider this in the following context:

- Establishing test meal composition for GE tests and glucose content
- Impact of patient diet and blood glucose concentration on GE rate
- Addressing patients with glucose-related conditions e.g diabetes

Alskär et a., 2015 were able to discover that duodenal glucose had an inhibtory effect on GE process which is important to consider when analysing GE in individuals. The semi-physiological semi-mechanistic model was superior to published empirical models which it was compared to and it was able to provide a better physiological understanding of the mechanisms regarding glucose absorption and the impact on GE. Although the model studied glucose absorption and GE only in patients affected by type 2 diabetes, model methodology and results can be applied to other semi-mechanistic PK models in the field GE, particularly focusing on patients affected by other diabetic/ glucose-related conditions e.g. type 1 diabetes, diabetes mellitus, diabetic gastroparesis etc. Understanding the fundamentals physiology in glucose absorption is vital before administering gastric-modifying drugs which affect GE in the body with focus on glucose absorption.

3.3. Pharmacokinetic Analysis

Pharmacokinetic (PK) Analysis involves the application of semi-mechanistic modelling in prescribing specific dosages of drugs for patients with related conditions. PK models are a fairly novel advancement in the field of medical/ biological modelling and enable unique modelling of individual patient cases rather than modelling an entire patient sample in one group. This is particularly relevant to GE as each individual will have a unique GE rate based on their lifestyle/ diet, underlying gastric conditions etc. Furthermore, PK models are able to analyse drug performance in the patient's body by monitoring plasma concentration after administration of the drug.

3.3.1. Optimal Design of Pharmacokinetic Studies

Ogungbenro & Aarons, 2009 investigated the mechanism for optimal design of PK studies and key features of such studies. They defined population PK studies as being governed by 'careful selection of the best combination of particular design factors' (Ogungbenro & Aarons, 2009):

1. Number of measurements to be taken
2. Location of measurements
3. Number of subjects to include in study

Efficient incorporation of these techniques in population PK studies will improve the efficiency and also result in reduced cost and time during drug development after the studies (Ogungbenro & Aarons, 2009). The paper covers different PK experiments which were carried out and provides a guide on what to do and what not to do based on the reviewed studies.

They summarise this based on 3 key features of population PK studies (Ogungbenro & Aarons, 2009):

1. Multiresponse experiments – this is where more than one response variable is measured. Examples of this include:
    - Monitoring concentration of an administered drug in more than one tissue or organ
    - Multi-drug studies where a combination of drugs is used in a study e.g. cancer and HIV
    - Balance of studies and whether responses are measured at the same time or there are missing measurements in specific times
2. Bayesian design – measures of uncertainty incorporated into model parameter estimates. Involves specification of parameter distribution to:
    - Give a measure of location and dispersion of parameters across data set range
    - Allows incorporation of uncertainty around parameters into design of model
3. Discrete data – used to characterise drug response and plasma concentration for pharmacodynamic variables. Examples of discrete data include:
    - Non-negative integers
    - Binary measurements e.g. yes/ no, success/ failure etc.
    - Ordinal measurements categorised based on categorical ordering e.g pain intensity: none/ mild/ moderate/ severe and pain relief: none/ a little/ medium/ a lot/ complete (Ogungbenro & Aarons, 2009)

3.3.2. Example Pharmacokinetic study

Ogungbenro & Aarons, 2014 went beyond their optimal design for PK studies paper and actually developed a PK model for valproic acid in adults and children. Valporic acid is an anti-convulscant drug used to treat different types of epilepsy in individuals by oral administration (Ogungbenro & Aarons, 2014). Ogungbenro & Aarons, 2014 developed a physiologically-based PK model to predict plasma and tissue/ organ behaviour in adults and children after oral and intravenous administration of valproic acid. They developed a ten-compartment model where the compartments were: the gut lumen, enterocyte, gut tissue, systemic blood, kidney, liver, brain, spleen, muscle and rest of body (Ogungbenro & Aarons, 2014). Dravet syndrome (type of epilepsy in children) was studied and model parameters were adjusted using age-dependant changes to change the initial model (which was designed for adults) to be applicable to children. In vitro and in vivo experiments were carried out previously in order to obtain specific system and drug performance parameters which were necessary for developing the PK model (Ogungbenro & Aarons, 2014).

Results show that the model was very successful in predicting plasma concentrations of valproic acid in both children and adults after drug administration. The model was also able to predict clearance in children which is important to ensure the drug is safely eliminated from the body in sufficient time and does not harm the body. It was concluded that the model could improve clinical applications in treatment of Dravet syndrome by optimisation of dosages for epilepsy patients and monitoring resultant response and plasma concentration (Ogungbenro & Aarons, 2014).

4. Discussion

The following will cover a discussion and a review of the various literature based around the research topic. Discussion will focus on variation in OBT techniques, methodology and modelling techniques relevant to both GE and modelling applications for other fields which could be implemented into the semi-mechanistic GE model.

As discussed in previous sections covering OBT, there have been discrepancies in the methodology for conducting OBT across different literature. Although the work done by Ghoos et al., 1993 was regarded as a breakthrough in OBT development and various authors referred to Ghoos et al., 1993 in their work, there were distinct issues with GE model developed by Ghoos et al., 1993. The main constraint with the model was that it relied on curve fitting/ estimation of GE parameters developed from OBT so that they are in line with scintigraphy results. Ghoos et al., 1993 proposed the use of a correction factor of '-66 minutes' to correct the OBT gastric emptying time ($t_{1/2,\,b}$) so that it is in line with half-emptying time obtained by scintigraphy method ($t_{1/2,\,s}$) (Ghoos, et al., 1993). Although this yielded good correlation with scintigraphy results, this limits the validity of the model as it must always be used in conjunction and rely on scintigraphy where the principle for developing the model was to move away from using scintigraphy. Furthermore, it is important to note the key limitation of the OBT method by Ghoos et al., 1993 where the rate of emptying of material in the stomach is equated to the rate of CO_2 exhaled in the breath from oxidised ^{13}C-octanoic acid. Sanaka et al., 2005 and Sanaka et al., 2010 both published papers invalidating this which covered the detailed kinetics of processes involved in gastric emptying i.e. absorption, metabolism, distribution and elimination via means other than breath. In the 'Letter to the Editor' by Sanaka et al., 2005, the main limitations with the method by Ghoos et al., 2005 were addressed based on the following questions (Sanaka, et al., 2005):

1. "Is GE really the rate-limiting step for $^{13}CO_2$ exhalation?
2. Why is the $^{13}CO_2$ recovery curve obviously remote from the scintigraphic GE flow curve?"

These questions and rest of the letter explained the reason for the discrepancies between OBT and scintigraphy where Ghoos et al., 1993 did not consider the impact of other processes on the GE rate. Based on this, it is likely that the correction factor of '-66 minutes' proposed by Ghoos et al., 1993 to correct $t_{1/2,\,b}$ to $t_{1/2\,s}$ was to take into account other processes in GE. The same issue is not encountered with scintigraphy as it involves direct measurement of GE rate whereas OBT involves indirect measurement of GE rate based on $^{13}CO_2$ exhalation rate. Not only do Sanaka et al., 2005 explain the reason for incorrect estimation of GE rate using ^{13}C-OBT, but they were also able to identify that the correction factor is individual based and would vary between each test. This limits the applicability of the method by Ghoos et al., 1993 where a new correction factor would be developed for each test to ensure parameters are coherent with scintigraphy.

Another limitation of the model developed by Ghoos et al., 1993 was the elimination of outlying data from individual patients. This is shown in Figure 13A and Figure 13B below which compares $t_{1/2,\,b}$ and $t_{1/2\,s}$ for all individuals after correction with outliers being omitted for Figure 13B. It is clear that the presence of outliers is significant to results where the correlation coefficient (r) increases by 0.1 when outliers are omitted which is very significant. Although the outliers are very distinct and not in line with curve trend, this still highlights the limitation of the method by Ghoos et al., 1993 where the method focuses on curve fitting of half-emptying time by OBT to scintigraphy and does not explain the reasons for these outlying data.

Bruno et al., 2013 evaluate the clinical applications of OBT and explain how different gastric conditions can severely impact the GE time and rate; it is likely in this case that these outliers were due to this.

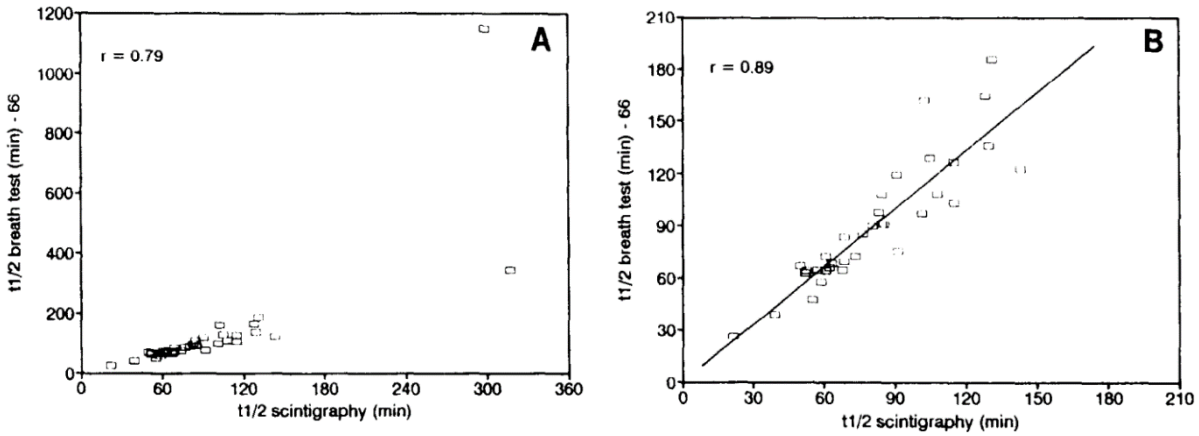


Figure 13 – Comparison of half-emptying times for OBT ($t_{1/2,\,b}$ which is corrected by -66 minutes) to scintigraphy ($t_{1/2,\,s}$). (A) includes all patient data; (B) omits 2 outliers

On the other hand, outliers may be due to lack of standardisation in conducting the OBT. As the method developed by Ghoos et al., 1993 was the first and background for many future OBT-based models, it is unfair to criticise Ghoos et al., 1993 for the lack of standardisation in their method. As mentioned in section 2.2.2, Seok et al., 2011 stated that one of the key limitations with GES is that lack of standardisation in the test meaning that there were too many variations for doing it and that it was important to develop a standardised normal range for carrying out GES. As a result, the American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine published a protocol for a standardised 'Egg Beaters' meal

which is a manual for GES tests that all researchers should follow when preparing tests meals for patients (Tougas, et al., 2000). It should be noted that this was not particularly revolutionary in the field of GES as there was incentive to steer away from using GES at this point due to its limitations (Amarri & Weaver, 1995):

1. The invasive nature of the test
2. Radiation burden of GES which restricts repeated testing on the same patient and prevents it from being used on pregnant women and children
3. Use of expensive equipment which requires competent operation and trained personnel

There has been some standardisation to OBT based on published protocols in 2002 by the Japan Society of Smooth Muscle Research (JSSMR) and Italian Group for Gastric Emptying Test (IGGET) (Sanaka & Nakada, 2010). This protocol is summarised in Table 7.

Table 7 – Standardised protocol for ^{13}C-octanoate breath test developed by the Italian Group for Gastric Emptying Test (IGGET) (Sanaka & Nakada, 2010)

Test meal: a manufactured muffin containing 378 kcal (EXPIROGer®, Sofar, SpA Milan)
[^{13}C]-conpound: 100 mg [^{13}C]-ctanoic acid
Sampling frequency and duration: at time 0 (preprandial baseline) and at 15-min intervals for 240 min
Parameter: $T_{1/2b}$
Reference range:$T_{1/2b}$ (upper limit; 146 min)
Note 1) The reference ranges are derived from 131 healthy volunteers (47 ± 18 years, 58 males and 73 females). The mean (SD) value is 89 (29) min. 2) $T_{1/2b}$ is calculated by the Ghoos's curve-fitting technique. 3) Age and gender do not influence the reference value.

Even with a released standardised protocol, published literature has still commented on the variation in OBT methodology and whether this affects test results. Standardised patient positioning is crucial to GE testing where a patient changing from lying down to sitting/ standing up could slow down GE by more than 50% in 1 hour (Szarka & Camilleri, 2009). Although Szarka & Cameilleri, 2009 were carrying out GES testing, the same standardisation for patients is required in OBT. None of the papers reviewed in this report cover patient posture as an important factor in GE testing. Bruno et al., 2013 state that patients must not sleep or conduct physical exercise after consuming the test meal; however, they do not explain why or state what the patients would be doing during the study which could impact the GE results. Perri et al., 2005 published a paper covering OBT methodology based on Ghoos et al., 1993 which did not comment on patient positioning during the test. Although Perri et al., 2005 were able to validate the same results developed by Ghoos et al., 1993, they were not able to explain physiological significance of GE parameters or go beyond the conventional curve-fitting/ correction approach developed by Ghoos et al., 1993. Furthermore, some of the correlations between OBT and scintigraphy results stated by Perri et al., 2005 were invalidated and proven to be wrong by the Mayo Clinic in the US who carried out an experiment to prove this (Perri, et al., 2005).

Another aspect of the OBT method that requires standardisation is the test meal. The most common test meal in OBT is an egg meal where the tracer compound (^{13}C-octanoic acid) is incorporated into the egg white which is separated from the yolk. However, variations in the

test meal have been shown across different literature. Tests investigating GE of liquid-based meals would vary significantly compared to solid-based meals as GE of liquids is significantly faster than solids (Szarka & Camilleri, 2009); this is because liquids require no trituration so can bypass digestion in the stomach directly and empty into the small intestine (Goyal, et al., 2019). Solid-based meals will have varying GE rates based on the meal content. Table 8 summarises meal choice and calorific content for different OBT experiments published by different sources:

Table 8 – Meal choice and calorific content for OBT experiments carried out by different papers

Paper authors	Meal choice	Calorific content
Standardised protocol developed by IGGET (2002), Sanaka et al., 2010	Manufactured muffin	378 kcal
Sanaka et al., 2008	Muffin meal spread with butter. Served with 250 ml water	320 kcal
Schommartz et al., 2006	Scrambled egg, margarine and toast. Served with 100 ml water	250 kcal
Ghoos et al., 1993	Scrambled egg with 5 g of margarine and 2 slices of white toast. Served with 150 ml water	250 kcal
Bruno et al., 2013	Scrambled egg with 5 g of margarine and 2 slices of white toast. Served with 100 ml water	250 kcal
Punkkinen et al., 2006	Test 1. Lactose and gluten-free cabbage casserole. Served with 100 ml water	220 kcal

Although a standardised protocol was developed in 2002, not all published literature adhered to this. It is noticeable that more literature followed the test meal methodology developed by Ghoos et al., 1993 over the standardised protocol developed by the IGGET. Although the IGGET protocol has less adherence in context of OBT experiments, it is the only method which has reference and parameter range for breath test half-emptying time ($t_{1/2,\,b}$; see Table 7). The generally accepted test meal is a scrambled egg meal served with 5 g of margarine, 2 slices of white toast and 100-150 ml of water (250 kcal). There is significant variation in intervals at which breath test samples are taken for different literature and this must be standardised across OBT experiments. It is obvious that using a different test meal with varying calorific content would impact GE results but limited analysis has been carried out for this. GE rate would vary with different meal components e.g. vegetables would digest faster and have an accelerated GE rate compared to carbohydrates and proteins. There is some variation in the volume of water provided with test meals in different studies. Although the variation is not significant, it is known that water eases digestion by softening food in the stomach and assists in the formation of chyme which would affect GE rate. Water volume in the test meal may not be a significant factor but there are no studies on the effect of water volume in test meals for OBT studies to go against this idea. The quantity of tracer compound (Carbon-label) in the test meal was different across many studies which would affect GE rate and breath test results.

Punkkinen et al., 2006 trialled different meal choices where they carried out an OBT/ scintigraphy study in an attempt to invalidate the correlations and methodology developed by Ghoos et al., 1993. Although Punkkinen et al., 2006 showed very weak correlation in their results, there were some distinct issues with their OBT study which limited the viability of any points they made:

1. OBT and scintigraphy experiments were conducted within 1 week of each other and not at the same time which would result in interindividual variability (IIV)
2. OBT test meal was cabbage casserole whereas scintigraphy test meal was a fried egg meal

As the OBT and scintigraphy experiments were not conducted at the same time and with the same test meal, any correlations and conclusions are invalidated as variables are not being controlled across the 2 experiments. Furthermore, use of different composition tests meals would result in very different GE rates.

There were more aspects of the OBT method which were not standardised/ repeated in all OBT studies carried out in reviewed literature. These were stated by Bruno et al., 2013 who developed a more up-to-date and concise version of the initial model by Ghoos et al., 1993 (Bruno, et al., 2013):

1. Fasting of patients at least 8 hours before the breath test is commenced to minimise the risk of false positive/ negative data (Bruno, et al., 2013)
2. Patients only allowed to drink non-carbonated water during this fasting period
3. Infrared mass spectrometry/ mass spectrometry used to analyse breath test samples to differentiate between $^{13}CO_2$ and $^{12}CO_2$ exhaled in the breath
4. Patients instructed to not do the following after consuming the test meal during the OBT study:
    - Drink
    - Eat
    - Smoke
    - Sleep
    - Carry out any physical exercise
5. Gastric conditions of individuals investigated in OBT study

OBT studies were done on both healthy individuals and individualised affected by specific gastric conditions chosen by the authors. However, most authors investigated different conditions to each other and very few chose a patient sample consisting of more than 2 different gastric conditions which limits the variation in their data.

Although there was minimal deviation from the model developed by Ghoos et al., 1993, some authors attempted to further expand the model to develop understanding of GE process and introduce new parameters and methodology. Perri et al., 2005 considered the clinic-pharmacological applications of the OBT model developed by Ghoos et al., 1993 and how the model could be developed into a pharmacokinetic (PK). By implementing PK features, the model could be used to diagnose precise dosages of gastric-modifying drugs to combat the inhibition/ acceleration by gastric conditions. Perri et al., 2005 only briefly discussed this mechanism but it was important to the overall model development as PK modelling was utilised by semi-mechanistic models developed in future literature (Ogungbenro & Aarons, 2011b).

Schommartz et al., 2006 investigated the application of new diagnostic parameters for OBT models: latency time (t_{lat}) and ascension time (t_{asc}). Definitions of these new diagnostic parameters are shown in Figure 14 where t_{lat} characterises initial delay in cumulative $^{13}CO_2$ curve and t_{asc} is the interval of time between t_{lat} and $t_{1/2}$ (denoted as $t_{0.5}$ here) (Schommartz, et al., 2006). Schommartz et al., also introduced mathematical equations for calculation of t_{lat} and t_{asc} and carried out statistical correlation analysis between the 2 parameters. This is shown in Figure 15. Although there is some signs of correlation in Figure 15. between t_{lat} and t_{asc}, the correlation is fairly weak due to variation in the results. Schommartz et al., 2006 propose that t_{lat} and t_{asc} are associated with disturbances in the GE process whether this is an increased lag phase, a delay in GE or both (Schommartz, et al., 2006). Further validation of the correlations between t_{lat} and t_{asc} is necessary before the parameters can be validated and implemented into OBT models. Schommartz et al., 2006 proposed the testing of these parameters using radioscintigraphy and to compare the results to OBT. However, this somewhat invalidates the principle for development of OBT in the first place which was to steer away from scintigraphy due to the radiation burden and invasive nature of the test. t_{lat} and t_{asc} have been implemented into other OBT and semi-mechanistic models in published literature.

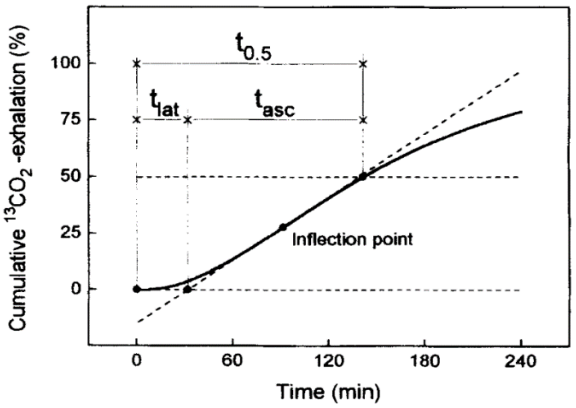


Figure 14 – Graph for evaluating ascension (t_{asc}) and latency time (t_{lat}) parameters in 13C-OBT (Schommartz, et al., 2006)

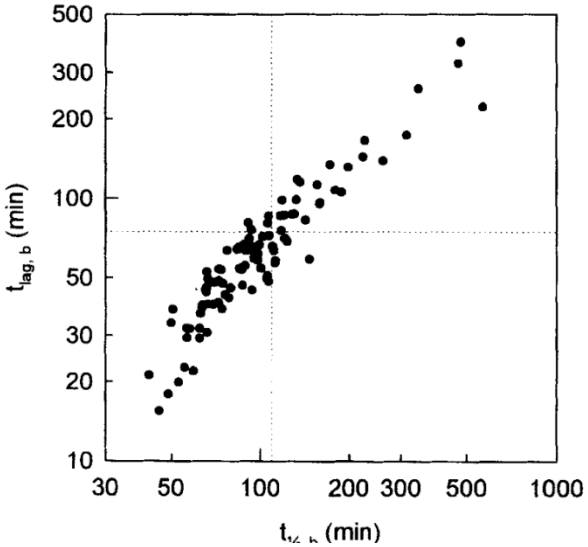


Figure 15 - Distribution of t_{lat} vs. t_{asc} based on statistical correlation analysis in OBT study (Schommartz, et al., 2006)

As mentioned previously in section 2.2.4.3, Sanaka et al., 2010 studied the detailed kinetics involved in GE of ^{13}C-octanoic acid once it is ingested in a test meal. This was important as it validated the discrepancy in the results of the model by Ghoos et al., 1993 between OBT and scinitgraphy where Ghoos et al., 1993 did not consider processes contributing to GE other than exhalation. Sanaka et al., 2010 cover a detailed explanation of the kinetics around absorption, metabolism, distribution and elimination via means other than breath (see section 2.2.4.3). This helped in the development of important rate constants and equations which were utilised in the semi-mechanistic models developed by Ogungbenro & Aarons, 2011b which the principle of this research topic is based on. Furthermore, the 5 compartment model developed by Ogungbenro & Aarons, 2011b was built on the foundation by Sanaka et al., 2010 which took into consideration the kinetics of ^{13}C-OBT in each compartment/ area of the body.

Markey & Shafat, 2013 investigated different mechanisms to determine the CO_2 production rate ($\dot{V}CO_2$) from the breath and the subsequent impact on GE results. They developed new parameters for the study:

1. $\dot{V}CO_{2DM}$ –direct measurement of rate
2. $\dot{V}CO_{2PR}$ –predicted rate from resting

3. $\dot{V}CO_{2BSA}$ – predicted rate from body surface area

Although this study could have revolutionised OBT mechanisms and saved time by , the results prevented it from doing so. There was limited correlations in the results by Markey & Shafat, 2013, particularly for $\dot{V}CO_{2PR}$ and $\dot{V}CO_{2BSA}$. The assumptions developed in order to calculate $\dot{V}CO_{2PR}$ and $\dot{V}CO_{2BSA}$ were inaccurate and assumed GE was primarily based on exhalation of breath which has been disproved by Sanaka et al., 2010. Markey & Shafat, 2013 even recommend caution against using a normalised body surface area predicted rate ($\dot{V}CO_{2BSA}$) in normal weight healthy adults as their study was on obese adults (Markey & Shafat, 2013). This further suggests that the research is not reproducible and the recommendation is by Markey & Shafat, 2013 is to use $\dot{V}CO_{2DM}$ which all OBT models do regardless.

Parameter estimation/ curve fitting is an important part of the model developed by Ghoos et al., 1993 where breath test half-emptying time is corrected to scintigraphy half-emptying time. Another limitation of the model developed by Ghoos et al., 1993 was the elimination of outlying data from individual patients. This is shown in Figure 13A and Figure 13B below which

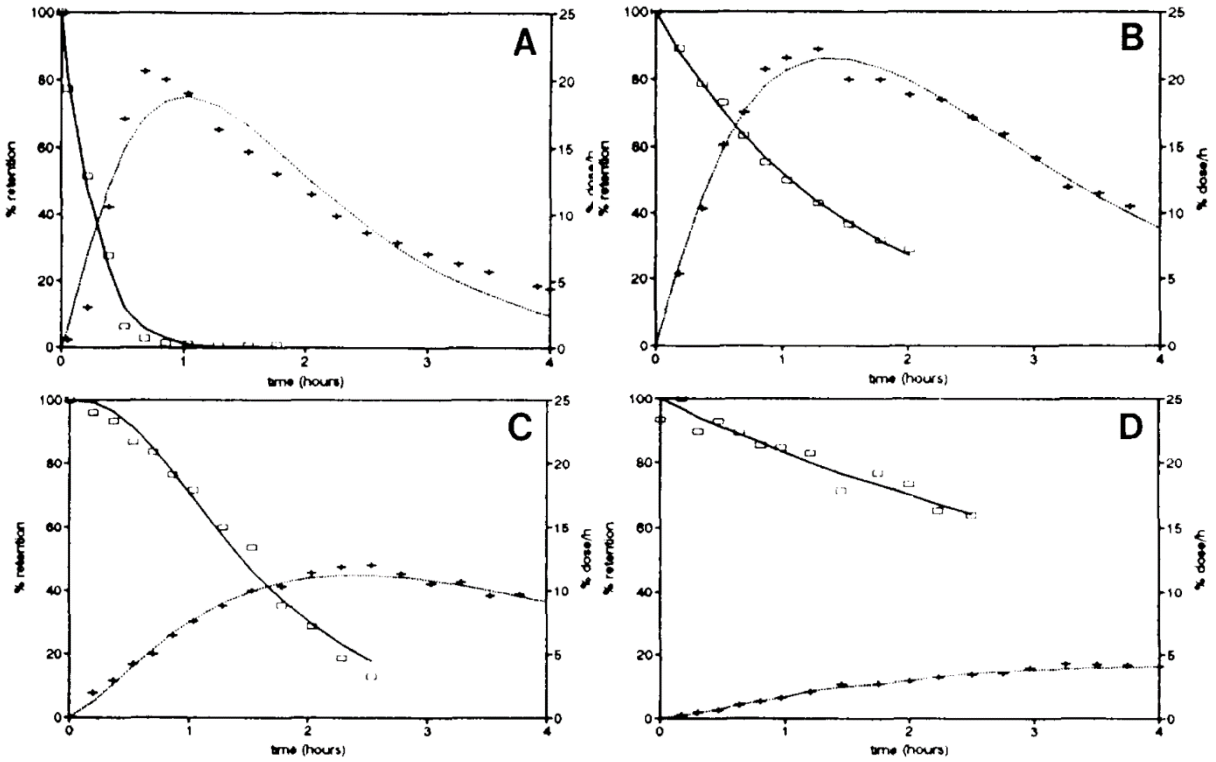


Figure 16 – Results curves of ^{13}C-OBT and scintigraphy for four patient cases: (A) Rapid GE ($t_{1/2,\,s}$ = 21 min.), (B) Normal GE curve ($t_{1/2,\,s}$ = 61 min.), (C) Delayed GE ($t_{1/2,\,s}$ = 81 min.) and (D) Extremely delayed ($t_{1/2,\,s}$ = 300 min.) (Ghoos, et al., 1993)

compares $t1_{/2,\,b}$ and $t1_{/2\,s}$ for all individuals after correction with outliers being omitted for Figure 13B. It is clear that the presence of outliers is significant to results where the correlation coefficient (r) increases by 0.1 when outliers are omitted which is very significant. Although the outliers are very distinct and not in line with curve trend, this still highlights the limitation of the method by Ghoos et al., 1993 where the method focuses on curve fitting of half-emptying time by OBT to scintigraphy and does not explain the reasons for these outlying data. Figure 16A-D show the GE pattern for OBT and scintigraphy in the same patients before correction is applied.

It is clear that the results between OBT and scintigraphy without correction are distinctly different. This is shown, not only in the comparison of $t_{1/2}$ between both methods but also for,

the Gastric Emptying Coefficient (GEC) which was also corrected by a similar correction factor to achieved desired correlation of OBT with scintigraphy results. Although parameter estimation/ curve fitting methodology yields accurate results in line with directly determined GE by scintigraphy, the model by Ghoos et al., 1993 fails to explain the physiological meaning of GE parameters and the significance of these variables to GE rate. This led to research into application of semi-mechanistic modelling to analyse the identifiability of different parameters in the GE model in order to identify outliers in patient data and develop significant curves which can explain the GE process and are not dependant on curve fitting alone.

Ogungbenro & Aarons, 2011a developed a semi-mechanistic PK model which produced favourable results with the best correlation relative to scintigraphic data compared to other OBT methods. Other methods used as basis for comparison were Modified exponential method, Ghoos and Wagner-Nelson; results are shown in Figure 17.

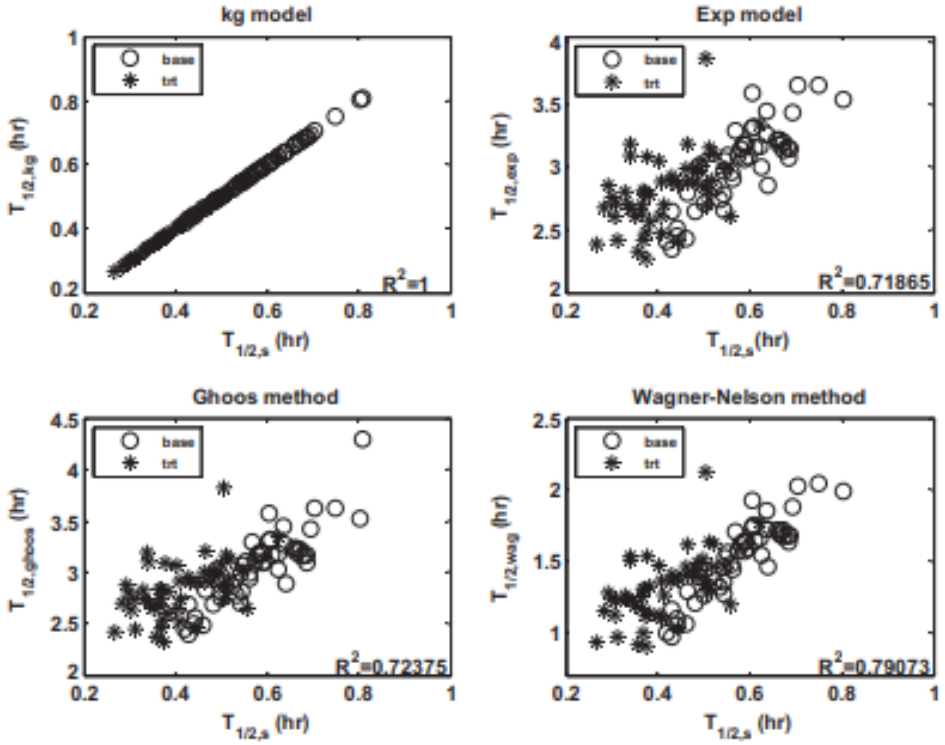


Figure 17 – Half-emptying times for the model developed by Ogungbenro & Aarons, 2011a, Modified exponential method, Ghoos method and Wagner–Nelson method against a scintigraphic equivalent. There is no variability on all parameters except kg and ka (Ogungbenro & Aarons, 2011a)

Absorption, distribution, metabolism and elimination by means other than breath exhalation contribute to the discrepancies between OBT and scintigraphy data for other models. This is shown in Figure 17 where the model by Ogungbenro & Aarons, 2011a had a correlation coefficient (r^2) of 1 while the other models ranged between 0.72 and 0.79. Discrepancies in the other models are due to invalidation of the OBT fundamental assumption where half of ^{13}C recovered in breath is not equivalent to half of ^{13}C emptied from the stomach due other processes occurring.

However, there were some key limitations with the model. The model involves a lot of variable adjustment/ correction for curve-fitting within simulating and does not fully explain the impact of other processes and rate constants on GE rate. This Based on this, it could be argued that the r^2 correlation value of 1 for the model by Ogungbenro & Aarons, 2011a is not very accurate as the model contains self-correction within itself so is designed to fit to scintigraphy curves.

There may be good positive correlation similar to the other models but perfect correlation is unlikely to be true. This curve fitting approach also results in parameters that have no physical/ biological significance so cannot explain the GE process.

It was found in Ogungbenro & Aarons, 2011c that when comparing a 4-compartmental model (stomach, intestine, central body and peripheral body) produced a more effective mode than a 3-compartmental model yield better results. In (Ogungbenro & Aarons, 2011b), it was then further found that the 5-compartmental model (stomach, intestine, central body, peripheral body and breath) proved to generate a superior model.

Furthermore, the model assumed a first order rate constant for GE and all other relevant processes. This is generally only applicable to estimating GE rate for liquids and must be further modified for solids by implemented a second order or higher model. Finally, the model is still not quite robust where some of the assumptions used for analysing GE in the first paper were not valid and must provide clearer and better understanding of the processes affecting GE rate.

An improved model was developed by Ogungbenro et al., 2014 on a specific PK application of semi-mechanistic modelling in GE based on profiles of Levodopa (gastric modifying drug). This model also suffers a similar issue to the GE OBT semi-mechanistic models where mechanisms for the GE, absorption, distribution and elimination mechanisms were assumed to be first-order rate constants without a justification for this approach. The paper also makes a questionable assumption about “predicting the stomach profile” when comparing OBT which is a poor justification for the simulation and data. Furthermore, the model is again based on curve-fitting and does not explain physiological significance of GE parameters. Finally, the data produced by Ogungbenro et al., 2014 was not compared to scintigraphic data where the authors assumed that their OBT results were reliable. Historical literature has shown that OBT is not able to accurately determine reliable GE results compared to direct estimation by scintigraphy and this raises a question with the validity of the results by Ogungbenro et al., 2014.

The future use of neural networks in the application of semi-mechanistic modelling produces excellent models than perform better and more effectively than non-linear black-box models. This proves that a future approach into the field, using neural networks may yield productive results, especially as they can interpret uncertainties around a system which is particularly relevant to understanding physiological significance of GE parameters. The main problem with neural networks is the requirement for sufficient process data to allow the model to be feasible, and ensuring that the data doesn’t generate a biased result.

5. Gap Identification & Areas to Investigate

On account of the research carried out and the literature on the semi-mechanistic modelling of gastric emptying based on OBT, it is clear that significant improvements are necessary in order to develop a fully functional and independent model. Based on the work done by Ogungbenro & Aarons in the first two papers trialling the semi-mechanistic model (2011) and the later paper investigating semi-mechanistic modelling in the application of levodopa (2014), it is apparent that the model is still not able to fully explain the gastric emptying process on a physiological

level. Although the model is able to accurately predict the gastric emptying rate in an OBT with results that as accurate as those obtained using radioscintigraphy, this is done using a curve fitting method where mathematical variables in the model are continuously corrected until a solution is obtained that is in line with radioscintigraphy results. On the other hand, the model is able to consider other processes taking place in the human body that contribute to the removal of ^{13}C-Octanoic acid in the body but do not actually represent the gastric emptying rate e.g. metabolism, elimination and distribution. Thus, the main issue is that the model works as it is designed to in determining the gastric emptying rate and can be used to diagnose gastric conditions; however, the model cannot be fully relied on in explaining the physiological parameters and processes that contribute to this modified gastric emptying rate due presence of a gastric condition affecting the GE process and rate.

As explained, further development of the model is required before it can be validated as an accurate and recognised mathematical modelling tool for monitoring the GE process on a physiological level. This would involve developing a model beyond the curve fitting approach with variables that are globally or even locally identifiable. This would enable the model to be applicable to many data sets for different patients and specific to how each individual's gastric system operates. Furthermore, this enables the identification of the non-specific variables which can be kept constant with each test to determine the main physical variables contributing to the GE rate objective function. In doing so, the simpler non-mechanistic curve fitting approaches can be disregarded, and a semi-mechanistic model can be developed which accurately describes the GE process based on physiologically significant variables. This also touches upon of Mixed Effects Parameter Estimation Modelling which is a new type of parameter estimation technique that can use semi-mechanistic modelling to enable the user to obtain better fits for data that is quite individualised, allowing for predicting GE between different individuals with varied data between them *e.g.* different gastric conditions. This enables all data to be analysed at once and any parameter estimates made to be globally/ uniquely identifiable.

In summary, the research gaps relevant to the semi-mechanistic modelling relate to the following areas:

1. Developing a model with globally/ unique identifiable parameters that does not rely on simple non-mechanistic curve-fitting
2. Utilising Mixed Effects modelling to separate individualised GE data
3. Identify parameters relating to other processes not specific to GE and using these to model physiological aspects of the rate of GE
4. Using semi-mechanistic modelling of GE rate to diagnose an unknown gastric condition in an individual

6. Conclusion

On account of the literature review and theoretical background covered for GE testing and semi-mechanistic modelling of OBT results to determine GE, there is a need to develop a semi-mechanistic PK model for determining GE. The main aim of the literature review was to carry out critique analysis of literature covering the use of semi-mechanistic PK modelling, non-linear mixed effects models and neural networks in the context of GE with some efforts being made to cover other applications of the models. This has been successfully done in the GE modelling and discussion sections which highlight the significance of developing a fully functioning semi-mechanistic model on GE testing.

More importantly, review of literature on modelling of GE systems and test results has shown that there is a distinct issue currently within the field of GE testing and this is the reliance on

parameter estimation/ correction factor techniques to solve the GE models. This has been shown by the fundamental papers in both OBT mathematical models and semi-mechanistic modelling by Ghoos et al., 1993 and Ogungbenro & Aarons, 2011a respectively. The principle of these models is to match the GE parameters by correcting OBT data to scintigraphy so valid GE results are obtained. However, such models fail to explain any physiological meaning to these parameters and their biological significance to the GE process. Furthermore, the models fail to identify parameters and their significance i.e. whether they are globally identifiable, locally identifiable or non-uniquely identifiable. This is important as developing a model with only globally and locally identifiable parameters where the model developer would know which parameters can be kept constant without affecting the GE function and which are significant to the model solution. This would allow differentiation between patient data in a large sample size and explain the behaviour of outliers which are usually omitted in published GE models. Outlying data could be a cause of testing variability but may also indicate abnormal GE behaviour due to presence of a gastric condition. Understanding this is integral in clinical applications of GE testing and diagnosis of patients with modified GE rate. This be further developed in application of PK modelling to prescribe specific drug dosages for patients with gastric conditions.

Referring to the aims and objectives in section 1.2 of the literature review, points 1-8 covering GES and OBT historical methodology, mathematical and statistical analysis and modelling have been extensively covered in this report through theoretical background and review of relevant literature. However, application of mixed effects parameter estimation (MEPE) in GE or other relevant areas has not been covered with the review merely stating that MEPE is effective in parameter identification. Reviewing published literature that tested MEPE modelling would have been beneficial to determine if MEPE modelling is feasible due to the complexity of such models. It would be difficult to develop a semi-mechanistic PK model incorporating MEPE and review of relevant literature on MEPE may have helped in analysing this. On the other hand, literature covering neural networks was found to be useful and presented a method for semi-mechanistic GE model optimisation using a technique other than MEPE.

Although aims and objectives covering theoretical background were met, some areas had an excessive amount of information which was provided. Complex kinetics based on GE were covered in section 2.2.4.3 at a level of detail well beyond the scope of this research project and literature review. Although it is necessary to understand the kinetics when developing the mathematical model of the GE process, the level of complexity may have been better directed towards reviewing of literature covering semi-mechanistic GE modelling and other applications.

References

Alskär, O. et al., 2015. Semimechanistic model describing gastric emptying and glucose absorption in healthy subjects and patients with type 2 diabetes. *Journal of Clinical Pharmacology,* 56(3), pp. 340-348. DOI:10.1002/jcph.602.

Amarri, S. & Weaver, L. T., 1995. 13C-breath tests to measure fat and carbohydrate digestion in clinical practice. *Clinical Nutrition,* Volume 14, pp. 149-154.

Bruno, G. et al., 2013. 13C-octanoic acid breath test to study gastric emptying time. *European Review for Medical and Pharmacological Sciences,* 17(2), pp. 59-64.

Chaudhuri, T. K., 1973. Use of 99mTc-DTPA for measuring Gastric Emptying time. *Journal of Nuclear Medicine,* 15(6), pp. 391-395.

Foster, C., 1994. Gastric Emptying During Exercise: Influence of Carbohydrate Concentration, Carbohydrate Source, and Exercise Intensity. In: N. A. o. Sciences, ed. *Fluid Replacement and Heat Stress.* s.l.:National Academies Press, pp. Section 6, 1-9. DOI: 10.17226/9071.

Ghoos, Y., Geypens, B. & Rutgeerts, P., 2002. Stable Isotopes and 13CO2 breath tests for investigating gastrointestinal functions. *Food and Nutrition Bulletin,* 23(3), pp. 166-168.

Ghoos, Y. et al., 1993. Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. *Gastroenterology,* 104(6), pp. 1640-1640. DOI: 10.1016/0016-5085(93)90640-x.

González, A. et al., 2000. Characterisation with stable isotopes of the presence of a lag phase in the gastric emptying of liquids. *European Journal of Nutrition,* Volume 39, pp. 224-228.

Goyal, R. K., Guo, Y. & Mashirmo, H., 2019. Advances in the physiology of gastric emptying. *Neurogastroenterology & Motility,* 31(4), pp. 1-14. DOI: 10.1111/nmo.13546.

Griffith, G. H., Owen, G. M., Campbell, H. & Shields, R., 1968. Gastric Emptying in Health and Gastroduodenal Disease. *Gastroenterology,* 54(1), pp. 1-7.

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

Heading, R. C., Tothill, P., Laidlaw, A. J. & Shearman, D. J. C., 1971. An evaluation of 113m indium DTPA chelate in the measurement of gastric emptying by scintiscanning. *Gut - British Society of Gastroenterology,* Volume 12, pp. 611-615.

Jacoby, H. I., 2017. Gastric Emptying. *Biomedical Sciences Reference Collection, Elsevier,* pp. DOI: 10.1016/B978-0-12-801238-3.64921-8.

Markey, O. & Shafat, A., 2013. The carbon dioxide production rate assumption biases gastric emptying parameters in healthy adults. *Rapid Communication in Mass Spectrometry,* Volume 27, pp. 539-545. DOI: 10.1002/rcm.6478.

NDDIC, 2017. *Your Digestive System and How It Works,* s.l.: National Institute of Diabetes and Digestive and Kidney Diseases,

O'Donovan, D., Feinle-Bisset, C., Jones, K. & Horowitz, M., 2004. Gastric Emptying. In: L. R. Johnson, ed. *Encyclopaedia of Gastroenterology.* s.l.:Elsevier, pp. 118-124, ISBN: 978-0-12-386860-2.

Ogungbenro, K. & Aarons, L., 2009. Optimal Design of Pharmacokinetic Studies. *Nordic Pharmacological Society. Basic & Clinical Pharmacology & Toxicology,* Volume 106, pp. 250-255. .

Ogungbenro, K. & Aarons, L., 2011a. A semi-mechanistic gastric emptying pharmacokinetic model for 13C-octanoic acid: An evaluation using simulation. *European Journal of Pharmaceutical Sciences,* 45(3), pp. 302-310: DOI: 10.1016/j.ejps.2011.11.020.

Ogungbenro, K. & Aarons, L., 2011b. Structural identifiability analysis of pharmacokinetic models using DAISY: semi-mechanistic gastric emptying models for 13C-octanioc acid. *J Pharmacokinet Pharmacodyn,* 38(2), pp. 279-292. DOI: 10.1007/s10928-011-9193-5.

Ogungbenro, K. & Aarons, L., 2014. A physiologically based pharmacokinetic model for Valproic acid in adults and children. *European Journal of Pharmaceutical Sciences,* Volume 63, pp. 45-52. DOI: 10.1016/j.ejps.2014.06.023.

Ogungbenro, K., Pertinez, H. & Aarons, L., 2014. Empirical and semi-mechanistic modelling of double-peaked pharmacokinetic profile phenomenon due to gastric emptying. *AAPS,* 17(1), pp. 227-236. DOI: 10.1208/s12248-014-9693-5.

Ogungbenro, K. et al., 2011c. A Semi-mechanistic Gastric Emptying Model for the Population Pharmacokinetic Analysis of Orally Administered Acetaminophen in Critically Ill Patients. *Pharm Res,* Volume 28, pp. 394-404. DOI: 10.1007/s11095-010-0290-8.

Owen, G. M., Griffith, G. H., Kirkman, S. & Shields, R., 1966. Measurement of rate of gastric emptying using chromium-51. *The Lancet,* Volume 1, pp. 1244-1245. DOI:10.1016/S0140-6736(66)90247-9.

Perri, F., Pastore, M. & Annese, V., 2005. 13C-octanoic acid breath test for measuring gastric emptying of solids. *European Review for Medical and Pharmacological Sciences,* 43(9), pp. 3-8. DOI: 10.1136/gut.43.2008.S28.

Punkkinen, J. et al., 2006. Measuring Gastric Emptying: Comparison of 13C-Octanoic Acid Breath Test and Scinigraphy. *Digestive Diseases and Sciences,* 51(2), pp. 262-267. DOI: 10.1007/s10620-006-3122-2.

Sanaka, M. & Nakada, K., 2010. Stable isotope breath tests for assessing gastric emptying: A comprehensive review. *Journal of Smooth Muscle Research,* 46(6), pp. 267-280. DOI: 10.1540/jsmr.46.267.

Sanaka, M., Yamamoto, T. & Kuyama, Y., 2005. Theoretical Flaws in the Gastric Emptying Breath Test: Why is it dubious? - Letter to the editor. *Digestive Diseases and Sciences,* 50(1), pp. 15-17. DOI: 10.1007/s10620-005-1270-4.

Sanaka, M., Yamamoto, T. & Kuyama, Y., 2008. Retention, Fixation, and Loss of the [13C] Label: A Review for the Understanding of Gastric Emptying Breath Tests. *Digestive Diseases and Sciences,* Volume 53, pp. 1747-1756. DOI: 10.1007/s10620-007-0103-z.

Schommartz, B., Ziegler, D. & Schadewaldt, P., 2006. Significance of Diagnostic Parameters in [13C]Octanoic Acid Gastric Emptying Breath Tests*. *Isotopes in Environmental and Health Studies,* 34(1-2), pp. 135-143, DOI: 10.1080/10256019708036341.

Seok, J. W., 2011. How to interpet Gastric Emptying Scintigraphy. *Journal of Neurogastroenterology and Motility,* 17(2), pp. 189-191.

Siegel, J. et al., 1983. Radiation dose estimates for oral agents used in upper gastrointestinal disease. *Journal of Nuclear Medicine,* 24(9), pp. 835-837.

Szarka, L. A. & Camilleri, M., 2009. Gastric Emptying. *Clinical Gastroenterology and Hepatology,* 7(8), pp. 823-827.

te Braake, H. A. B., Roubos, J. A. & Babuska, R., 1999. Semi-mechanistic modeling and its application to biochemical processes. *World Scientific Series in Robotics and Intelligent Systems,* 23(1), pp. 205-226. DOI: 10.1142/9789812815392_0010.

te Braake, H., van Can, H. & Verbruggen, H., 1998. Semi-mechanistic modeling of chemical processes with neural networks. *Engineering Applications of Artificial Intelligence,* 11(4), pp. 507-515. DOI: 10.1016/S0952-1976(98)00011-6.

Tougas, G. et al., 2000. Assessment of gastric emptying using a low fat meal: establishment of international control values. *The American Journal of Gastroenterology,* 95(6), pp. 1456-1462.

Appendices

See following pages for relevant appendices.

Appendix A. Meeting Minutes

A.1. 08/10/2020 – Introduction

Subject: Introduction	
Meeting Place: Online – Microsoft Teams	Meeting no: 1
Date and time: 08/10/2020 – 10 am	Minutes: Marwan Elnesr
Attendees: Michael Short, Ishanki De Mel, Johanna von Gerichten, Joe Prollins, Marwan Elnesr	Apologies: N/A

Student Input / Progress / Issues Raised

First meeting introduction
Discussed project outline and aims and what is required from us to do
Discussed some of the key papers relevant to the project and literature review
Talked about experimental data provided – where this came from, how to analyse, useful programs for analysis and confidentiality

Student Questions
Q: Rough project timeline over 2 semesters i.e. when to begin data analysis?
A: Not a lot of literature to review on the topic so can be done fairly quickly. Should be able to start data analysis and modelling in semester 1. For early stages, understand papers and what type of models would be best for an improved physiological model

Q: What software would be best to use? How to go about getting training on this?
A: Matlab, Python, Excel or any other non-linear programming software. Python is most recommended. Training available through online free CodeAcademy course. Recommendation to start learning early in semester 1

Agreed Actions / Plans and Other Supervisor Feedback

Look at 2 relevant papers minimum from file list on Teams and review them. Prepare notes and discussion for next meeting.

Start to have a look at experimental data spreadsheet and Python code.

Look into online resources for Python training courses

Sign-off

Student Signature(s)	Supervisor Signature	Date
		12/10/2020

A.2. 15/10/2020 – Progress Update

Subject: Progress Update	
Meeting Place: Online – Microsoft Teams	Meeting no: 2
Date and time: 15/10/2020 – 10 am	Minutes: Marwan Elnesr
Attendees: Michael Short, Johanna von Gerichten, Joe Prollins, Marwan Elnesr	Apologies: N/A

Student Input / Progress / Issues Raised

Discussing Literature Analysis deadline and papers to be read
Need to find own papers beyond reading list to be included in lit. analysis (at least 5 papers with min. 2 from reading list)
Try not to use the same papers as group partner to cover a wider range of work relevant to research topic
For end of semester 1 deadline of literature review – 40-50 papers total target

Student Questions:
Q. Issues with running Python Spyder Code of uploaded model?
A. Uploaded model may be using a different dated version of Python. Older model of Python 3.8 works but updated models seem to not work

Other Python modelling info/ advice:
Model looks at Hessian Matrix 2nd Order Derivatives to determine optimal point based on confidence
Remove lines 313-335 in code or leave as comments
Use ipopt solver instead of ipopt_sens and remove relevant functions as well
Could compile own version of ipopt if uploaded model version doesn't work

Q. Should we look into using DAISY program?
A. We are trying to develop a model that is better than that provided using Python which just fits data to the model and does not actually give an indication to the physical meaning of the variables or show how the mechanistic system model works.
We can make our own version of the model using DAISY – must show underlying equations and how this may be a better/ worse fit for the data

Q. Understanding different types of variables and what they mean?
A. Global/ uniquely identifiable – trying to fit over many data sets and does not vary across data sets e.g. kinetic constant is global parameter but T may vary
Locally identifiable – specific to how one individual's GE system works. Changing data set changes overall objective function
Unidentifiable – changing data set will make little difference to objective function

Parameters within data set are estimatable/ identifiable if data function is sensitive to changes in this parameter.
Parameters in the function which do not affect objective function are important to the model. It could be useful to fix these parameters to a specific value to allow the model to be solved rather than estimating the parameters. Other aspects can affect this but cannot fully explain why
Step 1 is to look for general trends in model and step 2 is to focus on the parameters

Q. Looking for own papers outside reading list
A. Look for more recent papers and info. on ones that have been peer-reviewed that cover major milestones in the field

Sources to find relevant papers – Scopus, WebOfScience, GoogleScholar, PubMed with targeted keywords/ specific searches

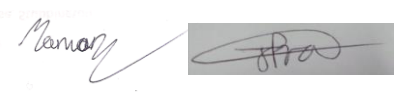
Agreed Actions / Plans and Other Supervisor Feedback

Look at 2 relevant papers minimum from file list on Teams and review them. Prepare notes and discussion for next meeting.

Look into online resources for Python training courses for Marwan – W3Schools, FreeCodeCamp video tutorial on YouTube

Discuss which papers will be used for literature analysis deadline between Marwan and Joe. Discuss points made to help each other with critical analysis

Sign-off

Student Signature(s)	Supervisor Signature	Date
		19/10/2020

A.3. 29/10/2020 – Progress Update

Subject: Progress Update	
Meeting Place: Online – Microsoft Teams	Meeting no: 3
Date and time: 29/10/2020 – 10 am	Minutes: Marwan Elnesr
Attendees: Michael Short, Johanna von Gerichten, Joe Prollins, Marwan Elnesr	Apologies: N/A

Student Input / Progress / Issues Raised

Discussion of queries related to papers used for literature analysis
Look for a nice balance of papers for lit. analysis e.g. 3 on OBT theory and 3 on semi-mechanistic PK modelling
Most papers related to topic talk about OBT background and not semi-mechanistic modelling itself – Look for papers that talk about semi-mechanistic modelling even if it is not relevant to GE modelling e.g. other fields
Don't need to do more than 5 papers
Don't need to include graphs/ diagrams unless critical to discussion or incorrect
If referring to graph, quote rather than actually include graph
Don't need abstract, intro, cover page etc. Just go straight into analysis
25% of write-up is novelty of paper (background, why it was chosen, findings etc.) and 75% is critique of paper (min. ½ to ¾ of page)
Important to make links between different papers analysed to support arguments

Q. What are the critiques of the paper done by Ghoos *et al*?
A. Method done by Ghoos is based on curve fitting of data for variables $t_{1/2}$ and t_{lag}

Agreed Actions / Plans and Other Supervisor Feedback

Look at an even number of paper topics i.e. 2-3 papers on OBT background in GE estimation and 2-3 papers on semi-mechanistic PK modelling of GE rate in OBT

Ensure links are made between different papers analysed

Collaborate with each other to see if new points are made for same papers analysed (Ghoos and Ogungbenro & Aarons). Useful to have opinion of writing of someone else from a different perspective

Sign-off

Student Signature(s)	Supervisor Signature	Date
		29/10/2020

A.4. 05/11/2020 – Progress Update

Subject: Progress Update	
Meeting Place: Online – Microsoft Teams	Meeting no: 4
Date and time: 05/11/2020 – 10 am	Minutes: Marwan Elnesr
Attendees: Michael Short, Johanna von Gerichten, Joe Prollins, Marwan Elnesr	Apologies: N/A

Student Input / Progress / Issues Raised

Literature review
40-50 papers for literature review between both of us – roughly 40-50 page report
Need to read more papers a week – from 2 papers to 3-4 papers each a week

Once each paper is read, write a short paragraph (5 sentences roughly, more for important papers to topic) to summarise the papers – this will be particularly useful when it comes to writing up the literature review as the information from the papers will still make sense rather than having to read the papers again

Outline analysis of work:

- Explain background of research using relevant papers (theoretical basis)
- Go into more detail on modelling aspect and PK analysis
- Explain links between modelling and theory and consider argument throughout paper
- Make links between papers

Make a succinct argument throughout the literature review and use papers to backup this argument coherently through the review
Example argument – We believe that semi-mechanistic PK modelling for 13C-OBT is the best method for estimating GE rate available today
Go through papers and analyse where this has/ hasn't been shown

Technical info.
Ghoos curves look completely different to those developed by semi-mechanistic PK modelling – this is due to there being a lot of outlying data.
Goal of the semi-mechanistic model is to identify these outliers and explain their physical meaning and behaviour based on the compartments of the model and the overall GE rate

PK modelling parameters represent physical parts of the GE process system. By changing different parameters, we are able to generate different curves. This raises the following questions:

- Why do these parameters vary so much for different groups and very individualistic?
- What could this individualistic parameter behaviour imply regarding the GE system and the rate of GE and metabolism?

It would be very useful to develop baseline model for future research work

Research topic
We can each look at different aspects of the research topic/ paper e.g. look at different models and analyse different things, look at different software modelling, develop different models for baseline and new model etc.

Research Proposal

Highlight where there is missing work/ gaps to fill in the research topic, what we're going to do to fill that gap, how we plan on doing it, time-scale, resource allocation, which data required to analyse, how many models will be developed, when modelling should be stopped and data analysis to begin etc.
Gantt chart is crucial
Identify which journal we want to write for, length and style of paper based on other papers in same research field, level of rigour required for publication etc

Agreed Actions / Plans and Other Supervisor Feedback

Start working towards main group literature review and project proposal deadlines – aim to have them done by week 11 before Christmas break so they can be proof-read over break
Look into papers that utilise PK/PD models that do not cover GE necessarily but look into other applications of semi-mechanistic modelling e.g. paper 5 on Marwan's literature analysis
Look into papers that go over theoretical background of GE – reader will not be an expert on the topic so would benefit from initial background and explanation

Look into Mixed Effects Modelling – can be used to group data in different sets based on curve fits and also get generalised fits for data sets. The main reason for this model is to identify physical aspects of GE system and model

Could be useful to look into developing a model using other software e.g. DAISY

Literature Analysis feedback to be emailed to us by Dr. Short to be reviewed in next research meeting on 12-Nov.
Upload any new and interesting papers found to Teams folder and review in next meeting with other project members

Sign-off

Student Signature(s)	Supervisor Signature	Date
		05/11/2020

A.5. 12/11/2020 – Progress Update

Subject: Progress Update	
Meeting Place: Online – Microsoft Teams	Meeting no: 5
Date and time: 12/11/2020 – 10 am	Minutes: Marwan Elnesr
Attendees: Michael Short, Johanna von Gerichten, Joe Prollins, Marwan Elnesr	Apologies: N/A

Student Input / Progress / Issues Raised

Upload new and interesting papers to Teams file to discuss in weekly meetings

Consider **Mixed Effects parameter estimation** – can be used as a backup in case the model doesn't work. Allows analysis of all the data at once and making parameter estimates that are globally identifiable and not specific to individuals

66 minutes correction factor for OBT data to scintigraphy

- Takes into account additional processes related to GE which OBT does not consider e.g. absorption, elimination (other than breath), metabolism
- Likely to be a specific variable which varies from individual to another based on their metabolism

Agreed Actions / Plans and Other Supervisor Feedback

Outline structure of literature review document for next supervisor meeting
Think of main argument throughout report and discuss with supervisors
Upload new paper to Teams folder for discussion in next week's meeting

Feedback from literature analysis reports sent back to each of us individually by email. Common **report writing related feedback** between both of us:

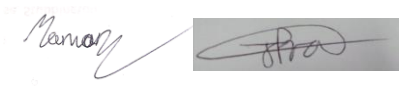
- Proof read to avoid silly spelling mistakes
- Lay out information well so report does not look unprofessional/ messy

Other **technical feedback** to **consider in future work** includes:

- Table of factors affecting GE rate
- Table of potential factors/ mechanisms in C-OBT
- Does 66 minutes correction factor have any physical meaning in 13C-OBT by Ghoos *et al*.? Accounts for processes not included in OBT model for GE
- Look into more papers covering PK modelling for other applications
- Look into papers covering Mixed Effects Modelling in PK applications

Seminar on Wednesday 18-Nov at 4 pm. for modelling of microbiomes – Await invite from Dr. Short

Sign-off

Student Signature(s)	Supervisor Signature	Date
		12/11/2020

A.6. 19/11/2020 – Progress Update

Subject: Progress Update	
Meeting Place: Online – Microsoft Teams	Meeting no: 6
Date and time: 19/11/2020 – 10 am	Minutes: Marwan Elnesr
Attendees: Michael Short, Ishanki De Mel, Joe Prollins, Marwan Elnesr	Apologies: N/A

Student Input / Progress / Issues Raised

Discussion on literature review structure and content of sections
Look through each other's conclusions in literature review to ensure coherence
Aim to complete work early to obtain feedback in time before winter break
Research project aim is to develop a model beyond curve fitting and investigating which parameters are constant and which can be adjusted between patients
Used to understand context behind data i.e. info about people with varying conditions and lifestyles
Explain curves don't fit to conventional methods (e.g. Ghoos) and why they don't

Agreed Actions / Plans and Other Supervisor Feedback

Literature review structure is very good and covers key sections and subheadings. Ensure theoretical background is covered to an acceptable level that explains research topic in detail to reader who is likely to not be knowledgeable of the research topic

For modelling section, discuss other modelling approaches and applications other than GE in sub-sections.
For modelling relevant to research topic, cover GE and physiological models

Submit a draft for sections in literature review and project proposal by Monday December 14th at the latest

Develop project aims and decide on individual proposals

Sign-off

Student Signature(s)	Supervisor Signature	Date
		19/11/2020

A.7. 03/12/2020 – Progress Update

Subject: Progress Update	
Meeting Place: Online – Microsoft Teams	Meeting no: 7
Date and time: 03/12/2020 – 10 am	Minutes: Marwan Elnesr
Attendees: Michael Short, Ishanki De Mel, Joe Prollins, Marwan Elnesr	Apologies: N/A

Student Input / Progress / Issues Raised

Q. Supervisor availability over winter break for feedback on drafts sent and any queries?
A. Dr. Short on leave officially 14th Dec – 4th Jan. May still be around to check emails and answer questions. Johanna and Ishanki on leave from 19th Dec.

Q. Best scientific journal to write towards for paper itself?
A. Based on which journals are most common from reading/ journals with highest prestige in field. Look at journal impact factors based on number of citations; high impact factor for journal means reviewer looks for high quality papers and they are overall better. Paper looks similar to ones that have been read before in field. **Nature journal is one of the best out there where it has 100 citations on papers**. Cater to reader e.g. more technical paper would not target nutritionists

Q. Are there any restrictions on what is stated in research proposal in comparison to actual research project?
A. Proposal is independent of research project. Think about perfect scenario (plan for this) and a worst-case scenario. Put this in proposal e.g. if by this stage, modelling is not working or data is not matching, we will aim to do this... Proposal is a tool to justify work being done and plan for this work, limitations and justification for investments into project.

Q. Should research proposal be open-ended?
A. **More specific is better** but some parts can be left open ended e.g. if we finish this early, we would like to go in this direction…

Q. Can we use university servers for neural networks and high CPU and RAM utilisation for next semester programming?
A. Not a lot of experimental data to crunch so could be run on a laptop. If issues encountered, there are alternative resources that could be provided to facilitate this by the University.

Can get more experimental data if required – data from patient experiment in University of Aberdeen and another one with same experiment and trial but different set of patients

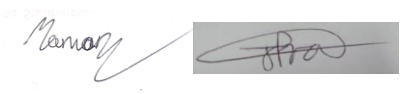
Review paper not just summary of papers – should have point at the end based on opinion of field. Good to use style of review papers online for our own but they would be significantly shorter than ours. They also assume things from readers and gloss over certain aspects which we should cover; our writing should cater to a general scientist reader who doesn't know much about the research topic

Literature review summary at front of our research review and should be used in final research paper writing up – carry out critique of work missing and why we are doing it

Agreed Actions / Plans and Other Supervisor Feedback

Finish section in literature review covering gaps in research review. Send to Dr. Short for feedback by next meeting on 10/12/2020

Sign-off

Student Signature(s)	Supervisor Signature	Date
		03/12/2020

A.8. 10/12/2020 – Progress Update

Subject: Progress Update	
Meeting Place: Online – Microsoft Teams	Meeting no: 8
Date and time: 10/12/2020 – 10 am	Minutes: Marwan Elnesr
Attendees: Michael Short, Ishanki De Mel, Joe Prollins, Marwan Elnesr	Apologies: N/A

Student Input / Progress / Issues Raised

State why each paper is relevant and how does this link to our work; maintain coherent argument throughout – put each paper into perspective
Diagram of digestive system – find paper describing this. Do not need to explain why we chose this source. Important for reader with minimal understanding of research topic to understand background and justification for research
Do more reading into parameter estimation – difference between semi-mechanistic model and curves
Curve fitting and parameter estimation are the same
More research into statistical methods (entry level papers) and discuss broadly in literature review when talking about parameter estimation, maths behind solvers – decide on what to convey to the reader, cannot include everything or bore them. **Have this in literature review**
Look into Mixed Effects Modelling papers and discuss on a broad scale

Mention that we have data available with outliers for OBT for semi-mechanistic modelling
Leave proposal aims as open-ended and do not make any false promises

Put in backups for research proposal goal in case main goal doesn't work out:

- Main goal – Get a fully functioning semi-mechanistic model that is able to identify outliers and with key parameter identification e.g. globally identifiable, locally identifiable, non-uniquely identifiable
- Backup goal – Get a fully functioning semi-mechanistic model similar to work done in Ogungbenro & Aarons paper

Definitions in experimental data:

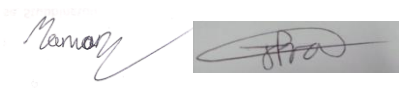
- PDR – Person Dose Response
- BSA – Body Surface Area
- ATE/ A%E – Atom % excess

Dr. Short available till Friday 18th December. Available on Monday and Wednesday if meeting required – no scheduled meeting during winter break

Agreed Actions / Plans and Other Supervisor Feedback

Make progress in paper writing throughout semester – notes of equations used in code, figures. Makes it easier to write up in end.

Sign-off

Student Signature(s)	Supervisor Signature	Date
		10/12/2020

Appendix B. Verification Plan

Check	Verified & Correct?	Initials
Spelling and grammar checked?	Yes	JP, ME
All tables and figures captioned?	Yes	JP, ME
All content is referenced correctly?	Yes	JP, ME
Presence of a table of figures, table of tables and table of symbols if relevant?	Yes	JP, ME
No “error referenced” errors?	Yes	JP, ME
Is the report coherent?	Yes	JP, ME
Is the report main text single-spaced typescript in Arial 11?	Yes	JP, ME
Are the report margins between 2~2.5 cm?	Yes	JP, ME
Is the report written in an appropriate style (i.e. no contractions nor colloquialisms)	Yes	JP, ME
Is there a contents list?	Yes	JP, ME
Is there a title page with the authors, URNs, supervisors etc.	Yes	JP, ME
Are all abbreviations, acronyms etc. defined on first use and placed in the table	Yes	JP, ME
Are all superscripts and subscripts applied correctly?	Yes	JP, ME
Has an introduction including a motivation for study, aims and objectives, and hypothesis etc. been included?	Yes	JP, ME
Have the meeting minutes been included?	Yes	JP, ME
Is it clear to read for an engineer not operating within the field with subject-specific terms defined?	Yes	JP, ME
Is the report comprehensive and appropriately covers the literature?	Yes	JP, ME
Have comment responses been reviewed and actioned upon if relevant?	Yes	JP, ME

B.1. Comment Responses

#	Section	Comment	Comment Response
		Michael Short – First Draft Intro and Research Gaps	
1	Introduction/ Background of OBT	Define GE	GE now defined in first instance and in acronyms, abbreviations and initialisms table
2	Introduction/ Background of OBT	This is a bit vague	Section has been clarified
3	Introduction/ Background of OBT	Random comment, but justified text generally looks a bit more professional	Text changed to justified
4	Introduction/ Background of OBT	Add years to each of your references. Often these authors have multiple papers, so this helps identify.	Dates added to references
5	Introduction/ Background of OBT	Since your other marker may be an engineer, a diagram of the digestive system (and perhaps compartments in the models) will be really useful. This might be better in the main body of text.	Diagrams added
6	Introduction/ Background of OBT	You might want to expand a bit on these in the main body of the literature review.	Further expansion applied in main body of literature review.
7	Introduction/ Background of OBT	Perhaps this should go into the main body?	Moved into main body.
8	Introduction/ Background of OBT	Good to contextualise and prepare for the reader's questions on relevance.	Contextualised and further detail provided

#	Section	Comment	Comment Response
9	Introduction/ Background of OBT	This is good. I would have liked to see a sort of "table of contents" of the sections. Something like: GE Basics – the body, why we study this Ghoos et al. and other models of this nature (with some images of what the data looks like and why more detail of why these are not great, mentioning the outliers in the data we have) Curve-fitting/parameter estimation (these are difficult to fit in, but necessary, maybe even just as a subsection) Semi-mechanistic modelling approaches in general (these are difficult to fit in, but necessary, maybe even just as a subsection) Semi-mechanistic modelling for GE, specifically.	Table of Contents added. Details cover sections mentioned added.
10	Gaps in research & Areas to investigate	Avoid informal phrases	Informal phrase removed
11	Gaps in research & Areas to investigate	Very good! We also want to use this model on data obtained at UoS. It will be good to mention this. If you need more details, give Johanna a shout.	No action required
12	Gaps in research & Areas to investigate	Not sure I follow this. The objective function is to minimise error.	References about the objective function removed and replaced
13	Gaps in research & Areas to investigate	Maybe a bit of confusion here over curve-fitting/parameter estimation. These are the same, however the curve is a simple, physically meaningless curve in Ghoos.	Section removed and rewritten.
14	Gaps in research & Areas to investigate	These are great!	No action required
15	Gaps in research & Areas to investigate	In your proposals, I would like it to also be clear what happens if you can't do this. What are the minimum expectations? Plans for if this fails, etc.	No action required relevant to this document

#	Section	Comment	Comment Response
		Ishanki De Mel - Draft 1 for Literature Review Report	
16	1.3 – Hypothesis	It would be better to present the hypothesis after some more background/context on GE, OBT, etc. This allows a reader who is not familiar with the areas to have a better understanding, before getting to why semi-mechanistic modelling is required and why it's the best at determining GE rates.	Further background and context provided.
17	1.4 – Papers Analysed – Table 1	I really like the idea of a summary table with all the key papers.	Section deleted. See comment 37
18	2.2 – Gastric Emptying and its Importance	Food for thought: Is there anyway you can include this section or a couple of sentences on this in the preamble? I think that it is critical that the reader gets the bigger picture from the start.	More context regarding GE and its importance added to preamble.
19	2.3 - Testing Methods for Determining Gastric Emptying Rate	If you have gathered this information from published articles, please reference them.	References added.
20	2.3 - Testing Methods for Determining Gastric Emptying Rate	Please add the years	Years added
21	2.3 - Testing Methods for Determining Gastric Emptying Rate	Can you please explain these a little more?	Further explanation now provided
22	2.4 - The 13C-Octanoic Acid Breath Test	This is a different referencing format, compared to the one used in the sub-sections above. I have seen your question on Harvard referencing; it's best to stick to one throughout the text.	Harvard referencing style applied throughout.

#	Section	Comment	Comment Response
23	2.4 - The 13C-Octanoic Acid Breath Test	This is repeated from the section above. Food for thought: is it possible to include this diagram or something similar in the section above? I saw that Michael had commented on this as well. It might be possible to merge this section with the section above, or include this as a sub-heading under "2.3. Testing Methods for Determining Gastric Emptying Rate" without too much repetition.	Repetition removed. Diagram added
24	2.4 - The 13C-Octanoic Acid Breath Test	Is it possible to rephrase this slightly more diplomatically? I understand that Ghoos has been more transparent than Perri (which is a valid point), but generally, we don't discuss whether a paper has included enough detail in the introduction or not, i.e. try to stick to analysing the methodologies/ formulations/ results, etc. as opposed to how the paper is written.	Focus placed on analysing the methodologies/ formulations/ results, etc. comment removed referring to writing style.
25	2.4 - The 13C-Octanoic Acid Breath Test	I've tried to improve this section, perhaps you can come up with something better as this sentence is now very long.	Sentence readjusted.
26	2.4 - The 13C-Octanoic Acid Breath Test	This is a long sentence. I have tried to split it up.	Sentence separated into smaller ones.
27	2.4 - The 13C-Octanoic Acid Breath Test	Is it possible to explain this a little bit more? Please keep in mind that the readers may not always be familiar with the biological aspects of your work.	Unit changed into SI unit and defined.
28	2.4 - The 13C-Octanoic Acid Breath Test	Please explain what they were trying to do here, rather than quoting them. It is always best to use quotes sparingly.	Quote removed.
29	2.5.1 - Ghoos et al, 1993	When presenting equations in a paper, it is common practice to include the equation in a bordeless table with a single row and two columns. I have demonstrated this above.	Practice now applied throughout

#	Section	Comment	Comment Response
30	3.3.4 - Measuring Gastric Emptying: Comparison of 13C-Octanoic Acid Breath Test and Scintigraphy	Food for thought: do you need an entire sub-section for this? Perhaps the key points from this paper can be included in Section 2.3	Sub-sections have been merged.
31	5 - Gap Identification & Areas to Investigate	Great work.	No action required
32	5 - Gap Identification & Areas to Investigate	Please rephrase.	Rephrased
33	5 - Gap Identification & Areas to Investigate	Spot on.	No action required
Michael Short - Draft 1 for Literature Review Report			
34	1.1 - Preamble	We need a lot more of an introduction here. What is the test, why are you studying it, what can it be used for? I would go for importance of human health and diet, understanding effects of changes of diet, lifestyle, etc. on human health, then the use of the test and potentially other uses. Try to make this entry-level and accessible to the average, non-expert.	Further information added covering all points mentioned.
35	1.3 - Hypothesis	I am not too sure how the 4 papers and the Ghoos paper really fit in here. Perhaps these should rather be in the 'preamble'? Perhaps it is almost better to rather merge 2.1 and 2.2 into a more complete introduction and in there also mention these 5 papers? Then in section 2 you can do a deeper dive from there without the need for too much background information?	4 papers section removed for irrelevance. A comprehensive introduction has been written with a deeper dive into section 2.
36	1.3 - Hypothesis	Stick with Harvard.	Harvard applied throughout

#	Section	Comment	Comment Response
37	1.4 - Papers Analysed	I think that this is OK to do, however this is only really done in perhaps more narrow applications. For example, you guys are looking at work from all over the place (but have these 5 – 10 core papers). In this case, would the table really be appropriate? I would only do this if you really think that this will make the readers' job easier. It works well for some papers, but this is up to you. When doing these larger literature surveys that are perhaps more structured or highly specific, these are used sometimes, but this is often for when you want to search for something specific in a large field (i.e. highly specific keywords that funnel information from the 10s of thousands to a manageable 100-200). Your call, but try to think of the readers.	Section removed
38	2.3 - Testing Methods for Determining Gastric Emptying Rate	Do this throughout	Adding years to references applied throughout
39	2.4 - The 13C-Octanoic Acid Breath Test	Try not to repeat too much from other sections	Repetitions reduced
40	2.4 - The 13C-Octanoic Acid Breath Test	This is all good, but could perhaps be improved by mentioning Ghoos through the lens of more than just one other paper? Maybe some abstract reading of some of the other studies will help make this section feel a little wider?	Several additional papers added making "section wider".
41	2.5 - Mathematical Analysis	It would be good to add intro paragraph/sentence underneath this heading	Paragraph added.
42	2.5.1 - Ghoos	Rather number equations on the side and also try to explain the variables and parameters as you go to make this easy to read.	Completed.

#	Section	Comment	Comment Response
	3.3.1 - Empirical and Semi-Mechanistic Modelling of Double-Peaked Pharmacokinetic Profile Phenomenon due to Gastric Emptying	Try to avoid speaking about a single paper in a section. Try to either talk about the approach holistically and then mention that the way this paper did it, or analyse multiple papers in the section, synthesising the similarities, differences, and overall issues.	A holistic approach has been adopted.
43	3.3.2. Semi-Mechanistic Modeling and its Application to Biochemical Processes	Same here. Introduce the section broadly and then go into paper details.	As above.
44	3.3.3. Semi-Mechanistic Modeling of Chemical Processes with Neural Networks	Every one of these starts the same way.	Approach style changed.
45	5 - Gap Identification & Areas to Investigate	This could be a bit clearer	Text has been clarified