Effects of Chronic Exercise Intervention on Gastric Emptying

Thesis submitted for the degree of Master of Science by

Research

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Abstract

Effects of chronic exercise intervention on gastric emptying

Katy Horner

Many studies have linked altered gastric emptying (the rate at which food empties from the stomach into the small intestine) to increased food intake and obesity. Although findings comparing lean and obese individuals are inconsistent, previous research in non-obese adults indicates an inverse association between gastric emptying rate and body mass index. Physical activity has been implicated as a modifiable lifestyle factor which may alter the delivery of nutrients to the small intestine. However this is based on limited cross sectional research comparing physically active and sedentary individuals. Surprisingly, no longitudinal studies could be found. In addition, gastric emptying has received little investigation as a potential mechanism which may explain changes in appetite and food intake with exercise. The aim of this thesis therefore is to determine changes in gastric emptying, appetite sensations and food intake with chronic exercise interventions.

Two longitudinal experimental studies are presented, the first involving a 7-week exercise intervention in a cohort of 19 adolescent schoolgirls, and the second a 10-week intervention study in a cohort of 12 Multiple Sclerosis patients. Gastric emptying was assessed using the $^{13}$C-octanoic acid breath test.

Increasing BMI was demonstrated to be significantly associated with slower gastric emptying for the first time in a population of female adolescents. A 7 week exercise intervention had no effect on gastric emptying or food intake at an ad libitum buffet meal but hunger sensations at 30 minutes after the test meal assessed by visual analogue scales were significantly reduced following intervention when compared to a control group.

Using heart rate variability analysis as an additional indicator of autonomic nervous system function in Multiple Sclerosis patients, no significant difference in changes in gastric emptying or heart rate variability were found following exercise intervention when compared to a control group. Three day food diary records indicated a significant increase in food intake following exercise intervention but significantly reduced hunger sensations were observed over 4 hours after the test meal following exercise intervention compared to changes in the control group.

The combined findings of this thesis suggest that chronic exercise intervention does not significantly alter gastric emptying in these populations but suppresses postprandial hunger sensations when compared to a control group. The accelerated gastric emptying previously observed by others in more active individuals may therefore be due to other lifestyle factors such as diet. Alternatively exercise interventions of increased weekly energy expenditure or duration may be needed to significantly alter gastric emptying. These studies provide the foundation to future work in this area.
Declaration of originality

I declare that the work contained in this thesis is original. The recruitment of participants, collection and analysis of samples, and the analysis and presentation of data were undertaken entirely by the author, unless otherwise stated. This work has not been submitted to any other University or higher education institution, or for any other academic award within this University.

Katy Horner

March 2010
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<th>Description</th>
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<tbody>
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<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
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<tr>
<td>EB</td>
<td>Energy Balance</td>
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<tr>
<td>EE</td>
<td>Energy Expenditure</td>
</tr>
<tr>
<td>EI</td>
<td>Energy Intake</td>
</tr>
<tr>
<td>GE</td>
<td>Gastric Emptying</td>
</tr>
<tr>
<td>GER</td>
<td>Rate of Gastric Emptying</td>
</tr>
<tr>
<td>GITT</td>
<td>Gastrointestinal Transit Time</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-Like Peptide-1</td>
</tr>
<tr>
<td>HR(_{\text{max}})</td>
<td>Maximum Heart Rate</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart Rate Variability</td>
</tr>
<tr>
<td>MCTT</td>
<td>Mouth to Caecum Transit Time</td>
</tr>
<tr>
<td>MVPA</td>
<td>Moderate to Vigorous Physical Activity</td>
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<tr>
<td>OCTT</td>
<td>Orocecal Transit Time</td>
</tr>
<tr>
<td>PA</td>
<td>Physical Activity</td>
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<tr>
<td>PP</td>
<td>Pancreatic Polypeptide</td>
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<tr>
<td>PYY</td>
<td>Peptide YY</td>
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<tr>
<td>(t_{\text{asc}})</td>
<td>Ascension Time</td>
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<tr>
<td>(t_{\text{lag}})</td>
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<tr>
<td>(t_{\text{lat}})</td>
<td>Latency Time</td>
</tr>
<tr>
<td>(t_{1/2})</td>
<td>Half Time</td>
</tr>
<tr>
<td>VO(_{2\text{max}})</td>
<td>Maximal Oxygen Uptake</td>
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Chapter 1 Introduction

This thesis focuses on the effects of chronic exercise on gastric emptying, appetite and food intake. Two longitudinal studies are presented, the first involving a 7 week exercise intervention in a cohort of 24 healthy adolescent schoolgirls, and the second a 10 week intervention in a cohort of 15 Multiple Sclerosis patients.

The role of exercise in increasing energy expenditure is well established. Exercise may also alter energy intake by influencing many aspects of the integrated regulatory process of appetite control including hormonal, neural and behavioural factors. The effects of exercise on physiological mechanisms controlling food intake is gaining increasing research interest and the role of the gut and peptides involved in signalling appetite sensations have been a particular focus. Surprisingly, despite numerous studies linking altered gastric emptying (the rate at which food empties from the stomach into the small intestine) to food intake and obesity, gastric emptying has received little investigation as a potential mechanism which may explain changes in appetite and food intake with exercise.

This may be partially attributable to the techniques involved in gastric emptying measurement as many early methods were invasive and scintigraphy, the most commonly used and considered ‘gold standard’ method, exposes subjects to radioactivity, thereby limiting research in subjects where GE tests would not normally be required. The development and validation of the $^{13}$C-octanoic acid breath test by Ghoos and colleagues (1993) has now made the assessment of GE feasible for research in a much wider group of subject populations. Involving the administration of $^{13}$C labelled test meals, collection of breath samples from subjects before and after meal consumption, subsequent breath sample analysis by isotope ratio mass spectrometry and the fitting of data to an established gastric emptying mathematical model, the method has been used in research of healthy children, adults and patients since its inception. Utilising this method makes the repeated measures studies presented in this thesis feasible. Further measurements used include visual analogue scales to assess feelings of hunger, fullness and desire to eat, ad libitum buffet meals to assess changes in food intake and physical activity recall questionnaires to assess changes in physical activity levels. Heart rate variability analysis, a technique that is gaining increasing use as an indicator of autonomic nervous system function (which plays an integral role in homeostasis) was used to explore underlying mechanisms behind changes in GE.
Understanding the effects of exercise interventions on mechanisms modulating energy intake and appetite is important both for the prevention and treatment of over- and under nutrition. In this regard, the influence of exercise on physiological variables such as gastric emptying rate that may play an important role in altering appetite sensations represents an area worthy of further investigation.

1.1 Thesis Aim
Using a longitudinal design, this thesis aims to determine changes in GE with chronic exercise, and the potential any such alterations may have to modulate EI and appetite sensations. Further understanding of the effects of exercise on GE, appetite and food intake will assist in designing more effective therapeutic strategies for the prevention and treatment of obesity and under-nutrition.

1.2 Thesis Outline
The next chapter of this thesis consists of a literature review. Two chronic exercise studies are then presented involving different subject populations; 1) adolescent females, and 2) Multiple Sclerosis (MS) patients. This is followed by a chapter summarising main findings and some suggestions for future study. The following outlines a summary of each chapter:

- Chapter 2 uses a thematic approach to assist in understanding the potential that GE may have to alter appetite and food intake with exercise. Various methodological issues are explored. Studies that have linked altered GE to obesity and the mechanisms proposed behind such changes are then discussed. To conclude, studies that have examined the effect of acute and chronic exercise on GE and mechanisms behind any changes are reviewed.

- Chapter 3 presents an experimental study conducted to investigate the effects of a 7 week exercise intervention on GE, appetite and food intake in healthy adolescent females. The association between BMI and GE is examined to assist in further understanding the potential role that GE may have in overweight/obesity.

- Chapter 4 presents an experimental study conducted to investigate the effect of a 10 week exercise intervention on GE, appetite and food intake in individuals with MS, a population in which prior research has
indicated delayed GE to be more prevalent. In addition, changes in heart rate variability are examined to assist in understanding the effects of exercise on other parameters of autonomic function in this population.

- Chapter 5 summarises the main findings from this thesis and provides some suggestions for future study.
Chapter 2 Literature Review

2.1 Introduction

Theoretically, if energy intake (EI) equals energy expenditure (EE) there should be neither gain nor loss of body weight, and energy balance (EB) should be maintained (Figure 2.1).

![Energy Balance](image)

**Figure 2.1: Energy Balance: EI = EE.**
*(BMR: Basal Metabolic Rate, PA: Physical Activity. Thermogenesis: Thermogenic effect of nutrients).*

Daily PA is a major factor in the EE side of the equation and one amenable to change. The benefits of PA are numerous. The World Health Organisation (2010) lists reduced risk of cardiovascular disease, stroke, type 2 diabetes, colon cancer, breast cancer, osteoporosis and hypertension and beneficial effects on body composition and mental and psychological health among the benefits of increasing levels of physical activity. Furthermore, PA without weight loss or energy restriction is associated with a substantial reduction in total and visceral fat and in skeletal muscle lipid, together with a significant improvement in fitness levels in obese individuals (Lee et al 2005).

Research has demonstrated a critical role for PA in long term weight maintenance (Klem et al 1997, Benshimon et al 2002, Catenacci et al 2008) and in the prevention of obesity (Wareham et al 2005). However, the role of PA in inducing weight loss is more controversial. The ability of PA to create a negative EB relies both directly on its impact on EE and indirectly on its potential to modulate EI (King et al 1997b). It has been proposed that

“Exercise may stimulate the appetite so that persons who exercise increase their eating and do not lose as much weight as expected,” (Epstein 1980).

While this statement implicates exercise as a relatively futile method of losing weight, it suggests exercise may be a beneficial appetite stimulatory treatment. Today, there is more evidence suggesting that EI is only weakly coupled to exercise-induced EE (Blundell & King 1998, Stubbs et al 2004). In fact, research has suggested that
exercise may have a beneficial role in short-term appetite control (Long et al. 2002; Martins et al. 2007). This interaction of EE with EI in determining EB is an area of research attracting increasing interest, and various mechanisms including changes in sensitivity to hunger and satiety signals (Blundell et al. 2003, Martins et al. 2007) have been proposed to account for alterations in EI with exercise. See Martins et al. (2008) for a review. However, the exact mechanisms still remain unclear.

Gastric emptying (GE) refers to the rate at which solids and/or liquids empty from the stomach into the small intestine. There is persuasive evidence that GE has a role in food intake (Sepple & Read 1989). In 1975, Hunt postulated that a more rapid GE and thus a shorter satiety period would lead to increased food intake and consequently contribute to the development of obesity. The association between rate of GE and obesity (discussed in section 2.5 of this thesis) is an area that has since received considerable investigation. However, findings are inconsistent.

A considerable body of research has focused on dietary factors that may alter GE. See Little et al. (2007) for a review. Surprisingly, the effects of exercise on GE and the associated consequences for appetite control and EI have received little attention. Instead, the majority of research concerning exercise and GE has focused on gastric symptoms and on methods of enhancing performance during exercise. Further understanding of the effects of therapeutic interventions such as PA on GE and the associated effects on food intake will assist in the design of more effective strategies for the treatment and prevention of obesity and malnutrition.

As little research has focused on changes in GE with exercise in relation to EI, this review of the literature will follow a thematic approach. Appetite control and the role of gastric function in food intake will first be discussed. Gastric emptying will then be looked at in more detail, and various methodological aspects explored to assist in the interpretation of studies cited and methods used in this thesis. This will be followed by a review of the literature linking GE to obesity; to assist in understanding the potential role that GE may have in altering food intake. Studies that have investigated changes in GE with exercise are then reviewed, and the mechanisms proposed behind any such changes will be discussed in detail to further understand the control mechanisms of GE that may be influenced by exercise. The final section of this literature review will discuss how changes in GE that have been observed with acute and chronic exercise may relate to previous findings regarding changes in food intake with exercise.
2.2 Appetite Control

2.2.1 Introduction to Appetite Control

Appetite is important in considering EB since it modulates the energy intake (EI) side of the equation. Before considering the mechanisms involved in appetite control, it is necessary to distinguish between satiation and satiety. Satiation refers to the process that controls the size of a meal by terminating the period of eating, whereas satiety can be described as the state following a meal during which hunger is dampened and the urge to consume food is inhibited (Blundell 1991). The regulation of food intake is a complex interplay of gastrointestinal signals (hormones, neuropeptides, autonomic nervous system), metabolic cues from hormones, peptides and absorbed nutrients, and hypothalamic brain centers (Mathus Vliegen et al 2005).

Blundell & Halford (1994) conceptualised appetite control to represent three levels of events and processes.

1) Psychological (hunger perception, cravings, hedonic sensations) and behavioural events (meals, snacks, energy and macronutrient intakes)

2) Physiological and Metabolic Events

3) Neurotransmitter and Metabolic Interactions in the brain

Although it is acknowledged that food intake is influenced by numerous factors including environmental, psychological, social and cultural stimuli (De Castro et al 1996), this review will focus primarily on the physiological control of appetite, and specifically the role of gastric function.

2.3 Gastric Function and Appetite control

The stomach can be separated into 5 main anatomical regions: cardia, fundus, antrum, corpus and pylorus. The proximal part of the stomach consists of the fundus and part of the gastric corpus, and its main function is to act as a reservoir for the ingested meal. The antrum and distal part of the gastric corpus comprise the distal part of the stomach. The main function of this part of the stomach is to generate motor activity which will modify the ingested meal into a consistency and solution suitable for emptying into the small intestine for further digestion (Tack 2005).

Feelings of hunger and satiety have long been associated with gastric motor and sensory functions and the role of the stomach in appetite control has been of interest for
at least a century (Park & Camilleri 2005). Gastric distension in combination with the presence of intestinal nutrients is considered to contribute to satiety (Geliebter et al 1988, Read et al 1994).

### 2.3.1 Gastric Distention and Volume

Early studies observed that mechanical distension of the stomach with a balloon could elicit a feeling of fullness (Hertz 1911, Boring 1915), thus linking sensory functions from the stomach to motor function. In support of this contention, Santangelo et al (1998) found a significant association between antral area and fullness sensations ($r^2 = 0.46$, $p = 0.004$), confirming that antral distension plays a part in the regulation of eating behaviour. Beckoff et al (2001) objectively measured food intake at a buffet meal and found that food intake was inversely related to the amount of gastric contents remaining of a glucose preload. This led the authors to suggest that it was gastric distension that modified food intake. Although the mechanisms remain unclear, Park & Camillieri (2005) in their review of gastric function in obesity suggested that distending the stomach stimulates gastric stretch receptors, triggering vagal discharges that activate hypothalamic neurons (Anand & Pillai 1967), inducing the feeling of fullness (Deutsch 1978).

As a result, gastric capacity has been implicated to have a role in appetite control as it seems plausible that a large gastric capacity may require a bigger meal to trigger early postprandial fullness (Geliebter et al 1992). However, in general the literature shows that the capacity of the stomach is normal in obese people (Camillieri & Grudell 2007), suggesting that it may be the feedback signals inducing meal termination that are impaired in obese individuals (Ranganath et al 1996, Matsuda et al 1999, Jeanrenaud et al 2001, English et al 2002). This is supported by studies finding that when energy density of food is decreased over a more prolonged period of time by dilution of food with non-nutritive ingredients, smaller but more frequent meals are consumed such that overall EI remains relatively constant (Adolph 1947, Smith & Duffy 1955). These findings suggest that volume detection does not play a major role in the longer term regulation of EB.

### 2.4 Gastric Emptying

Gastric emptying (GE) refers to the emptying of solids and/or liquids from the stomach into the small intestine. GE, through the delivery of nutrients to the small intestine is a component of gastric function considered critical in appetite control (Park & Camillieri
2005) and has a role in both satiation and satiety. Gastric distension, nutrient stimulation of intestinal chemoreceptors and metabolism of absorbed substrates all contribute to meal termination (satiation). Once the meal is emptied, intestinal stimulation subsides, and the need for more food is signalled influencing the interval to the next meal (satiety) (Jackson et al 2007).

GE is a complex process, resulting from the coordinated motor activity of the stomach, pylorus, and duodenum (Yin & Chen 2005), regulated by central and enteric autonomic neural activity, and by local and systemic peptides (Minami & MacCallum 1984). GE is influenced by a variety of factors. These include volume (Mitchell & Voss 1991), composition, energy content (Moore et al 1984) and temperature of the meal (Sun et al. 1988), age (Moore et al 1983), sex (Datz et al 1987), physical activity (Marzio et al 1991) and position of the subject (Moore et al 1988), drug intake (Chaudhrui et al 1990), and time of the day (Goo et al 1987). Meal properties are an important factor to consider in comparing findings from GE studies. Intake of a large volume has been shown to accelerate GE (Hunt & Stubbs 1975, Mitchell & Voss 1991). A linear increase in amount emptied has been observed with an increase in gastric content (Hunt & Spurrel 1951) up to a maximum of 600ml of ingestate (Costill & Saltin 1974). In addition, solids and liquids follow different emptying patterns. Noncaloric liquids are emptied in an exponential fashion. As the calorie content and viscosity increases the pattern of emptying becomes more linear than exponential (Park & Camilleri 2005). After a lag time in which food is triturated into smaller particles, solids empty in a linear fashion (Park & Camilleri 2005).

2.4.1 Methods of measurement

In interpreting findings from GE studies, it is important to consider the measurement technique used to assess GE. As GE is often exponential it is important that the measurement technique used reflects the kinetic nature of GE over time. However, this is not often the case. Neufer et al (1989b), for example compared gastric residues at 15 min after moderate exercise to gastric residues after 20min seated rest and observed a significantly accelerated GE with exercise. In contrast, Feldman & Nixon (1982) who observed no change in GE with exercise reported the amount emptied at 120 minutes post meal. Contradictory interpretations may therefore occur when trying to compare results between studies using different time points.
Many methods have been used to assess GE, from early studies involving the ingestion of radiopaque markers followed by abdominal screening to more recent use of magnetic resonance imaging and electrical impedance tomography. The most commonly used methods in the literature to date including intubation and aspiration of gastric contents, scintigraphy, ultrasonography, the paracetemol absorption test and the $^{13}$C-octanoic acid breath test ($^{13}$C-OBT) are discussed in more detail below. Each method has its own strengths and weaknesses. In general, the best method of measurement may depend on the study characteristics such as the type of test meal and the subject population involved.

2.4.1.2 Intubation/Aspiration of Gastric Contents

Early studies such as that by Hunt & Spurrell (1951) used an intubation technique to study the physiology of gastric emptying. This method involves ingestion of a non-absorbable marker such as phenol red and the complete aspiration of gastric contents at a particular time point postprandially. A limitation of studies using a single sample technique is that often gastric contents at one time point only were determined. George (1968) introduced a double sampling technique allowing serial measurements of gastric contents. Unlike other methods such as scintigraphy which require the subject to remain still, this technique can be conducted even during intense exercise and has been widely used by exercise physiologists. However, a major limiting factor of this method is that it can only be used to measure GE of liquids. Furthermore, subjects may find the introduction of a nasogastric tube uncomfortable.

2.4.1.3 Scintigraphy

Scintigraphy is considered the ‘gold standard’ method for measuring GE. The technique involves radiolabelling part of a meal (often eggs), and the subsequent detection of radiation emitted from the radionuclide inside the body usually using a gamma camera. With appropriate labelling of the test meal components and appropriate corrections applied to the images obtained, it is the method of choice for clinical investigation of disturbed emptying patterns and can be applied to solid or liquid meals (Maughan & Leiper 1996). However, there are also limitations to the method. Inaccuracies in the estimation of GE parameters may arise from anterior intragastric movement making it necessary to correct for certain factors such as differences in the depth of gastric content, yet the accuracy of the methods used to correct such factors are unknown (Meyer 1983). In addition, the method requires the facilities of a nuclear medicine
department and the unnecessary exposure of healthy subjects who would not otherwise undergo a GE test to radiation can be criticised.

2.4.1.4 Ultrasonography
Bateman & Whittingham (1982) developed a non-invasive technique for measuring GE using ultrasound. GE rates measured by ultrasonography have been shown to correlate well with those measured by scintigraphy (Bolondi et al 1986, Holt et al 1986). Additional strengths of this method include the non-invasiveness and imaging of gastric function. Limitations include the need for the subject to remain still and operator expertise.

2.4.1.5 Paracetemol Absorption Test
Another non-invasive method is based on paracetemol absorption kinetics. As paracetemol is rapidly absorbed from the small intestine but not from the stomach, it is considered a model for GE (Heading et al 1973, Petring et al 1990). Heading et al (1973) observed a significant correlation between GE measured by scintigraphy and the time taken to reach plasma paracetemol concentration. A major advantage of the paracetamol absorption test is that it requires no special equipment. Further advantages include real volume estimation and the detailed evaluation of emptying dynamics (Medhus et al 1999). Limitations include individual differences in metabolism and excretion not being taken into account and drug interactions such as the oral contraceptive which is reported to increase paracetemol clearance by up to 50% (Medhus et al 1999).

2.4.1.6 $^{13}$C-octanoic acid Breath Test
Ghoos et al (1993) developed a carbon labelled breath test called the $^{13}$C-octanoic acid breath test to measure GE of solids, based on the characteristics of octanoic acid. The test is based on the principle that octanoid, once it has left the stomach, is absorbed in the duodenum, metabolized in the liver and excreted in the expired air (Figure 2.2).
Figure 2.2 Sequential metabolic steps after ingestion of the 13C-labeled baked egg yolk. The rate limiting step of the breath $^{13}$CO$_2$ excretion is represented by the gastric emptying of the yolk. From Perri et al (2005).

Ghoos et al (1993) concluded that this method described GE of a standard meal (250kcal labelled egg and bread meal) in an accurate and reliable way. In addition the method was found to describe the biphasic nature of GE and the day to day variability of GE was comparable to that of scintigraphy (Ghoos et al 1993). A subsequent study by the same group found that the breath test was sensitive enough to detect pharmacological influences on GE (Maes et al 1994).

The test is not without its limitations as Choi et al (1998) point out that the variability in the rate of absorption, metabolism, and excretion of the marker between individuals must be considered in the interpretation of data. However, the advantages of the test are numerous, particularly in research where subjects would not normally require a GE test, as the $^{13}$C-OBT provides a non-invasive, non-radioactive alternative to scintigraphy. In particular, it has found favour with intervention studies where consecutive measurements may be required (Mansi et al 2000, Peracchi et al 2000).

2.4.1.7 Outcome Measures

The outcome measures that are reported are another varying factor between studies and an important consideration in evaluating study findings. Traditionally, the primary outcome measure reported in scintigraphic studies is half time ($t_{1/2}$) referring to the time it takes for half the meal to empty from the stomach. Lag time or phase ($t_{lag}$) is reported in some but not all studies and is generally defined as the time it takes from meal ingestion to the onset of emptying, although the definition can vary slightly from study to study.
For studies using the $^{13}$C-OBT, when values are derived from the gastric emptying model defined by Ghoos et al (1993), $t_{1/2}$ refers to the time taken for 50% of the $^{13}$C dose to be excreted in the breath and $t_{lag}$ refers to the time taken to maximal $^{13}$CO$_2$ excretion. Using regression equations additionally defined by Ghoos and colleagues (1993), these values can be corrected to scintigraphy equivalent values of $t_{1/2}$ and $t_{lag}$.

Nevertheless, the significance of $t_{lag}$ from $^{13}$C-OBT studies using the methods defined by Ghoos and colleagues (1993) is considered by some to be controversial (Punkinnen et al 2006), as both half and lag times use largely the same formulas, making it difficult to distinguish between for example, a delayed initial emptying but an accelerated subsequent emptying. This is important as a number of studies have indicated no relationship between a prolonged lag phase and a delayed emptying (Ziessman et al 2007, Efron et al 1993). With regard to breath $^{13}$C-OBT parameters, this prompted Schommartz et al (1998) to propose the parameters latency time ($t_{lat}$) and ascension time ($t_{asc}$) in order to try and more accurately reflect the different phases of GE. $t_{lat}$ is defined as the initial delay in the cumulative $^{13}$C curve and $t_{asc}$ the time course between the latency phase and half excretion time, representing a period of high $^{13}$C excretion rates. These parameters are outlined in Figure 2.3 below.

![Figure 2.3](image)

**Figure 2.3** Representation of GE parameters similar to that depicted by Schommartz et al (1998). This graph has been modelled based on a data set collected from chapter 3 of this thesis. $t_{1/2}$ is that defined by Ghoos et al (1993) and $t_{lat}$ and $t_{asc}$ defined by Schommartz et al (1998). (The solid line represents the cumulative $^{13}$C exhalation curve. $T_{lat}$ is calculated as the intersection of the tangent at the inflection point at the x-axis).
Since the original methods for the $^{13}$C-OBT devised by Ghoos and colleagues (1993), a number of additional $^{13}$C-OBT parameters have been proposed and different methods have been used for curve fitting and calculation of the time related breath test parameters. Examples include the Viramontes method and the Wagner Nelson method by Sanaka et al 2006. However, the original gastric emptying model devised by Ghoos et al (1993) remains the most commonly used method in the literature to date and these methods along with the parameters defined by Schommartz et al (1998) are reported in experimental studies in this thesis.

2.5 Gastric Emptying in obesity

Although it has been postulated that retention of food and gastric distention serve as a signal for satiety and that rapid GE may predispose to obesity, findings from studies investigating whether GE is accelerated in obese subjects are far from conclusive. See the review paper by Clegg & Shafat (2009) for a comprehensive overview of studies that have measured GE in lean and obese individuals. Studies have shown similar (Mathus-Vliegen et al 2005; Roque-Vasquez et al 2006, Hutson et al 1993, Verdich et al 2000, French et al 1993), accelerated (Tosetti et al 1986, Wright et al 1983, Zahorska-Markiewicz et al 1986, Naslund et al 1998, Mathus-Vliegen et al 2005, Roque-Vasquez et al 2006) and delayed (Maddox et al 1989, Jackson et al 2004, Horowitz et al 1986) GE in obese compared to lean individuals (Table 2.1). This inconsistency in findings has generally been attributed to differences in methodologies and some methodological limitations.

2.5.1 Inconsistency in Findings

Some studies did not account for other factors influencing GE such as menstrual cycle or smoking (Maddox et al 1989, Horowitz et al 1986). In studies using scintigraphy to measure GE, lack of correction for Compton scatter and attenuation are limiting factors (e.g. Wright et al 1983). Information on factors known to affect GE such as prior food intake is also often not available (e.g. Verdich et al 2000). An additional varying factor between studies is the subject population tested. Roque-Vasquez et al (2006) found similar rates of solid GE in lean and obese but they did not include obese weighing more than 137kg due to safety considerations regarding gamma camera specifications. However, other studies finding accelerated rates of GE found so in the morbidly obese (Tosetti et al 1996) indicating that the severity of obesity of the subject population tested may influence findings. Varying age and sex of subjects may be another reason
for inconsistency in findings. Mathus Vliegen et al (2005) recruited 45 weight stable obese subjects and found a significantly faster rate of liquid emptying in obese compared to their reference subjects. However, their reference subjects consisted of 10 significantly younger subjects, a factor shown to influence rate of GE (Horowitz et al 1984).

Furthermore, different test meal properties between studies may contribute to the inconsistency in findings. It is evident from Table 2.1 that all studies used different test meals with radionuclide labels being added to pancakes (Mathus-Vliegen et al 2005), eggs (Roque-Vasquez et al 2006), soups (French et al 1993) and mashed potatoes (Naslund et al 1998) for example. Some studies solely measured rate of solid emptying (Wright et al 1983, Naslund et al 1998, Tosetti et al 1996, Verdich et al 2000) while others tested both solids and liquids (Hutson et al 1993, Horowitz et al 1983, Maddox et al 1989). However, no consistent pattern emerges when only comparing solid meal GE studies or liquid GE studies as faster, normal and delayed GE has been found in the obese for both liquids and solids. The size of the test meal is an important factor which may influence findings, especially when considering an obese population. The 190kcal test meal used by Wright et al (1983) would be considered very small for obese individuals. Tosetti et al (1996), on the other hand, used a 644 kcal test meal consisting of a 99m Tc-labelled hamburger and potatoes and a non-labelled crème caramel dessert. A criticism of this method, however is that it could theoretically lead to an estimation of the emptying rate of the first part of the meal rather than the overall emptying rate of the whole meal (Chiloiro et al 1999).

These studies highlight the critical importance of considering study methodologies and factors such as menstrual cycle, age, sex and weight status of subjects before generalising on study findings.

### 2.5.2 Association of Body Mass Index and GE

Interestingly, in contrast to the highly contradictory findings of studies comparing GE in lean and obese individuals, studies that have correlated GE times with BMI in subjects with BMIs in the normal to overweight range have generally demonstrated a positive association (i.e. individuals with a higher BMI have a slower rate of GE)\(^1\). Lavigne et al (1978) found a significant positive association between GE time and body surface area and between GE time and body weight. Using scintigraphy to assess GE, Brogna et al

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\(^1\) Positive association between GE **time** and BMI is sometimes referred to as an inverse association between **rate** of GE and BMI.
(1998) observed a significant positive association between GE $t_{1/2}$ and BMI in 12 healthy male adults ranging in BMI from 18.5 to 31.9 kg·m$^{-2}$ (Figure 2.4), supporting the findings of Lavigne et al (1978).

Figure 2.4: Correlation between body mass index (BMI) and gastric emptying half time ($t_{1/2}$) in 12 healthy males mean (SD) age 42±12 years (range 23-54 years) and mean (SD) BMI 26.6±4.2 kg·m$^{-2}$ (range 18.5-31.9 kg·m$^{-2}$). From Brogna et al (1998).

In a group of 62 subjects (including obese individuals), Maddox et al (1989) similarly observed a significant correlation between BMI and gastric emptying time ($r = 0.44$, $p < 0.01$), such that increasing BMI was associated with longer GE time. Horowitz et al (1983) supported these findings in obese individuals reporting that increased duration of gastric emptying lag phase was significantly correlated with excess body weight. However, no correlation between GE and body weight in the control subjects was found. More recently, using the $^{13}$COBT to assess GE in a large scale study of 90 adults, Hellmig et al (2005) observed no statistically significant correlation between GER and BMI but did observe a trend towards slower GE of solids with increasing BMI. The large variability in participant characteristics such as age and gender may account for the lack of statistical significance.

It has been suggested that the positive association between GE time and BMI may break down at extremes of the body mass spectrum (thinness and obesity) (Brogna et al 1998). This contention that GE may become deregulated at extremes of BMI represents an additional plausible explanation for the inconsistency in findings of studies comparing GER in lean and obese individuals evident in Table 2.1.
Table 2.1 The variability in findings and methodologies of studies that have compared gastric emptying in obese and lean individuals.

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Meal</th>
<th>Subjects</th>
<th>Finding in Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mathus-Vliegen et al (2005)</td>
<td>Scintigraphy</td>
<td>Pancake (410 kcal) &amp; 150ml 10% glucose drink</td>
<td>45 weight stable obese (9 male, 36 female)</td>
<td>Liquids Accelerated Solids similar</td>
</tr>
<tr>
<td>Roque-Vasquez et al (2006)</td>
<td>Scintigraphy</td>
<td>Scrambled egg, bread, milk (296 kcal)</td>
<td>73 normal, overweight or obese males &amp; females</td>
<td>Liquids Accelerated Solids similar</td>
</tr>
<tr>
<td>French et al (1993)</td>
<td>Scintigraphy</td>
<td>High and low fat soups</td>
<td>8 obese &amp; 7 lean</td>
<td>Similar</td>
</tr>
</tbody>
</table>
2.5.3 Potential Mechanisms behind altered GE in the Obese

Most experiments comparing GE in lean and obese individuals have not been designed to investigate underlying physiological mechanisms mediating differences in GE in lean and obese individuals. However, a number of theories and differences in control mechanisms have been hypothesised to try and account for why GE may be altered in obese individuals and how this may relate to food intake.

2.5.3.1 Accelerated GE

Hunt (1975) proposed that an enhanced or accelerated rate of GE, and hence a shortened satiety period might predispose to overeating and obesity. Sepple & Read (1989) demonstrated a significant correlation between postprandial increases in hunger ratings and the time for 90% of the meal to empty (r = 0.75) supporting this contention that faster GE would lead to a shorter satiety period and an increase in energy intake. Alterations in mechanisms involved in the control of GI motility have been observed in obesity further supporting the contention that GE is accelerated in the obese. Teff et al (1999) suggested that increased activity in the parasympathetic efferent simulation of the stomach might be the mechanism behind accelerated GE in obese subjects. The vagus nerve plays a critical role in the regulation of gastric emptying. Vagal afferents innervate the gastrointestinal lumen and the circular smooth muscle fibers of the gastric wall and receive information regarding the extent of gastric distension and the size and nutrient composition of the ingested meal. A decreased vagal activity has been proposed as an explanation for the slower gastric emptying that has been observed in women (Teff et al 1999). Dysfunction of the autonomic nervous system, decreased CCK sensitivity due to high fat diet (French et al 1993) and lower plasma concentrations of somatostatin and neurotensin have all been observed in obese compared to normal weight subjects (Wisen & Hellstrom 1995). These alterations in control mechanisms all favour accelerated GI transit.

2.5.3.2 Delayed GE

Jackson et al (2004) used the $^{13}$Coctanoic acid breath test to assess GE in lean and obese individuals in a study designed to minimise the effects of potential confounding factors such as age, gender and physical activity level. These factors had not been well controlled for in previous studies. In their study of 16 lean and 16 obese subjects, delayed GE in both lag time and half time measures was found in obese subjects. Reasons postulated by the authors to account for their findings included a slower distribution of the meal through the stomach (caused by a reduction in fundal tone
(Horowitz et al 1983)), altered sensitivity of the stretch receptors (Maddox et al 1989) or a change in antral area or mixing (Chiloiro et al 1999) in obese individuals. In addition, the authors postulated that delayed GE may contribute to the pathogenesis of obesity through the inactivation of GI satiety signals and a subsequent increase in food intake. They proposed that a lack of nutrients in the duodenum as a result of delayed GE lag time leads to a delay in the release of satiety signals responsible for the regulation of appetite. “Without these hormones, the feedback mechanism is interrupted, and satiety signals are not sent to the brain. Appetite is therefore unchecked and food and calorie intake may increase resulting in weight gain and obesity,” (Jackson et al 2004). Although, the contention that a delayed initial emptying exists in the obese is plausible, it is not a consistent finding as others have found an accelerated initial emptying in obese (Naslund et al 1998).

2.5.4 Summary of GE in obesity and role in food intake

Findings from the studies listed in Table 2.1 along with other prior literature (Park & Camilleri 2005) suggest no consistent effect of obesity on GE, which may be attributable to methodological inconsistencies and the possibility that GE becomes deregulated at extremes of BMI. From the literature reviewed, the complex role of changes in gastric emptying on satiation, satiety and consequently food intake is also evident.

It is evident that a delayed GE prolongs satiety (Sepple & Read 1989) by prolonging the retention of food in the stomach and thus increasing and prolonging distention (Rayner et al 2000). However, the initial interaction of nutrients with the small intestine is critical to the release of satiety signals (Jackson et al 2004) and the inhibition of GE (Meyer et al 1994, Meyer et al 1999). Therefore, while a delayed GE may serve to increase satiety by prolonging distention, it also delays the onset of satiety signals initiated by delivery of nutrients to the small intestine. As findings are equivocal regarding the role of GE in obesity and plausible mechanisms have been put forward for both accelerated and delayed GE to explain an increased food intake in the obese, further well-controlled, validated studies are needed to clarify the potential role of altering the stomach's function as a means of controlling food intake (Park & Camilleri 2005). The following section will discuss the role of exercise as an intervention strategy which may alter gastric emptying and consequently influence food intake.
2.6 Can Exercise Alter Gastric Emptying?

2.6.1 Introduction

Reports of the first experiment investigating the effect of exercise on GE dates back to the 13\textsuperscript{th} Century and a study conducted by Holy Roman Emperor Frederick II. His experiment involved feeding two prisoners, ordering one to do strenuous exercise and the other to bed. The men were then killed and disembowelled, and Frederick observed that the stomach of the rested man was empty, whereas that of the exercised man was full (McDowall 1955). This finding that strenuous exercise delayed GE was confirmed by the early 20\textsuperscript{th} century studies of Hellenbrandt & Tepper (1934) and Campbell (1928) albeit using different experimental methods to that of Frederick, involving barium impregnated meals and aspiration of gastric contents respectively. These studies also found moderate exercise to accelerate GE. Since these early studies, the relationship between GE and exercise has been widely investigated. However, the majority of studies are limited to the acute exercise setting, by researchers looking to enhance performance during exercise. GE is thought to be the major determinant of the availability of ingested drinks during exercise (Maughan 1991) and is therefore important for enhancing fluid homeostasis and thermoregulation as well as supplementing CHO stores during exercise (Ryan 1998).

The aim of this section is to review the research available concerning the relationship between exercise and GE. The experimental studies in this thesis are focused on chronic exercise. As there is limited research to date on this topic, acute exercise studies will first be reviewed as this may yield further information regarding mechanisms behind how GE might be altered with exercise and how this may relate to changes in food intake.
### 2.6.2 Acute Exercise and Gastric Emptying

Table 2.2 illustrates study characteristics and outcomes from studies that have investigated the effect of an acute bout of exercise on GE.

**Table 2.2 Studies that have investigated the effect of acute exercise on GE**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Technique*</th>
<th>Meal</th>
<th>Methods</th>
<th>Intervention</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campbell (1928)</td>
<td>-</td>
<td>GE, nasogastric tube</td>
<td>Solid meal</td>
<td>Pre</td>
<td>Moderate &amp; strenuous ex</td>
<td>Mod: Accelerated&lt;br&gt;Stren: Delayed</td>
</tr>
<tr>
<td>Hellenbrandt &amp; Tepper (1934)</td>
<td>-</td>
<td>GE</td>
<td>Solid</td>
<td>Pre</td>
<td>Moderate &amp; strenuous ex conditions</td>
<td>Mod: Accelerated&lt;br&gt;Stren: Delayed</td>
</tr>
<tr>
<td>Feldman &amp; Nixon (1982)</td>
<td>5 Untrained males &amp; 1 female</td>
<td>GE, nasogastric tube</td>
<td>Solid</td>
<td>Pre</td>
<td>45 min rest; 45 min cycle at 50% or 70% VO\textsubscript{2max}</td>
<td>Mod: Unchanged&lt;br&gt;Stren: Unchanged&lt;br&gt;Stren: Delayed</td>
</tr>
<tr>
<td>Fordtran &amp; Saltin (1967)</td>
<td>4 males &amp; 1 female</td>
<td>Liquid</td>
<td>During</td>
<td>1 hr rest or 1 hr treadmill exercise at 71% VO\textsubscript{2max}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Costill &amp; Saltin (1974)</td>
<td>15 males</td>
<td>GE, nasogastric tube</td>
<td>Liquid</td>
<td>Pre</td>
<td>Cycle exercise at 40-70% VO\textsubscript{2max} and rest</td>
<td>40-70% VO\textsubscript{2max}: Unchanged&lt;br&gt;Stren: Delayed compared to lower intensities</td>
</tr>
<tr>
<td>Ramsbottom &amp; Hunt (1974)</td>
<td>6 subjects</td>
<td>GE</td>
<td>Glucose</td>
<td>Pre</td>
<td>20 min Cycle ergometer ex</td>
<td></td>
</tr>
<tr>
<td>Keeling et al (1990)</td>
<td>21 females</td>
<td>MCTT, expired H\textsubscript{2}</td>
<td>Liquid</td>
<td>Pre</td>
<td>Rest or treadmill walk at 5.6 km/hr up 2% grade</td>
<td>Mod: Accelerated</td>
</tr>
<tr>
<td>Neufer et al (1986)</td>
<td>21 male and 4 female trained runners</td>
<td>GE, nasogastric tube</td>
<td>Liquid</td>
<td>Pre</td>
<td>15 min treadmill running at 50-70% VO\textsubscript{2max} or 15 min rest</td>
<td>Mod: Accelerated</td>
</tr>
<tr>
<td>Neufer et al (1989a)</td>
<td>10 physically fit males</td>
<td>GE, nasogastric tube</td>
<td>Liquid</td>
<td>Pre</td>
<td>Six 15 min exercise bouts (3 walking &amp; 3 running at different intensities) and 15 min rest</td>
<td>Mod: Accelerated&lt;br&gt;Stren: Delayed compared to lower intensities.</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Methodology</td>
<td>Type</td>
<td>Phase</td>
<td>Protocol</td>
<td>Findings</td>
</tr>
<tr>
<td>-------------------------------</td>
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<td>--------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Neufer et al (1989b)</td>
<td>10 physically fit males</td>
<td>GE, nasogastric tube</td>
<td>Liquid</td>
<td>Pre</td>
<td>Five 15 min bouts treadmill exercise ~50% VO$_2$-max, at 18˚,35˚,49˚ C euhydrated and hypohydrated.</td>
<td>Mod + hot environment: Delayed compared to neutral environment. Mod + hypohydrated: Delayed Unchanged</td>
</tr>
<tr>
<td>Carrio et al (1989)</td>
<td>10 male marathon runners</td>
<td>GE, scintigraphy</td>
<td>Solid</td>
<td>Pre</td>
<td>During 90 min rest. During 90 minute run at 4-4.5 mins/km covering 20-22.5 km</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Rehrer et al (1989)</td>
<td>8 trained cyclists, 8 untrained</td>
<td>GE, nasogastric tube</td>
<td>Solid</td>
<td>Pre</td>
<td>80 min rest, 80 min cycle exercise at 50% Wmax, 80 min cycle exercise at 70% Wmax. Untrained cycle conditions lasted 60 min.</td>
<td>Mod &amp; Stren: Delayed initial emptying of CHO Untrained vs Trained: Unchanged</td>
</tr>
<tr>
<td>Rehrer et al (1990)</td>
<td>15 male trained runners</td>
<td>GE, nasogastric tube</td>
<td>Liquid</td>
<td>Pre</td>
<td>4 conditions, dehydration and rest, dehydration and ex., euhydration and rest, euhydration and ex. 40 min ex. or rest following 2hr euhydration or dehydration regime.</td>
<td>Dehydration: Delayed Dehydration + Ex: Delayed</td>
</tr>
<tr>
<td>Van Nieuwenhoyen et al (1999)</td>
<td>10 well trained males</td>
<td>GE, $^{13}$C acetate breath test, MCTT, Breath H$_2$</td>
<td>Liquid</td>
<td>Pre</td>
<td>2 conditions: rest, rest or rest, ex, rest at ratio 6 min:90min: 210min. Ex: 70% Wmax on a cycle ergometer. GE measured from t=40 to t = 90.</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Cammack et al (1982)</td>
<td>1 male, 6 females</td>
<td>GE, scintigraphy, MCTT, Breath H$_2$.</td>
<td>Solid</td>
<td>Pre</td>
<td>2 conditions: 1) Rest; 2) cycle ergometer pedalling at 33rpm for 5 out of 10 mins for a total of 6 hours.</td>
<td>Mod: Accelerated GE, Unchanged MCTT</td>
</tr>
<tr>
<td>Moore et al (1990)</td>
<td>10 males</td>
<td>GE, scintigraphy</td>
<td>Solid</td>
<td>Pre</td>
<td>3 conditions: 1) stood at rest, 2) walked on treadmill at 3.2 km/hr or 3) at 6.4 km/hr</td>
<td>Mod: Accelerated (6.4 km/hr treadmill speed sig. faster t$_{1/2}$ than 3.2 km/hr)</td>
</tr>
<tr>
<td>Marzio et al (1991)</td>
<td>17 subjects</td>
<td>GE, ultrasonography or scintigraphy</td>
<td>Liquid</td>
<td>Post</td>
<td>3 conditions: 1) rest 2) 30 min treadmill exercise at 50% HR$<em>{max}$ and 3) at 70% HR$</em>{max}$</td>
<td>Stren: Delayed Mod: Accelerated</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Method</td>
<td>Interventions</td>
<td>Outcomes</td>
<td></td>
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<td>------------------------</td>
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<td>-----------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Houmard et al (1991)</td>
<td>10 well trained male and tri and bi-athletes</td>
<td>GE, nasogastric tube</td>
<td>Liquids: Water &amp; CHO. During 60 min rest or 60 min cycle ergometer or 60 min treadmill at 75% mode specific VO$_2$max.</td>
<td>Stren: Water Delayed, CHO unchanged. Running vs Cycling: Unchanged.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mudambo et al (1997)</td>
<td>18 male soldiers</td>
<td>GE, $^{13}$C acetate breath test</td>
<td>Liquid. During, Post 6 conditions: 1) rest 2) rest &amp; extra fluid 3) ex 4) ex &amp; extra fluid 5) recovery 6) recovery &amp; extra fluid. Ex consisted of 3 hr walk/run over 16km obstacle course in the heat (39°C).</td>
<td>During: Ex + fluid: Accelerated Post Ex: Delayed compared to rest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leiper et al (2001)</td>
<td>8 males</td>
<td>GE, orogastric tube</td>
<td>Liquid. Pre 3 conditions: 1) 60 min rest, 2) 60 min steady state cycle ergometer ex at 66%VO$_2$max or 3) during intermittent ex at 75%VO$_2$max.</td>
<td>Stren: Delayed compared to other conditions.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clegg et al (2007)</td>
<td>8 recreationally trained males</td>
<td>GE, $^{13}$C octanoic acid breath test. MCTT, Breath H$_2$</td>
<td>Solid. Post 1 hour cycle ergometer moderate exercise at 60% predicted HRmax.</td>
<td>Mod: Unchanged.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GE: Gastric Emptying, MCTT: Mouth to Caecum Transit Time, CHO: carbohydrate, Mod: Moderate (<70% VO$_2$max), Stren: Strenuous (≥ 70% VO$_2$max), Ex: Exercise, HRmax: maximum Heart Rate, -: Information not located.

* Orogastric, nasogastric tube: refers to different intubation techniques for either single or serial time point aspiration methods or the double sampling aspiration technique for GE measurement.

** Time Ingested in relation to exercise or rest condition.
In discussing the aetiology of gastrointestinal disturbances during endurance events, Brouns (1991) states that GE “is not influenced at moderate exercise intensities, but will be inhibited at maximal exercise intensities or during a state of dehydration”. This contention that GE is (a) inhibited at higher exercise intensities, is supported by the studies of Neufer et al (1989) and Rehrer et al (1989), (b) inhibited during a state of dehydration by Rehrer et al. (1990) and (c) unchanged at moderate intensities by the studies of Feldman & Nixon (1982), Costill & Saltin (1974), Ramsbottom & Hunt (1974), Van Nieuwenhoyen (1999), Houmard (1991) and Clegg et al (2007).

However, studies are not unanimous on contention (c) that GE is unaffected by lower intensity exercise. A number of studies have in contrast demonstrated accelerated GE at moderate intensity exercise (Campbell 1928, Hellenbrandt & Tepper 1934, Keeling et al 1990, Neufer et al 1989, Neufer et al 1986 (Figure 2.5), Cammack et al 1982).

Figure 2.5 Data from Neufer et al (1986) (adapted by Foster 1994) demonstrating the increase in GE with moderate exercise (50-70% VO2max) for both water (H2O) and carbohydrate drinks containing maltodextrin (MD), glucose (G), and fructose (F). The slower emptying of glucose is also evident in this data.

Moses (1990), in reviewing the effect of exercise on the GI tract, states that “It is generally accepted that light exercise accelerates liquid emptying, vigorous exercise delays solid emptying and has little effect upon liquid emptying until near exhaustion”.

As Table 2.2 indicates the inconsistency in findings may be attributable to the wide variability of methodologies employed by researchers. Along with different techniques used for measuring GE and the type of subject population used, there are a number of factors which may account for this variability in findings. Moses (1990) lists
calorie count, meal osmolality, meal temperature and exercise conditions as factors impacting GE during exercise.

2.6.2.1 Meal Properties
Numerous studies have highlighted the influence of test meal properties on GE (see Section 2.2.2). Meal size and composition, including nutrient concentration, osmolality and particle size, are all strong modulators of GE (Rehrer & Gerard 2000). Increasing the CHO content will generally increase the energy delivery to the duodenum (Hunt & Knox 1968, Hunt & Stubbs 1975, Foster et al 1980). Gastric volume is influenced by the pattern of meal ingestion, a factor which can markedly affect GE of both fluids and CHO (Noakes et al 1991). While the majority of studies in Table 2.2 involve ingestion of a single bolus, others involve a serial pattern of fluid ingestion (Houmard et al 1991). A serial pattern of fluid ingestion of large volumes, leading to a greater degree of gastric distention will lead to a faster GER. Table 2.2 illustrates the wide variability that exists in meal properties used.

Two studies finding GE to be unchanged during moderate intensity exercise involved the ingestion of substantial solid test meals (Feldman & Nixon 1982, Clegg et al 2007), suggest that moderate intensity exercise may only accelerate liquid emptying as postulated by Moses (1990). However, this is not supported by Cammack et al (1982) who found moderate exercise to significantly accelerate emptying of a sausage, beans, and potato meal and Moore et al (1990) who found moderate intensity exercise to accelerate GE of a beef stew meal. The test meal was ingested 45min prior to exercise in the Feldman & Nixon (1982) study therefore the effect of exercise on initial emptying was not examined. In Clegg et al (2007), the test meal was particularly high in fat (68% energy) and as a result the meal composition may have been an overriding factor in inhibiting the effects of exercise on GE.

2.6.2.2 Timing of Meal Ingestion
Another variable factor in study methodologies is the timing of meal ingestion. Test meals were ingested immediately prior to exercise or during exercise in the majority of studies listed in Table 2. Four studies involved subjects ingesting the test meal immediately post exercise, among which 3 found exercise to slow subsequent GE (Leese et al 1995, Mudambo et al 1997, Marzio et al 1991). In the studies of Leese et al (1995) and Marzio et al (1991), exercise consisted of treadmill exercise at 70%VO$_2$$_{max}$, while in Mudambo et al (1997) the exercise condition consisted of a 3hr walk/run.
These 3 studies involved the ingestion of liquid meals and a higher intensity exercise to that of Clegg et al (2007), who found no difference in GE of a high fat pancake meal ingested after 60% HR_{max} cycle exercise. Marzio et al (1991) in addition demonstrated that treadmill exercise at 50%HR_{max}, resulted in a faster GE of a meal ingested immediately post exercise. These studies, showing similar findings to those that measured GE during exercise (delayed GE after higher intensity exercise, accelerated GE at lower intensities) suggest that exercise induced changes in GE are not solely due to the mechanical “bouncing” of fluid in the stomach during exercise, as altered GE also appears to carry over to the post exercise recovery period. Therefore, exercise appears to have similar effects on GE irrespective of the timing of meal ingestion (i.e. immediately before, during or after immediately exercise).

2.6.2.3 Exercise Conditions
The exercise conditions including duration, intensity and type of exercise may also account for some of the variance in findings. The exercise bout in Cammack et al (1982) involved prolonged intermittent exercise over a 6 hour period whereas other studies have involved an acute exercise bout of as little as 10 minutes (Brown et al 1994). Moore et al (1990) found that walking at 6.2km/hr significantly accelerated emptying compared to walking at 3.2km/hr, leading the authors to conclude that the amount of exercise is a physiological factor that alters solid meal GE rates. In general, accounting for the variability in study methodologies, the findings with regard to intensity of exercise are consistent with those of Emperor Frederick, suggesting that GE of liquids and solids appear to be unchanged or slightly accelerated at lower exercise intensities while above 70% VO2max, GE is certainly delayed.

2.6.2.4 Mechanisms
Various mechanisms which may account for the changes in GE observed with exercise have been speculated upon since the time of Emperor Frederick.

The increased availability of non-invasive methods has permitted further investigation into the effects of exercise on gastric contractility. Brown et al (1994) used ultrasound imaging to investigate the role that the pylorus and antral area may play in the delay in GE observed with strenuous exercise. Contraction frequencies and antral areas were significantly reduced after 10 minutes cycling at 85% VO2max compared to the non-exercise condition. Additionally, closure of the pylorus and tubular narrowing of the gastric antrum was reported after exercise. The authors concluded that these
alterations may be important in explaining the slowed GE that occurs with strenuous exercise.

Two studies in recent years have used electrogastrography (EGG) to investigate changes in gastric electroactivity that may explain changes in GE occurring at moderate intensity exercise (Lu et al 2000, Kato et al 2004). EGG is a non-invasive method of measuring gastric myoelectrical activity, involving the placement of electrodes on the abdominal skin. Gastric myoelectrical activity is composed of electrical control activity (slow waves) and electrical response activity (spike potentials). Slow waves are considered to determine the maximal frequency of gastric contractions, while antral contractions have been directly associated with spike potentials superimposed on the slow wave (Jebbink et al 1994). Lu et al (2000) and Kato et al (2004) both demonstrated more powerful and stable postprandial gastric myoelectrical activity following exercise compared to rest and suggested that this may partially explain the accelerated GE that has been observed after light to moderate exercise. Although contractile activity in the stomach and pylorus is known to be controlled by a complex integration of neural (mainly vagal in origin) and hormonal factors (Meyer et al 1987), the exact mechanisms underlying the enhanced EGG by exercise are unknown (Kato et al 2004). Nevertheless, changes in gastric myoelectrical activity represent a plausible explanation for alterations in GE with exercise as a growing body of evidence has suggested an association between abnormalities in gastric myoelectrical activity and gastric motor dysfunction (Hasler et al 1995, Kohagen et al 1996, Lin et al 1999).

2.6.2.4.1 Stress Response

It has been proposed that one mechanism whereby exercise influences GE is through some aspect of a stress response to exercise (Keeling et al 1990, Brouns et al 1991). Both physical and mental stresses are known to alter GI function (Barclay & Turnberg 1988) and therefore emotional or mental strain induced by exercise may alter GI function during exercise (Brouns 1991). Cannon (1929) suggested that the variable physiological responses to psychological stress depend on the relative dominance of parasympathetic or sympathetic tone. Cammack et al (1982) further suggested that the same applies to the physical stress of exercise. This contention is supported in relation to exercise by the findings of Robinson et al (1966), who showed that “withdrawal of parasympathetic restraint on the sinoatrial node” occurred early in exercise whereas sympathetic activity increased later and became more pronounced as the exercise intensity increased. The accelerated GE observed with moderate intensity exercise may
therefore be partly attributable to a dominant parasympathetic tone, and the delay in GE observed with strenuous exercise to a dominant sympathetic tone.

2.6.2.4.2 Heat Stress
The indirect effect of heat stress on GE during exercise may also play a role in altering gastric function during exercise. Mudambo et al (1997) found exercise to accelerate GE during a 3-hour walk/run in soldiers when extra fluid was ingested during exercise. Rehrer et al (1990) and Neufer (1989) similarly highlighted the role that hydration status may have in altering GE during exercise. Both studies found GE to be slowed when exercise and dehydration were combined. Rehrer et al (1990) investigated GE during 4 conditions; at rest and during exercise, following a euhydration or dehydration regime. Although dehydration at rest slowed GE, the dehydration-exercise condition slowed GE significantly compared to all other conditions. The authors concluded that the effects of dehydration and exercise were additive in slowing GE. Collectively these findings clearly indicate that both dehydration and exercise slow GE.

2.6.2.4.3 GI Blood Flow
One mechanism whereby exercise and dehydration may both impact GE is through a decrease in gut blood flow, resulting in a slowed GE (Neufer et al 1989a). Blood flow is instead directed to skeletal muscle during exercise and into the central blood volume (Rowell et al 1964). Konturek et al (1973) suggested that GI blood flow may decrease by more than 50% during exercise, while a reduction in splanchnic blood flow of 80% has been reported during maximal exercise compared to rest (Clausen 1977). Reduced splanchnic blood flow may cause a reduction in intestinal absorption leading to a build up of nutrients in the intestine (Neufer et al 1989), thereby triggering inhibitory feedback mechanisms to slow GE (Minami & MacCallum 1984, Rehrer et al 1994). Limited GI blood flow may also result in a diminished availability of oxygen and energy sources (Moses 1990) and this local hypoxia of the GI tract may result in enhanced secretion of some gastroenteropancreatic hormones (discussed below) and subsequently delayed GE (Brouns 1991).

2.6.2.4.4 Hormonal Mechanisms
As GI function is partially under hormonal control, exercise induced changes in GI hormones and peptides may play a role in altered GE with exercise. Many of the physiological effects of exercise are thought to be mediated by catecholamines (Bannister et al 1972, Galbo et al 1975), hormones known to delay GE (Jenkinson et al
1967, Vizi et al 1969, Rees et al 1980) and therefore may be partially responsible for the delayed GE observed with strenuous exercise.


Table 2.3 Gut Hormones involved in the regulation of nutrient ingestion. From Crowell et al (2006)

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Origin</th>
<th>Site of Action</th>
<th>Physiological Actions</th>
<th>Effects on Satiety</th>
<th>Effects on Hunger</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK</td>
<td>L cells of upper small intestine</td>
<td>CCK-A receptors on the gallbladder, pancreas, smooth muscle, and peripheral nerves</td>
<td>Stimulates gallbladder contraction and pancreatic secretion</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>Stomach, most abundant in oxyntic cells</td>
<td>Growth hormone secretagogue receptor, most prominent in the pituitary and hypothalamus</td>
<td>Growth hormone release, Possible prokinetic effect on the stomach</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Peptide YY</td>
<td>L cells in small intestine and colon</td>
<td>Neutrophil YY2 receptors (Y2R) in hypothalamus</td>
<td>Inhibits vagally stimulated gastric acid secretion and motor function</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>GLP-1 and -2</td>
<td>L cells mainly in jejunum, ileum, and colon</td>
<td>GLP receptors (member of the class II G-protein-coupled receptors) in CNS, GI tract, and pancreas</td>
<td>Stimulates insulin secretion, suppresses glucagon secretion, inhibits gastric emptying and small bowel motility</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>GIP</td>
<td>K cells in the duodenum and proximal jejunum</td>
<td>Secretin/VIP family of receptors found on duodenal, terminal ileal, pancreas, stomach, brain, and small intestine</td>
<td>Increases nutrient absorption and triglyceride synthesis, Increases intestinal secretion</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>CRF</td>
<td>Hypothalamus</td>
<td>CRF1 and CRF2 receptors in the stomach, colon, adipocytes, and brain</td>
<td>Inhibits gastric emptying, stimulates food- and meal-initiated activity</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Neuropeptide</td>
<td>Hypothalamus</td>
<td>CRF2 receptors in the stomach, intestine, and brain</td>
<td>Inhibits gastric emptying</td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>

Hilstead et al (1980) reported a significant increase in fasting PP levels after 3 hours of cycle exercise at 40% VO₂max compared to the resting condition in male marathon runners. Greenberg et al (1986) showed similar findings after 45 min cycle ergometer exercise at 50% VO₂max, but in the postprandial state. PP is known to inhibit GE, decrease hunger and increase satiety (Crowell et al 2006), therefore changes in the release of this peptide may be involved in both changes in GE and changes in food intake with exercise. Observations that GE is unchanged or accelerated with mild to
moderate exercise however is paradoxical as PP is known to delay GE, suggesting that other peptides or mechanisms may have an overriding influence.

The same authors recently reported increased GLP-1 fasting levels during a 2 hour treadmill run compared to rest (O’Connor et al 2006). Martins et al (2007) found GLP-1 to also be altered by exercise in the postprandial state. Normal weight sedentary men and women participated in 1 hr cycle exercise at 65% VO2max. Along with GLP-1, both PP and PYY levels were found to be significantly increased by exercise, compared to a resting condition. Of note is that all three peptides are known to inhibit GE. Ghrelin, an orexigenic hormone, on the other hand was reported to be unaffected by exercise in the same study.

Increased CCK levels (another hormone known to inhibit GE) have also been reported after an acute exercise bout. Bailey et al (2001) found that a maximal exercise test increased CCK from 10.7 ± 4.5 to 50.6 ± 34.7 pmol/l (p < 0.05) when compared to rest. Sliwowski et al (2001) reported CCK and gastrin to be increased after maximal exercise in both fasted and fed conditions and that CCK remained elevated at 2 hours post exercise compared to basal levels. The authors postulated that this may be due to an alteration in GE and gastric adaptive relaxation but that this requires further study. Nagamatsu et al (2003) investigated the effects of 30minutes moderate intensity (60% VO2max) cycling on GE and CCK using positron emission tomography to measure GE. Slower GE along with increased CCK was reported during exercise compared to control. The authors concluded that the depressive effect of exercise on GE may be partially influenced by CCK. CCK is known to inhibit GE, decrease hunger and increase satiety (Crowell et al 2006). This hormone may therefore have a critical role in the delayed GE that has been observed with strenuous exercise.

Collectively, the evidence strongly implicates changes in hormonal mechanisms as a contributing factor to altered GE with exercise. Furthermore, many of these peptides are known to influence satiety and hunger sensations and therefore the complex interactions of alterations in hormones involved in the regulation of nutrient ingestion and concomitant changes in GE may contribute to changes in food intake with exercise.

2.6.2.4.5 Mechanical Effects

In considering the possible mechanisms that may alter GE with exercise, the simple mechanical effects of the “bouncing of the gut” during exercise must not be overlooked, and this has been considered by several researchers (Neufer et al 1986, Neufer et al
1989, Leiper et al 2001, Rehrer 1991). Since the magnitude of accelerations of the body are more than twice as high during running compared to cycling, it could be expected that this would result in an increased GER during running (Rehrer & Meijer 1991). Neufer et al (1986) suggested that the actual movement of fluids around the pylorus during treadmill exercise may contribute to an enhanced GER. In addition, the authors suggested that the contractile activity of the abdominal muscles during treadmill exercise enhances movement of the stomach, thereby increasing intragastric pressure and GE. This theory would mean that when the abdominal muscles are more relaxed, GE would be expected to be similar to at rest and is supported by the studies of Costill & Saltin (1974) and VanNieuwenhoven (1999) finding GE to be unchanged by moderate intensity cycle exercise compared to rest. However, this is not a consistent finding, as others have found GE to be accelerated by varying intensities of cycle exercise (Cammack et al 1982, Lieper et al 2001, Brown et al 1994, Rehrer et al 1989). Two studies have since directly compared the effects of running vs cycle exercise, showing no difference in GER to exist due to mode of exercise (Houmard et al 1991, Rehrer et al. 1990b). Leiper et al (2001) pointed out that despite the limited upper body movement during cycling, intra-abdominal pressure may still be increased due to the greater recruitment of abdominal muscles to aid respiration during bouts of intense exercise (Aliverti et al 1997), which may partly account for the findings of similar GE during cycling and running. The evidence thus remains equivocal as to the impact of the bouncing of the gut during exercise on GI function and GE specifically.

2.6.2.5 Summary
In summary, GE of liquids and solids appears to be unchanged or accelerated by an acute bout of moderate intensity exercise, while at intensities above 70% VO$_2$max the evidence suggests GE is certainly delayed. Although a number of mechanisms have been suggested to account for these findings, they remain poorly understood. For example, increased catecholamines (known to delay GE) do not explain the findings of increased GER during moderate intensity exercise, while the mechanical effects of exercise resulting in an enhanced emptying do not account for the delayed GE observed with strenuous exercise. The different mechanisms may therefore have roles of varying relative importance depending on certain aspects of experimental methodology such as the test meal or exercise conditions. For example it could be postulated that during intense exercise elevated catecholamine levels (Galbo et al 1983) may override the mechanical advantage in GE that has been associated with more moderate intensity

2.6.3 Does Gastric Emptying Adapt to Chronic Exercise?

2.6.3.1 During Exercise

Brouns and Beckers (1993) proposed that the GI tract does not adapt to the physiological stresses imposed by exercise – that is it does not respond to chronic exercise with enhanced function or structural changes. Various studies that have found GE to be altered by exercise in trained individuals (Neufer (1986, 1989), Rehrer (1989, 1990) in Table 2.2) support this contention. However, other evidence suggests that a training adaptation does occur. Van Nieuwenhoven et al (1999) found GE to be unaltered during exercise in 10 well trained males. Carrio et al (1989) similarly observed no change in GE during an acute bout of running in marathon runners when compared to rest. It is possible therefore that an adaptation to regular exercise maybe one explanation for these findings. Further study is needed to examine whether any adaptation in GE during exercise exists in regular exercisers and to examine the influence of training mode and intensity.

2.6.3.2 At Rest

Carrio and colleagues (1989) additionally observed that the marathon runners in their study had a faster basal rate of GE of an egg and bread meal compared to a group of sedentary controls (Figure 2.6), and considered this a direct effect of training on neural control of the stomach. With athletic training the resting bradycardia is caused by a decrease in the influence of cardiac sympathetics on the pacemaker with no change in the vagal influence (Badder 1975) thus producing a greater parasympathetic predominance. Carrio et al suggested that this enhanced parasympathetic tone as a result of athletic training may be one explanation for their findings of accelerated GE in marathon runners. Further the authors suggested that low basal catecholamine concentrations in athletes may be involved, as these hormones are known to delay GE.
Figure 2.6 Mean (SD) % gastric retention values for the solid component of the meal at 30 min intervals over 90 min following meal ingestion. Comparison between sedentary individuals and marathon runners. From Carrio et al (1989) p. 153.

Shimamoto et al (2002) using the $^{13}$C acetate breath test to assess GE of a 250ml liquid meal at rest in 7 active and 7 inactive elderly individuals found that time to peak $^{13}$CO$_2$ excretion was more delayed in inactive compared to active individuals. Reduced postprandial gastric myoelectrical activity in inactive individuals was proposed by the authors as a potential mechanism which may account for their findings. However, the authors point out that a limitation of their study was that the 2 groups were not accurately matched for age and this may also have contributed to their findings.

Harris et al (1991) observed a faster orocecal transit time (OCTT) in more active individuals. However, the association in their study was drawn between differing energy intakes associated with varying PA levels and OCTT. They observed a significant negative association between OCTT and daily EI in men representing a wide range of PA levels and energy intakes. OCTT of a liquid meal ranged from ~200min to below 50min for 2 individuals with daily energy intakes of ~1300 and ~4500 kcal/d, respectively. In the same study, no difference in intestinal absorption was observed, leaving the authors to conclude that the hyperphagia associated with chronic exercise is linked to an apparent difference in digestive strategy: food transit to the colon is more rapid, without compromised absorption. These findings suggest that changes in OCTT with regular PA may be secondary to an alteration in diet. However, in interpreting these findings in relation to GE it must be considered that OCTT includes both GE and small intestinal transit times, and therefore a faster OCTT may not necessarily reflect a faster rate of GE.
A limitation of the previous studies discussed assessing the relationship between chronic exercise and GE or OCTT is that they are cross sectional and therefore cognitive or lifestyle factors associated with regular exercise may contribute to results. Few intervention studies exist. Koffler et al (1990) investigated the effects of a 13 week total body strength training intervention in untrained elderly men and observed no change in OCTT. Cordain (1986) investigated the effect of a 6 week aerobic training programme on gastrointestinal transit time (GITT) in 9 untrained men and 8 controls and observed a significantly faster GITT in the exercise group and no significant change in the control group. The authors proposed mechanical jarring during running, enhanced parasympathetic tone and changes in hormonal regulation as potential mechanisms which may account for their findings. GITT refers to the whole time it takes for solids or liquids to move through the digestive system and therefore a major limitation in considering GITT studies in relation to GE is that over 90% of GITT is within the large intestine (Emmanuel & Roy 2007). Nevertheless, the mechanisms proposed by the authors to account for the changes in their study may also be responsible for an adaptation in GE with chronic exercise, as the same mechanisms have been speculated to account for changes in GE with acute exercise.

No intervention studies could be found that have investigated the effect of chronic exercise intervention on GE.

2.6.4 Effects of Exercise on Appetite, Food Intake and Relation to GE

2.6.4.1 Acute Exercise
A number of studies investigating the effects of exercise on appetite sensations have observed a marked suppression of hunger following intense exercise > 60% VO₂max (Thompson et al 1988, Kissileff et al 1990, King et al 1996, Westerterp-Plantenga et al 1997), a phenomenon that has been termed ‘exercise induced anorexia’. Even with a large increase in exercise induced energy expenditure of 4.6 MJ in one single day, no compensatory increase in energy intake tracked over the following 2 days has been observed (King et al 1997). Although several mechanisms have been proposed to explain ‘exercise induced anorexia’, the exact reasons behind this phenomenon remain unknown (Martins et al 2007). It is evident from the acute exercise studies reviewed in this thesis that there is a consistent finding of delayed GE with vigorous exercise. Delayed GE by increasing and prolonging gastric distension could maintain the sensation of satiety for longer, delaying the desire to eat and therefore may well be
implicated in the phenomenon of exercise induced anorexia. Kissileff et al (1990) found significantly less EI after high intensity exercise compared to moderate intensity exercise in non-obese women after 40 minutes cycling. Others have similarly found no effect of moderate intensity exercise on hunger scores or EI (Imbeault et al 1997, Pomerleau et al 2004). Observations of no alterations in hunger or appetite with moderate intensity exercise further support the contention that changes in GE may play a role in mediating changes in EI with exercise as in the literature reviewed moderate intensity exercise has been shown to either accelerate or have no effect on GE.

However it must be acknowledged that these findings are not unanimous. Others have shown a suppression of hunger during 60 min cycle exercise at 65% VO$_{2}$max (Martins et al 2007), an intensity of exercise which has been found by others to accelerate GE (Neufer et al 1986). Similarly, vigorous exercise has not always resulted in a suppression of hunger during exercise (e.g. Maraki et al 2005). Furthermore, it is important to note that significantly decreased hunger sensations with exercise have not always translated into concomitant reductions in energy intake as the suppression of hunger has generally been demonstrated to be transient (i.e. only increasing during and for a short period after intense exercise) (Thompson et al 1988, King et al 1995, 1997). Others have shown changes in energy intake despite no significant changes in appetite sensations. Pomerleau et al (2004), for example observed an increase in energy intake following high intensity (70% VO$_{2}$max) exercise but no change in hunger scores. Differences in cognitive factors such as the common behaviour of using food as reward for exercising (Martins et al 2007) may be one explanation. Furthermore, as evident in the review of acute exercise and gastric emptying studies, differences in methodologies such as whether exercise was performed in the fasted or fed state, the timing of measurement of the parameters of interest and exercise conditions such as intensity of exercise may account for some of the variability in findings. In addition, gender of subjects is a contributing factor to differing changes in energy intake with exercise as evidenced and discussed in detail by Hagobian & Braun (2010) in their recent review. Findings of no change in food intake following strenuous exercise in obese women but a reduced food intake in lean women (Kissileff et al 1990) further suggests that individual characteristics play a role in mediating changes in food intake with exercise. Earlier work has also shown that obese individuals tended to be less responsive than non-obese individuals to physiological challenges to energy balance regulation in a variety of circumstances (Spitzer et al 1981).
Collectively these data suggest that vigorous exercise is associated with a transient suppression of hunger while exercise at lower intensities has little effect on hunger sensations. Gastric emptying may be one mechanism contributing to these changes. Subject characteristics and other methodological aspects are a potential confounding factor mediating the differing responses in terms of appetite and food intake that have been observed with exercise.

2.6.4.2 Chronic Exercise

In terms of changes in daily EI with chronic exercise, cross sectional studies have demonstrated that active individuals compensate to match their higher EE with higher EI (Horton et al 1994, Maughan et al 1989, Saris et al 1989). However, the cross sectional nature of these studies provide no insight into the temporal pattern of adaptation and therefore such changes may only occur after a long period of regular exercise. In reviewing the effects of chronic exercise intervention on energy intake Blundell & King (1999) reported 65% of studies reviewed to show no change in EI after exercise and only 19% showed an increase. Whybrow et al (2008) concluded that in contrast to acute and short term exercise intervention studies, as exercise continues day to day EI begins to track EE over the course of 1-2 weeks. However, it is essential to recognise that not all individuals respond the same as research has shown a large inter-individual variability in EI response to 12 weeks of supervised exercise (King et al 2008).

Cross sectional (Long et al 2002) and intervention studies (Martins et al 2007) have demonstrated an increased accuracy of short term regulation of food intake in response to preloads of different energy content. These findings suggest that exercise may have a beneficial effect on appetite regulatory processes, and changes in insulin and satiety signalling sensitivity have been proposed as potential mechanisms. See Martins et al (2008) for a review.

To further understand mechanisms mediating changes in EI with chronic exercise, in recent years studies have examined changes in peptides implicated in appetite control with exercise. Decades of investigations have documented CCK as one of the most biologically potent satiety peptides controlling food intake (Moran et al 2004). Hirschberg et al (1994) showed that female long distance runners at rest demonstrate a reduced CCK response to a mixed meal compared to age, sex and weight matched controls. Further the authors observed elevated subjective ratings of hunger in the athletes. Jurimae et al (2007) in a cross sectional study found that physically active
girls (aged 11-16yrs) had significantly higher mean plasma ghrelin (an orexigenic peptide) levels than physically inactive girls. The authors suggested that regular PA increases plasma ghrelin concentrations to stimulate appetite and food intake to cover the higher energy expenditure. McKelvie et al (2007) investigated the response of the acylated form of ghrelin to 5 days of exercise in adolescent boys and found a significant increase after exercise which was correlated with an increase in markers of appetite. These changes in peptide levels with chronic exercise all favour an increase in EI and notably also favour an acceleration of GE, as CCK is a major regulator in inhibiting GE and ghrelin and acylated ghrelin more specifically are known to accelerate GE. However, others have observed increases in peptides GLP-1 (Chanoine et al 2007), PP (Hurley et al 1991) and deacetylated ghrelin (Kim et al 2006) following exercise interventions ranging in length from 5 days (Chanoine et al 2007) to 12 weeks (Kim et al 2006). These changes would favour a delayed GE with regular exercise.

In general, although limited the evidence from the literature reviewed indicates that more active individuals have a faster GE (Carrio et al 1989, Shimamoto et al 2002) and OCTT (Harris et al 1991). A faster GE and therefore a shorter satiety period (Sepple & Read 1989), leading to an increase in meal frequency may represent a plausible mechanism for the increase in EI that has been observed in chronic exercisers (Horton et al 1994, Maughan et al 1989, Saris et al 1989). However, evidence that chronic exercise intervention does not necessarily increase EI (Blundell & King 1999) suggests that changes in appetite control mechanisms with chronic exercise intervention may be different, highlighting the necessity for longitudinal exercise intervention studies examining changes in GE to assist in understanding the mechanisms mediating changes in EI with exercise.

As a number of studies have established that dietary modification may influence gastric emptying (Cunningham et al 1991, Corvilain et al 1995, Horowitz et al 1996) and gastric motor function (Andrews et al 1998) in healthy young adults, the suggestion by Harris et al (1991) that differences in digestive strategy in more active individuals are related to the hyperphagia associated with chronic exercise is plausible. As no intervention studies could be found on GE and food intake, the causal nature of this relationship requires further investigation. It could also be speculated that rather than a higher EI being the cause of a faster GE, other mechanisms such as increased parasympathetic tone proposed by Carrio et al (1989) or increased gastric electroactivity proposed by Shimamoto et al (2002) in more active individuals causes a faster GE and
thus leads to a greater EI. Further research is needed to simultaneously assess changes in appetite sensations, gastric emptying and food intake to establish the role of alterations in gastric emptying in mediating changes in EI, appetite sensations and appetite control with exercise.

2.7 Summary of Literature Review

The role of the stomach in appetite control has been of interest for at least a century and gastric emptying is considered to have a critical role (Park & Camillieri 2005). However, much still remains to be learned as findings are far from conclusive. As evident from studies investigating GE in lean and obese individuals, plausible mechanisms have been proposed to explain an increase in food intake with both delayed and accelerated GE. Therefore, further studies are needed to fully elucidate the association of GE with body mass index and the relative roles of gastric distension and intestinal nutrients in both satiation and satiety.

Surprisingly, limited research exists that has examined the influence of exercise on GE in relation to EI. The literature reviewed clearly indicates a consistent finding of delayed GE with acute vigorous exercise, which may be the result of changes in control mechanisms such as hormonal and neural factors. Cross sectional studies indicate a faster GE in more active individuals. Delayed GE may contribute to the suppression of hunger that has been observed with acute strenuous exercise, while an accelerated GE that has been observed in chronic exercisers may explain why EI may be increased in these individuals. However, as both delayed and accelerated GE have been implicated in increased food intake, studies simultaneously measuring GE, appetite and energy intake are needed. Furthermore, as evident from this chapter a multitude of methodological and subject characteristics influence both changes in GE and changes in EI with exercise. Future studies employing similar methodologies would allow for an easier comparison of findings. Furthermore, repeated measures studies would eliminate many of the inter-individual factors known to influence GE.

Intervention studies may help to increase understanding of the mechanisms modulating changes in EI and appetite with exercise and consequently increase understanding of how exercise may be more effectively used in the treatment and prevention of over and under nutrition. In this regard, the influence of exercise on physiological variables such as gastric emptying rate, a factor consistently implicated to play a critical role in appetite control represents an area worthy of further investigation.
This is particularly pertinent in populations prone to physical inactivity such as female adolescents (Neumark-Sztainer & Hannan 2000) and in patient populations such as those with Multiple Sclerosis known to have both altered GE (El-Maghraby 2005) and sedentary lifestyles. Such studies may help in devising more effective treatment methods to promote a better regulation of EI and thereby enhance wellbeing in these individuals.
Chapter 3 The effect of a 7 week exercise intervention on gastric emptying and appetite in adolescent girls

Abstract

Background/Objectives: Gastric emptying (GE) plays a role in appetite regulation and has been implicated in the pathogenesis of obesity. Physical activity (PA) may be one factor which moderates GE. The objectives of this study were to investigate the effect of a 7 week exercise intervention on GE, appetite and food intake in adolescent girls. In addition, the association between body mass index (BMI) and GE was investigated.

Subjects/Methods: Nineteen healthy schoolgirls (BMI 22.1±2.1 kg·m⁻²; age 16.3±0.3 years) attended the laboratory on 2 occasions, at week 0 and week 8. GE was assessed by ¹³C octanoic acid breath test, subjective appetite sensations by visual analogue scales and food intake at a buffet meal. During weeks 1 to 7 subjects attended three classes per week of either a moderate intensity exercise class (exercise group; (EXE): n = 9) or a non-exercise class (control group (CTL); n = 10). Statistical Analysis was conducted using Pearson correlations and Mixed ANOVA.

Results: Mixed ANOVA indicated no significant time by group interactions for GE or food intake, but a significant reduction in 30min postprandial hunger ratings in EXE compared to CTL over time (p = .032). GE half time significantly increased in both EXE and CTL over time (main effect, p = .043). Pearson correlations showed that at baseline, increasing BMI was significantly associated with slower GE lag (r = .68, p = .001), latency (r = .62, p = .005) and half times (r = .62, p = .005).

Conclusion: Findings indicate increasing BMI is associated with slower GE in adolescent girls. A 7 week PA intervention reduced 30 minute postprandial hunger ratings but did not significantly influence GE compared to a control group.

Key Words: Gastric Emptying, Body Mass Index, Exercise, Appetite.
3.1 Introduction

In simple terms, obesity can be considered an imbalance between energy intake and energy expenditure. Rate of gastric emptying refers to the rate at which nutrients are emptied from the stomach into the small intestine and has been implicated in the pathogenesis of obesity. Hunt et al (1975) postulated that rapid GE and hence a shortened satiety period may contribute to the development of obesity (Hunt et al 1975). In contrast, delayed GE has also been proposed to lead to increased food intake due to reduced nutrients in the small intestine and consequently a delay in the release of satiety signals controlling appetite (Jackson et al 2004). Slower GE has been demonstrated to correlate with increasing BMI in non obese individuals (Brogna et al 1998) supporting the latter contention. However, findings from studies that have compared GE in lean and obese individuals are highly inconsistent as delayed (Horowitz et al 1983, Jackson et al 2004), similar (Hutson et al 1993, Verdich et al 2000) and accelerated (Wright et al 1983, Naslund et al 1998) emptying have been reported in obese subjects. Nevertheless, GE is considered to play a critical role in appetite control (Park & Camilleri 2005) and persuasive evidence indicates GE influences energy intake (Sepple & Read 1989).

Physical activity may be one modifiable lifestyle factor which alters GE as prior cross-sectional research has demonstrated an accelerated rate of GE in active compared to sedentary individuals (Carrio et al 1989, Shimamoto et al 2002). Understanding the mechanisms by which dietary factors and physical activity, or their interaction, influence the development of obesity has been identified as critical to understanding the relationship between physical activity (PA) and obesity (Jebb & Moore, 1999). Although the effect of exercise on energy balance is usually associated with increased energy expenditure, regular exercise may also influence energy balance indirectly by altering energy intake. See Blundell & King (1998) and Martins et al (2008) for reviews. Higher fasting levels of the orexigenic peptide ghrelin (known to accelerate GE) have been reported in physically active compared to sedentary adolescent girls (Jurimae et al 2007). The authors suggested that regular physical activity (PA) increases plasma ghrelin levels to stimulate appetite and food intake to cover the increased energy expended. Others have reported beneficial effects of physical activity on appetite control, whereby more active individuals display better short term energy intake compensation to preloads of different energy content (Long et al 2002, Martins et al 2007, Van Walleghan et al 2007). Recently, Chanoine et al (2007) reported increases in postprandial GLP-1, a short term satiety signal following just 5 days of exercise.
intervention in normal weight and overweight adolescents. The release of these peptides is known to be under both neural and endocrine influence as well as being stimulated directly by intestinal nutrients (Huda et al. 2006). As GE is considered to play a critical role in appetite control (Park & Camilleri 2005), by influencing both gastric distension and the presence of nutrients in the small intestine, GE may therefore play a key role in mediating changes in appetite sensations and energy intake with exercise. However, as plausible mechanisms have been put forward whereby both accelerated and delayed GE increase food intake (Hunt 1975, Jackson et al 2004), studies simultaneously measuring appetite sensations, food intake and GE are needed to fully understand the effects of exercise on GE and the implications for energy intake and appetite control. Furthermore, while the effect of exercise on GER has received considerable investigation, it is largely limited to acute exercise bouts by researchers investigating methods of supplementing carbohydrate stores and enhancing fluid homeostasis during exercise. See Murray (2006) for a review.

As adolescence is a key period critical for the later development of metabolic complications (Artz et al 2005), a better understanding of the effects of therapeutic interventions such as increasing physical activity on GER and appetite will assist in the understanding and implementation of more effective strategies for the prevention of obesity. The primary aim of the current study therefore was to investigate the effect of a 7 week exercise intervention on GE, appetite and food intake in healthy non-obese adolescent girls. In addition, the association between body mass index and GE was examined.
3.2 Methods

The exercise intervention presented in this chapter was conducted in collaboration with Deirdre Harrington (PhD student, University of Limerick) as part of her PhD study designed to promote physical activity in sedentary adolescent females using activities known to appeal to this population. The recruitment, design and randomisation for the intervention were undertaken by Deirdre. All exercise classes were designed by Deirdre and the majority of classes were led by Deirdre or other trained instructors. The author of this thesis assisted the instructors in the delivery of classes. Accelerometry data presented in this thesis was undertaken by Deirdre. All other data reported was collected and analysed solely by the author of this thesis and is presented solely in this thesis.

Participation in physical education classes only (no other physical activity classes or sport during or after school and less than 30 minutes accumulated PA on most days of the week apart from Physical Education) was the inclusion criteria for all subjects in the aforementioned study. Thirty six adolescent females who fit inclusion criteria volunteered to participate. These students were randomly assigned to either an exercise (EXE) or control (CTL) group using a random number table. Students in the EXE group were assigned to three forty minute exercise classes per week as part of their school timetable. Students assigned to the CTL group were assigned to an alternative non-exercise class. Throughout the intervention period, participants in both groups (EXE and CTL) had Physical Education classes as normal (two forty minute classes per week).

For the present study 24 subjects were recruited from this cohort of 36 volunteers and outcome measures were collected at baseline and following 7 weeks of intervention. The following description is of the methods of the present study.

3.2.1 Subjects

Twenty four adolescent schoolgirls (age 16.3±0.3 years, BMI 22.1±2.1 kg·m⁻²) volunteered to participate in the outcome measures involved in the present study. Thirteen students (n = 13) had been previously randomly assigned to the EXE group and eleven students (n = 11) to the CTL group. None had a history of gastrointestinal disease or surgery, significant illness (including diabetes mellitus) nor was taking any medication. The study protocol was approved by the University of Limerick Physical Education and Sport Sciences Research Ethics Committee (Appendix A). The school principal, students and their parents signed informed consent forms.
3.2.2 General Study Protocol

Testing was completed during 2 visits of the students to the laboratory. Each testing day involved testing 2-3 students at the same time. The first testing session took place during week 0 (baseline) and the second during week 8 (post-intervention). Both testing sessions followed an identical protocol. To reduce inherent variability, subjects were instructed to refrain from moderate to heavy exercise and consuming alcohol for 24 hours beforehand. In addition, all subjects travelled by car to the laboratory on the morning of testing and arrived between 9 and 9.30am, following a standard 12 hour overnight fast.

3.2.3 Anthropometry

Height was measured without shoes, to the nearest 1mm using a freestanding stadiometer (Holtain Ltd, Great Britain). Weight was measured in light clothing without shoes on platform scales (SECA, Germany) to the nearest 0.01kg. BMI was calculated as weight in kilograms divided by the square of height in meters.

3.2.4 Gastric Emptying Measurement

Gastric emptying parameters were calculated using the $^{13}$C-octanoic acid breath test ($^{13}$C-OBT), a method which has been validated and described in detail previously (Maes et al 1998). Breath samples were obtained in duplicate at baseline and every 15 minutes for 4 hours after ingestion of the test meal, a test protocol used in previous GE studies of children and adolescents (Maes et al 1995, Hauser et al. 2006). At each breath collection time point, expired breath was collected via a straw into a 10ml Exetainer tube (Labco Ltd., High Wycombe, England). The study period was spent in sedentary activities such as reading and watching television. Subjects were not allowed to eat or drink anything other than the standard test meal during this time. Room temperature was controlled at 23°C on all study days.

3.2.4.1 Test Meal

To prevent an olfactory response to the test meal, the test meal was prepared in a separate room. The egg yolk of the standardized pancake breakfast meal (250 kcal; 26g CHO, 13g PRO, 11g Fat; Table 1) was labelled with 100mg $^{13}$C-octanoic acid (Eurisotop, Saint-Aubain, France). The meal was ingested within 10 minutes together with 150ml of water.
The test meal was selected for a number of reasons. A solid test meal is considered more sensitive to detecting changes in GER than a liquid meal (Malmud et al. 1982). As in Maes et al (1995), it was chosen over the egg and bread meal to improve the palatability of the meal for adolescents. A pancake test meal has also previously been used among children and adolescents in scintigraphic and $^{13}$C-OBT studies (Montgomery et al 1998, Maes et al 1995, Symonds et al 2003, Hauser et al 2006). In addition, the macronutrient distribution and total calorie content is also very similar to that of Maes et al (1995), Hauser et al (2006) and Ghoos et al (1993).

Table 3.1 Ingredients and nutrient composition of standard test meal

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight (g)</th>
<th>Energy (kcal)</th>
<th>CHO (g)</th>
<th>PRO (g)</th>
<th>FAT (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cream Flour (Odlums)</td>
<td>25.5</td>
<td>85.4</td>
<td>19.5</td>
<td>2.7</td>
<td>0.3</td>
</tr>
<tr>
<td>1 large egg (Dunnes Stores)</td>
<td>65.0</td>
<td>95.5</td>
<td>0.0</td>
<td>8.1</td>
<td>7.0</td>
</tr>
<tr>
<td>Free Range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole Milk (Dunnes Stores)</td>
<td>50.0</td>
<td>32.0</td>
<td>2.35</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>Nutella</td>
<td>7.0</td>
<td>37.1</td>
<td>3.92</td>
<td>0.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Totals</td>
<td>147.5</td>
<td>250.1</td>
<td>25.7</td>
<td>13.0</td>
<td>11.3</td>
</tr>
</tbody>
</table>

3.2.4.2 Stable isotope $^{13}$C breath test data analysis
$^{13}$C-isotopic enrichment of breath samples was measured using continuous flow isotope ratio mass spectrometry (ABCA; Europa Scientific, Crewe, UK). The results were expressed relative to Pee Dee Belemnite, the internationally agreed standard of known $^{13}$C concentration. Breath $^{13}$CO$_2$ enrichment data were fitted to the established gastric emptying model (Ghoos et al 1993) to derive the outcome measures lag time (the time taken to maximal $^{13}$CO$_2$ excretion) and half excretion time (the time taken for 50% of the $^{13}$C dose to be recovered). These parameters were corrected to scintigraphy equivalent values using the regression equations as defined by Ghoos et al (1993), giving the parameters $t_{\text{lag}}$ and $t_{1/2}$. The parameters latency time ($t_{\text{lat}}$, the initial delay in the cumulative $^{13}$C excretion curve) and ascension time ($t_{\text{asc}}$, the time course between the latency phase and $t_{1/2}$, representing a period of high $^{13}$C excretion rate), defined by Schommartz et al (1998) were also calculated. For all the data $r^2$ coefficient between the modelled and raw data was calculated and $r^2>0.90$.

3.2.5 Physical Activity
Participants completed the self report 7 day recall Physical Activity Questionnaire for Adolescents (PAQ-A; Appendix B) (Kowalski et al 1997) at week 0 and week 8. The scoring system consists of a 5 point scale from 1 – low PA to 5 – high PA level. In
addition, participants were given an ActivPAL (PAL Technologies Ltd, Glasgow, Scotland) accelerometer to wear on the midline of the thigh for 7 days during week 0 and week 7 of the intervention. The ActivPAL is a single unit uniaxial accelerometer and samples bodily accelerations at 10Hz which are converted to counts. Valid data was considered as a minimum of 4 full days of data including one weekend day. Average steps per day and time spent in bouts of moderate to vigorous PA (MVPA) at least 10 minutes long were calculated.

3.2.6 Appetite
Measures of hunger, desire to eat and fullness were assessed with 100mm visual analogue scales (VAS) before (t -10min) and at 30 minute intervals after (t 0, 30, 60, 90, 120, 150, 180, 210, 240, 270 min) the pancake test meal on gastric emptying study days. The questions asked were: How strong is your desire to eat (very weak (0mm) to very strong (100 mm))? How hungry do you feel (not at all (0mm) to very hungry (100mm))? How full do you feel (not at all (0mm) to very full(100mm))? The area under the time curve (AUC) for each of the sensations was calculated using the trapezoidal rule. Variables thirst, coldness and tiredness were used to distract subjects from their satiety status.

3.2.7 Food Intake
Immediately after completion of the gastric emptying study (t 240 min), each subject was offered a standard ad libitum buffet-style meal consisting of a variety of lunch type foods (sandwiches, salad, butter, mayonnaise, fruit, yogurt, cake, biscuits, crisps; Table 3.2) and invited to eat as much as they wished over 30 minutes. The protein: fat: carbohydrate ratio was approximately 13:38:49 percent energy, a similar composition to buffet meals used in previous investigations of the effects of exercise on energy intake (Long et al 2001, Martins et al 2007). Buffet food was prepared in excess of what would normally be eaten (3250 kcal) and weighed before and re-weighed after each subject had finished eating. Subjects ate alone in a cubicle and were not told that their food intake was to be measured. Subjects were told the buffet was a lunch meal and they could eat as much as they wished over 30 minutes. Subjects were also told they could take any food item not consumed within this time period with them and any food items taken were noted by the investigator. Total amount (g and kcal) and macronutrient content of food consumed during the 30 minutes was calculated from manufacturers’ data.
### Table 3.2 Amount, type and nutrient composition of food served at ad libitum buffet lunch meal

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Amount served (g)</th>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>PRO (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread (4 slices) Brennans</td>
<td>150</td>
<td>315</td>
<td>2.1</td>
<td>64.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Brown bread (4 slices) Brennans wholegrain</td>
<td>150</td>
<td>310</td>
<td>2.2</td>
<td>61.3</td>
<td>13.8</td>
</tr>
<tr>
<td>Chicken sliced (Dunnes Stores Wafer Thin Chicken)</td>
<td>100</td>
<td>143</td>
<td>5.8</td>
<td>3.6</td>
<td>19.1</td>
</tr>
<tr>
<td>Ham sliced (Dunnes Stores cooked Ham)</td>
<td>100</td>
<td>99</td>
<td>2.9</td>
<td>0.7</td>
<td>17.6</td>
</tr>
<tr>
<td>Cheese sliced (St. Bernard Mature Red Cheddar Slices)</td>
<td>100</td>
<td>390</td>
<td>32.0</td>
<td>0.2</td>
<td>26.0</td>
</tr>
<tr>
<td>Tomato sliced</td>
<td>100</td>
<td>13</td>
<td>0.1</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Cucumber sliced</td>
<td>100</td>
<td>10</td>
<td>0.1</td>
<td>1.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Cadbury mini rolls</td>
<td>50</td>
<td>240</td>
<td>12.4</td>
<td>30.4</td>
<td>2.6</td>
</tr>
<tr>
<td>1 strawberry yogurt (Yoplait)</td>
<td>125</td>
<td>120</td>
<td>3.3</td>
<td>18.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Cookies (maryland)</td>
<td>166</td>
<td>448</td>
<td>20.8</td>
<td>60.0</td>
<td>5.6</td>
</tr>
<tr>
<td>Apple (each apple = approx. 110g) (Dunnes Gala)</td>
<td>110</td>
<td>55</td>
<td>0.1</td>
<td>13.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Banana - freddy fyffes</td>
<td>70</td>
<td>66</td>
<td>0.2</td>
<td>16.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Orange juice (St. Bernard Orange Juice Drink 250ml x2)</td>
<td>500</td>
<td>195</td>
<td>0</td>
<td>50.5</td>
<td>0</td>
</tr>
<tr>
<td>Water (Riverock)</td>
<td>500</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Coca cola</td>
<td>500</td>
<td>210</td>
<td>0</td>
<td>53.0</td>
<td>0</td>
</tr>
<tr>
<td>Butter (Dunnes Stores Taste of Butter)</td>
<td>30</td>
<td>162</td>
<td>17.7</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Mayonnaise (Hellmans)</td>
<td>30</td>
<td>217</td>
<td>23.6</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Crisps - cheese and onion (1 bag Tayto)</td>
<td>25</td>
<td>130</td>
<td>8.6</td>
<td>11.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Crisps - salt &amp; vinegar (1 bag Tayto)</td>
<td>25</td>
<td>130</td>
<td>8.6</td>
<td>11.5</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Totals:</strong></td>
<td><strong>3253</strong></td>
<td><strong>140.6</strong></td>
<td><strong>398.9</strong></td>
<td><strong>104.4</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Total kcal</strong></td>
<td><strong>1265.0</strong></td>
<td><strong>1596.0</strong></td>
<td><strong>417.0</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>% Energy</strong></td>
<td><strong>38.8%</strong></td>
<td><strong>49.0%</strong></td>
<td><strong>12.8%</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### 3.2.8 Exercise Intervention

During week 0, participants in the EXE group attended a lifestyle education class on PA and current PA guidelines. The exercise intervention (from week 1 to week 7 inclusive) consisted of three forty minute moderate intensity exercise classes per week, in addition to normal Physical Education classes. Because activities at or above moderate intensity...
expend approximately 30 kJ/min (7.17 kcal/min) in adolescent girls (Schmitz et al 2005) this would translate into approximately 860 kcal per week of additional energy expenditure in the EXE group. To monitor compliance, attendance at all classes was recorded. Moderate intensity PA was described to participants as “working hard enough to raise your heart rate and break a sweat, yet still being able to carry on a conversation” as described by the American College of Sports Medicine and American Heart Association (Haskell et al. 2007), and used as a guide to exercise intensity when heart rate monitors were not available. All participants wore heart rate monitors (Polar S410i, Tampere, Finland) during the first class to familiarize themselves with exercising at 65-75% of their age predicted maximum heart rate (220-age in years). Classes included instructor led aerobics, dance and circuits classes, two classes in a local gym where participants undertook various forms of cardiovascular exercise such as cycling, treadmill and stair climbing exercise and one aqua aerobics class.

3.2.9 Statistical Analysis
Statistical analysis was carried out using SPSS for Windows (version 16.0, SPSS Inc., Chicago, IL, U.S.A.). Values are reported as mean +/- standard deviation. All variables were checked for normal distribution using the Shapiro-Wilk test. Independent Student’s t tests were used to compare baseline descriptive characteristics and percentage change over the intervention between EXE and CTL groups. Mixed ANOVA was used to examine main effects [group (EXE vs. CTL), time (pre vs. post exercise)] and their interaction comparing pre and post exercise variables for PA level, body composition, gastric emptying, appetite and food intake. Pearson’s correlations were used to test for correlations between variables. Due to low n numbers in individual groups (EXE (n = 9) and CTL (n = 10) individual group correlations were not undertaken. Therefore correlations reported refer to the group as a whole (n = 19) unless otherwise stated. A retrospective power analysis was also conducted. Statistical significance was set at p ≤ 0.05.
3.3 Results

3.3.1 Sample Characteristics

Twenty four subjects were recruited and completed baseline measurements. Five subjects (EXE (n =4), CTL (n=1)) did not complete the study, three due to illness and two due to other school commitments. There were no significant differences in their baseline characteristics compared with the adolescents who completed the study. Characteristics of participants (n = 19) who completed the study were not significantly different between groups at baseline (Table 3.3).

Table 3.3 Participant’s baseline characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EXE (n = 9)</th>
<th>CTL (n = 10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>16.4 ± 0.3</td>
<td>16.2 ± 0.4</td>
<td>.320</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.3 ± 6.8</td>
<td>163.2 ± 6.2</td>
<td>.721</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.9 ± 10.0</td>
<td>59.4 ± 7.9</td>
<td>.917</td>
</tr>
<tr>
<td>BMI (kg·m$^{-2}$)</td>
<td>22.1 ± 2.9</td>
<td>22.2 ± 1.4</td>
<td>.903</td>
</tr>
<tr>
<td>Physical Activity Score (PAQ-A)</td>
<td>2.4 ± 0.6</td>
<td>2.0 ± 0.5</td>
<td>.142</td>
</tr>
</tbody>
</table>

Data are means ± SD. All p values refer to Independent Student’s t tests.

3.3.2 Association between variables at baseline

There was no significant association between age or PAQ-A score and GE (p > .05). Pearson correlations indicated a significant association between baseline BMI and GE lag ($r = .68$, $p = .001$), latency ($r = .62$, $p = .005$) and half times (Figure 3.1), all indicating increasing BMI was associated with slower gastric emptying. The association between BMI and ascension time was approaching significance ($r = .433$, $p = .064$).
3.3.3 Effect of Exercise Intervention

All subjects in EXE completed >/= 65% of exercise classes. Means and s.d. for anthropometric, gastric emptying, PA and food intake parameters and percentage change from baseline to post-intervention in EXE and CTL are presented in Table 3.4.
Table 3.4 Anthropometric, physical activity, gastric emptying and food intake measures at baseline, post-intervention and % change in CTL (n=10) and EXE (n=9) groups.

<table>
<thead>
<tr>
<th>Measure</th>
<th>CTL (n = 10)</th>
<th>Baseline</th>
<th>Post</th>
<th>% change</th>
<th>EXE (n = 9)</th>
<th>Baseline</th>
<th>Post</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg) †</td>
<td></td>
<td>59.4 ± 7.9</td>
<td>57.2 ± 8.2</td>
<td>-3.8 ± 2.7</td>
<td>59.9 ± 10.0</td>
<td>60.2 ± 9.9</td>
<td>0.7 ± 2.7*</td>
<td></td>
</tr>
<tr>
<td>BMI (kg·m⁻²) †</td>
<td></td>
<td>22.2 ± 1.4</td>
<td>21.3 ± 1.8</td>
<td>-4.4 ± 3.5</td>
<td>22.1 ± 2.9</td>
<td>22.2 ± 2.9</td>
<td>0.6 ± 2.5*</td>
<td></td>
</tr>
<tr>
<td>PAQ-A (score)</td>
<td></td>
<td>2.0 ± 0.5</td>
<td>2.2 ± 0.6</td>
<td>11.7 ± 16.8</td>
<td>2.4 ± 0.6</td>
<td>2.5 ± 0.7</td>
<td>10.8 ± 43.0</td>
<td></td>
</tr>
<tr>
<td>T₁/₂ (mins) ††</td>
<td></td>
<td>81.5 ± 17.5</td>
<td>91.4 ± 21.7</td>
<td>13.5 ± 21.9</td>
<td>77.8 ± 17.8</td>
<td>84.3 ± 14.0</td>
<td>12.2 ± 26.6</td>
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</tr>
<tr>
<td>Tlag (mins)</td>
<td></td>
<td>34.4 ± 9.5</td>
<td>37.3 ± 9.0</td>
<td>11.8 ± 24.3</td>
<td>30.7 ± 11.1</td>
<td>35.1 ± 8.5</td>
<td>27.3 ± 11.8</td>
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<tr>
<td>Tasc (mins)</td>
<td></td>
<td>121.4 ± 15.9</td>
<td>131.9 ± 20.5</td>
<td>9.3 ± 16.2</td>
<td>121.0 ± 13.9</td>
<td>122.4 ± 10.7</td>
<td>2.1 ± 13.5</td>
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</tr>
<tr>
<td>Tlat (mins)</td>
<td></td>
<td>35.9 ± 7.5</td>
<td>36.5 ± 5.9</td>
<td>5.4 ± 24.8</td>
<td>32.1 ± 10.3</td>
<td>36.9 ± 7.9</td>
<td>23.7 ± 39.3</td>
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</tr>
<tr>
<td>Buffet EI (kcal)</td>
<td></td>
<td>1066 ± 215</td>
<td>1091 ± 244</td>
<td>3.5 ± 26.2</td>
<td>1248 ± 214</td>
<td>1113 ± 347</td>
<td>-13.2 ± 17.0</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD. 95% confidence intervals for mean % change are indicated in brackets in mean % change column.

* Percentage change significantly different to CTL, p < .01. † Significant time by group interaction p < .05.
†† Main effect of time p < .05.
3.3.3.1 Physical Activity

Physical activity levels as assessed by self-report questionnaire did not significantly change in either group between baseline and post-intervention. There were no main effects of time or group (p > .05) or time x group interaction for PAQ-A score (p > .05) (Table 3.4).

Full accelerometry data sets were obtained for 4 individuals in the CTL group and 6 individuals in the EXE group at baseline and therefore were not statistically analysed. Accelerometry data from week 7 indicated a mean (SD) of 10060 (3253) and 7902 (2327) steps per day for EXE (n = 8) and CTL (n = 8) groups respectively. Time spent in MVPA was 23.4 (23.2) and 9.1 (7.9) min per day for EXE and CTL respectively. However, differences were not statistically significant as indicated by independent t test (p > .05).

3.3.3.2 Anthropometric Characteristics

There was a significant time x group interaction for weight F (1, 17) = 11.79, p = .003 (Table 2). The CTL group had a mean -3.8 ± 2.8% reduction in weight compared to a 0.7 ± 2.7% increase in the EXE group (p = .02). The time x group interaction for BMI was also significant F (1, 17) = 12.05, p < .05 (Table 3.4). The CTL group had a -4.4 ± 3.5% reduction in BMI compared to a 0.6 ± 2.5% increase in the EXE group.

3.3.3.3 Gastric Emptying

The exercise intervention did not significantly alter GE as there was no significant time x group interaction F (1, 17) = .226, p > .05) for \( t^{1/2} \). However there was a main effect of time F (1, 17) = 4.8, p = .043, indicating a significant increase in \( t^{1/2} \) in the group as a whole at post-intervention compared to baseline (p = .036). As evident in Table 3.4, both groups had similar percentage increases in \( t^{1/2} \) of 13.5 ± 21.9% and 12.2 ± 26.6% in the CTL and EXE groups respectively. The main effect of time on \( t_{lag} \) followed a similar pattern and was approaching significance (F (1, 17) = 3.74, p = .07). Main effects of time and group and their interaction for \( t_{lat} \) and \( t_{asc} \) were not significant (p > .05).

Retrospective power analysis indicated that based on the standard deviation of the response variable of 15.9 minutes, the sample size of 19 subjects was sufficient to detect a 21.8 minute difference in change in GE \( t^{1/2} \) between exercise and control groups with a power of 80% and a significance level of 5% (Motulsky 1995). For lag time, based on the standard deviation of 7.9 minutes, the sample size was sufficient to detect
an effect of the exercise intervention of 10.9 minutes compared to the control group with a power of 80% and a significance level of 5%.

3.3.3.4 Buffet Meal Food Intake

The exercise intervention did not significantly influence energy intake at the buffet meal. There were no significant main effects of time (p = .124) or group (p = .602) and no significant time x group interaction (p = .219) for total EI at the buffet meal (Table 3.4). In addition, there were no significant differences in macronutrient intake at the buffet meal between baseline and post-intervention in either group (Figure 3.2).

![Figure 3.2 Macronutrient selection at the ad libitum buffet meal served at t 240min following the standardised test meal in A) the control group and B) the exercise group. CHO – carbohydrate, PRO – protein.](image_url)

Retrospective power analysis indicated that based on the standard deviation of the response variable of 278 kcal, the sample size of 19 subjects was sufficient to detect a difference between control and exercise groups with a power of 80% and a two sided
5% significance level, if the true difference between the groups was 380 kcal (Motulsky 1995).

### 3.3.3.5 Subjective Appetite Sensations

Absolute ratings of hunger (Figure 3.3), fullness and desire to eat are presented (Appendix C). The exercise intervention had no significant effect on total 4 hour area under the curve ratings (main effects of time and group and time x group interactions for hunger, fullness and desire to eat AUC: p > .05). There was a trend towards an increase in baseline hunger ratings in the EXE group following intervention compared to a slight decrease in the CTL group (F (1, 17) = 3.47, p = .08, Figure 3.3). There was a significant time x group interaction for postprandial hunger at 30 minutes F (1, 17) = 5.46, p = .032. The EXE group showed a reduction in 30 min postprandial hunger from baseline to post-intervention of -13.44 ± 17.97 (95%CI: -27.26 - 0.38) mm compared to a slight increase in the CTL group (4.85 ± 16.17 (95%CI: -6.7 – 16.4) mm) (Figure 3.3). Changes in desire to eat followed a similar pattern and the time x group interaction at 30min approached significance (F (1, 17) = 3.65, p = .07). There were no time x group interactions approaching or reaching significance at any other time points for hunger (Figure 3.3) fullness, or desire to eat (p > .05).
Figure 3.3 Mean ± SD (error bars) absolute ratings of subjective hunger at baseline (unfilled markers) and post-intervention (filled markers) in A: control (CTL; n = 10) and B: exercise (EXE; n = 9) groups. Breakfast was consumed between t = -10 and t=0 min. An ad libitum buffet lunch meal was consumed between t = 240 and t=270 min. † significant time x group interaction (p = .032).

3.3.3.6 Association between variables at post-intervention

There were no significant associations between any variables at post-intervention. Figure 3.4 illustrates steps per day assessed by accelerometry in week 7 plotted against GE half time (p > .05).
Figure 3.4 Steps per day assessed by accelerometry plotted against GE half time at post-intervention for the whole group which provided valid accelerometry data sets (n = 16; r = .03, p = .91). For illustration purposes subjects in the EXE group are identified by ♦ and subjects in the CTL group by □.

The association between BMI and GE in the whole group (n = 19) that was evident at baseline was smaller and non-significant at post-intervention (t_{lag}: r = .37, p = .119; t_{half}: r = .388, p = .100 (Figure 3.5); t_{lat}: r = .277, p = .252; t_{asc}: r = .325, p = .175; Appendix D).

Figure 3.5 Relationship between gastric emptying half time (t_{1/2}) and BMI at post-intervention for the whole group (n = 19; r = .388, p = .100). For illustration purposes subjects in the EXE group are identified by ♦ and subjects in the CTL group by □.
3.3.4 Associations between changes in variables

There were no significant correlations between changes in any variables from baseline to post-intervention. Change in self report physical activity scores and BMI were not associated with changes in any of the 4 GE outcome measures (p > .05, Appendix D). Figure 3.6 illustrates change in BMI between baseline and post-intervention plotted against change in gastric emptying half time (in minutes) for the whole group (n = 19) (r = .005, p = .984). Figure 3.7 illustrates change in BMI plotted against change in latency time (r = .231, p = .340). In addition, there were no significant correlations between changes in GE and changes in EI at the buffet meal (Appendix D).

![Figure 3.6](image1.png)

Figure 3.6 Change in GE half time (t\(_{1/2}\)) plotted against change in BMI (kg m\(^{-2}\)) from baseline to post-intervention for the whole group (n = 19); r = .005, p = .984. For illustration purposes subjects in the EXE group are identified by ◣ and subjects in the CTL group by ■.

![Figure 3.7](image2.png)

Figure 3.7 Change in GE latency time (t\(_{lat}\)) plotted against change in BMI (kg m\(^{-2}\)) from baseline to post-intervention in the whole group (n = 19); r = .231, p = .340. For illustration purposes subjects in the EXE group are identified by ◣ and subjects in the CTL group by ■.
3.4 Discussion

Prior cross sectional research has indicated a faster rate of GE in chronic exercisers compared to their sedentary counterparts (Carrio et al 1989, Shimamoto et al 2002). No studies in the existing literature have investigated the effect of chronic exercise intervention on gastric emptying. The current longitudinal study was conducted to test the hypothesis that increasing physical activity levels through a 7 week exercise intervention would accelerate GE of a meal at rest, and alter appetite sensations and food intake at a buffet meal. The major finding of this study was that a seven week moderate intensity chronic exercise intervention did not significantly alter GE when compared to a control group of adolescent schoolgirls.

One possible confounding factor which must first be acknowledged before interpreting findings of this study is that the control group had a modest but significant weight loss compared to little change in bodyweight or BMI in the exercise group. As a recent meta-analysis concluded that school based exercise interventions did not improve BMI although they had other beneficial effects (Harris et al. 2009), the lack of significant change in body composition in the exercise group is not surprising. However, the significant changes in the control group are unexpected. One possible explanation is a Hawthorne effect (Holden et al 2001) whereby participants in the control group modified their behaviour as a result of being enrolled in the study. Self report physical activity data indicated no significant change in physical activity level in the control or exercise groups between baseline and post-intervention. However, accelerometry data from week 7 indicated lower steps and time spent in moderate to vigorous physical activity per day in the control group compared to the exercise group. Although not statistically significant, this data suggests that the improvements in body composition in the control group were mediated by behavioral changes other than physical activity.

Either as a result of diet or exercise or a combination of both, the change in energy balance causing the weight loss in the control group is important to consider as there was a significant increase in half time in the group as a whole from baseline to post-intervention. Although quantifying the change in \( t_{1/2} \) that constitutes clinical significance is equivocol in the literature, due to the large intra-individual coefficient of variation of \(~13\%\) (Cremonini et al 2002) changes in GE \( t_{1/2} \) of > 20-30min have been previously considered by others clinically significant (Berthold et al 2008). Therefore,
despite statistical significance the physiological relevance of the increase in $t_{1/2}$ in both groups must be considered minimal as mean increases were 9.9 and 6.5 minutes for the control and exercise groups respectively. It is therefore unlikely that the change in energy balance in the control group created a possibility of incorrectly rejecting the null hypothesis that the exercise intervention would have no effect on GE. In addition, retrospective power analysis indicated that the sample size was sufficient to detect a treatment effect of 24 minutes in GE half time further indicating the non-significant effect of the exercise intervention on GE was not likely to be the result of a type II error. Therefore it can be reasonably concluded that the exercise intervention had no significant effect on GE.

This may be due to several reasons. Research indicating an accelerated GE in more active individuals is cross sectional and therefore differences in lifestyle and cognitive factors between chronic exercisers and sedentary individuals are not accounted for. These factors represent a large potential difference for feeding behaviour. Harris et al (1991) observed a faster orocecal transit time (OCTT) in more active individuals but suggested that it was the high energy intakes associated with chronic exercise that was associated with faster OCTT (i.e. not the exercise directly). High energy intakes by enhancing gastric distension may accelerate GE. However, the causal nature of the association between physical activity, energy intake and GE or OCTT cannot be determined from correlational analyses. Furthermore, the cross sectional nature of these studies means that an adaptation of accelerated GE may only have occurred after a long period of time of high intensity exercise. The majority of exercise intervention studies reviewed by Blundell & King (1999) showed that an increase in food intake did not automatically follow an increase in physical activity. However, more than half of correlational studies indicated a positive relationship between EI and physical activity suggesting that at some time point a balance is achieved between the two (Blundell & King 1999). It is possible therefore that GE may only adapt to exercise or reach a new steady state after a longer period of time than the 7 week intervention in the present study.

The increase in weekly energy expenditure may also not have been sufficient to significantly alter GE. The lowest compliers in the present study participated in 65% of exercise classes meaning an increase of eighty minutes moderate intensity exercise or approximately 573kcal energy expended (Schmitz et al 2005) per week. In contrast,
studies showing improvements in appetite control following 6 weeks intervention had a mean exercise time of approximately 187 minutes per week (Martins et al 2007). A recent 12 week supervised intervention demonstrated changes in appetite regulatory processes in previously sedentary individuals following exercise designed to expend 2500kcal per week (King et al 2009). Collectively therefore the finding that the 7 week moderate intensity exercise intervention did not significantly alter GE compared to a control group suggests various possibilities. It is possible that the acceleration in GE that has been previously observed in chronic exercisers is due to other associated lifestyle factors such as habitual diet. An alternative possibility is that a higher increase in weekly energy expenditure and/or increased duration of exercise intervention may be necessary to significantly alter GE.

Consistent with no significant effect of exercise intervention on GE, energy intake at the ad libitum buffet meal was not significantly altered by exercise intervention compared to changes in the control group. This is consistent with previous findings of no change in energy intake following 5 weeks of aerobic exercise in females (Epstein & Wing 1978). However, the method of assessing food intake was by food diary in that study. For the sample size in the present study minimum mean effect sizes would have to be \( \geq 380 \) kcal to detect an effect of intervention. Therefore, despite a mean reduction in total EI of 135kcal in the EXE group compared to a mean increase of 25kcal in the CTL group, the lack of statistical significance is not surprising. In a recent repeated measures study of 15 healthy males Nair et al (2008) reported that to detect a treatment effect minimum mean effect sizes for buffet meal EI would have to be \( \geq 219 \) kcal. Although, the sample size in the present study was slightly larger, restrained eaters were not excluded which may have influenced buffet EI findings as eating behaviours such as restraint are known to influence energy intake compensation (Ruderman 1986). Therefore, future studies with larger sample sizes and screening for restrained eaters may be needed to detect an effect of exercise intervention on energy intake at a buffet meal.

Despite no significant difference in gastric emptying or EI following exercise intervention compared to changes in the control group postprandial 30 minute hunger ratings were reduced. Reduced hunger sensations in the initial postprandial period could be important in preventing overconsumption at meals and further supports the hypothesis that exercise may have a beneficial effect on appetite control (Long et al 2002, Martins et al 2007). Interestingly, the exercise group also displayed a trend
towards an increased fasting hunger rating. Therefore, when considering the baseline rating was higher and the 30 minute postprandial rating was significantly reduced, the same test meal induced a significant suppression of hunger in the exercise group following intervention compared to changes in the control group. This dual process action of exercise on appetite control has recently been reported by King et al (2009) whereby exercise increases the drive to eat but concomitantly increases the satiating efficiency of a fixed meal. However, as GE was not significantly altered other mechanisms must be responsible for the observed changes in the present study. Evidence that implicates insulin sensitivity in the regulation of appetite is well reported (Haber et al 1977, Holt et al 1992) along with evidence to support an increase to insulin sensitivity in response to exercise (Aldred et al 1995, Poehlman et al 2000). Short term satiety gut peptides have also been suggested to play a role in changes in appetite control with exercise including ghrelin, cholecystokinin (CCK), peptide YY (PYY), and pancreatic polypeptide (PP) (Martins et al 2007). Recently, increased postprandial levels of GLP-1 were reported at 30 minutes but at no other time points following a 5 day exercise intervention in adolescent boys (Chanoine et al 2007). As GLP-1 is known to increase satiety (Crowell et al 2006) changes in the release of this signal may be another plausible mechanism accounting for the change in hunger sensations at this time point in the present study. However, as GLP-1 is known to delay GE (Camilleri 2009) changes in this signal were not likely as GE was not significantly different between exercise and control groups. Gastric emptying was the primary focus of this thesis and other physiological mechanisms involved in appetite control were not measured. Further understanding regarding the effects of exercise on tonic and episodic signals involved in appetite control is needed.

An additional interesting finding from the present study was the significant association between BMI and GE evident at baseline. This significant association of slower GE with increasing BMI in adolescent girls is a novel one, and confirms previous findings in other populations (Lavigne et al 1978, Brogna et al 1998). Hellmig et al (2005) observed no statistically significant correlation between GER and BMI in 90 adults using the $^{13}$COBT but did observe a trend towards slower GE of solids with increasing BMI. The lack of statistical significance in their study may be attributable to the large variability in participant characteristics such as gender (male and female) and age (20 -90). Madsen & Graff (2004) similarly demonstrated no association between BMI and GE but also in a population of both males and females of a large age range. As GE is influenced by a variety of factors such as volume (Mitchell & Voss 1991),
composition, energy content (Moore et al. 1984) and temperature of the meal (Sun et al. 1988), age (Moore et al. 1983), sex (Datz et al. 1987), physical activity (Marzio et al. 1991) and position of the subject (Moore et al. 1988), drug intake (Chaudhrui et al. 1990), and time of the day (Goo et al. 1987), this may account for the lack of significant association between GE and BMI in some studies. In contrast, all of these factors were controlled for in the present study.

Brogna et al. (1998) observed a significant positive association between GE half time and BMI in twelve healthy male adults ranging in BMI from 18.5 to 31.9 kg·m⁻², and suggested that this association may break down at extremes of the body mass spectrum (thinness and obesity). This may be one plausible explanation for the inconsistency in findings of studies comparing GER in lean and obese individuals. The present results suggest that it is important not to solely focus on comparing extremes of BMI. A slower GER with increasing BMI, even within the non-obese BMI range may potentially be a factor predisposing some adolescents to overweight or obesity in adulthood. However while correlations provide more insight into the association between BMI and GER, they do not provide a causal relationship, and it could also be speculated that a slower GER may be a consequence of increasing BMI. As adolescence may be a particularly vulnerable period for the development of obesity (Daniels et al. 2005), the causal nature of this relationship represents an area worthy of future study.

Interestingly, the association for the whole group between BMI and GE that was evident at baseline in the present study was weaker and non-significant at post-intervention. The small numbers in the different treatment groups (exercise (n = 9) and control (n = 10)) do not allow for meaningful individual group correlations at post-intervention. Nevertheless as the whole group association was weakened at post-intervention it could be speculated that the exercise intervention may have disturbed the association between BMI and GE evident at baseline. Figures 3.5 and 3.6 indicate no association between changes in GE half or latency times and changes in BMI in the whole group. As changes in BMI were relatively modest (range: ~ -2 to 1.5 kg·m⁻²), future exercise intervention studies involving larger sample sizes and inducing a significant reduction in BMI may yield further information on what could be a complex interactive association between BMI, GE, energy intake and exercise. This was beyond the scope of the current thesis but presents an area worthy of future study.
The present study has several limitations. Compliance with the intervention of 65% was relatively low. Compliance is an inherent difficulty in conducting PA interventions both within the school environment as classes were sometimes missed due to other school commitments and also more specifically with adolescent girls as issues known to reduce participation in PA such as body image, self esteem and negative stereotypes are more prevalent in this population (Neumark-Sztainer & Hannan 2000). Activities known to appeal to adolescent girls (McCallion 2004) were selected in an attempt to make the classes as appealing as possible.

The primary aim of the current study was to test the hypothesis that increasing PA levels would alter GE. However, the exercise intervention did not significantly increase physical activity levels. This is a consistent finding in school based exercise intervention studies in female adolescents despite other health benefits being reported (Brown et al 2009). The study was designed to objectively assess change in physical activity levels using accelerometry, however only a small number of valid data sets were obtained at baseline. Therefore, self report questionnaires completed by all subjects on the gastric emptying study days were instead used to assess change in PA. The questionnaire used is a method that has generally had strong correlation coefficients with other PA measures compared to other recall measures (Kowalski, Crocker, & Faulkner, 1997; Kowalski, Crocker & Kowalski, 1997) and has previously been used to investigate the relationship between PA and health outcomes (Bailey et al 1999; MacKelvie et al (2001b). However measuring change in PA by self report may not be sensitive enough to detect small changes in habitual PA that could have important health consequences. Accelerometry data from week 7 of the intervention indicated the exercise group were undertaking a mean 23.4 minutes moderate to vigorous physical activity per day compared to 9.1 minutes in the control group. Although the aspects of physical activity that have a protective effect against obesity have not been clearly defined the length of time spent at higher intensity physical activity may be a key factor (McMurray et al 2000). Large standard deviations indicate a much larger individual variability in daily PA in the exercise compared to the control group. Similarly self report data indicated a larger variability in subject’s percentage change in PA in the exercise compared to control group. It is possible that some subjects may have compensated for the increase in EE during exercise classes, by reducing activity during the rest of the day while others may have increased PA outside of exercise classes. Future studies with a greater number of participants would allow for the characterisation
of individual responses to PA and associated effects on parameters influencing food intake.

Another limitation of the current study is the lack of habitual food intake data, meaning it is not possible to quantify changes in daily EI in response to the intervention. Three day food diaries were administered to subjects prior to baseline and post-intervention testing. However on analysis the poor quality of records kept by subjects was deemed to give an inaccurate reflection of EI. Under reporting of EI is particularly problematic in adolescents and the interpretation of links between intake and health has been based often erroneously on the assumption of validity (Livingstone et al 2004). Consequently this data is not reported and the lack of habitual food intake data is instead recognised as a limiting factor of this study. Nevertheless food intake at an ad libitum buffet meal indicated no significant difference in changes in energy intake and macronutrient preferences from baseline to post-intervention between exercise and control groups.

There are also a number of strengths to the current study. Use of a homogenous subject population of similar age, gender and ethnicity minimized potential confounding factors of GE. In the intervention study, a 7 week intervention was conducted so that subjects were studied in the same phase of menstrual cycle on both test sessions, thus minimising the effect of cycle phase on EI (Buffenstein et al 1995). Furthermore, integrating the intervention into the school timetable meant the prescribed exercise was supervised.

Methodologically, there is a disconcertingly large variability in GE study protocols making it difficult to compare findings. The test meal used was similar to previous studies of children and adolescents (Maes et al 1995; Hauser et al 2006) and a protocol that has been validated against scintigraphy (Eradi et al 2006). Furthermore, a 250 kcal pancake meal in size resembles a normal breakfast for the subject population and a solid test meal is also more sensitive to detecting changes in GER than a liquid meal (Malmud et al 1982). An advantage of the $^{13}$C-OBT to measure GE over the widely considered ‘gold standard’ scintigraphy is that it does not expose subjects to radioactivity, and therefore it makes repeated measures studies involving healthy participants, such as the present one feasible. The test protocol was well tolerated by all participants, providing encouragement for future intervention studies in this population.
In summary, for the first time in a population of female adolescents a positive association between GE times and BMI was observed. Future studies involving larger sample sizes should examine the causal nature of this relationship. In addition, when compared to a control group no significant difference in GE or buffet meal energy intake but a significant reduction in postprandial 30 min hunger ratings was observed after a 7 week exercise intervention in adolescent girls. These findings support the contention that along with the many other reported benefits of PA, exercise may also improve appetite control. Exercise interventions of longer duration and/or increased weekly energy expenditure may be required to significantly alter GE. More integrative studies of gastrointestinal physiological responses to exercise may yield further information on the mechanisms behind changes in EI and appetite with exercise.
Chapter 4 The effect of a 10 week exercise intervention on gastric emptying, appetite, food intake and cardiac autonomic function in Multiple Sclerosis

Abstract

Background/Objectives: Autonomic dysfunction is common in Multiple Sclerosis (MS) patients and delayed gastric emptying (GE) has been previously reported. The aims of this study were to investigate the effect of a 10 week exercise intervention on GE, heart rate variability (HRV; as an indicator of autonomic balance), appetite sensations and food intake in MS patients.

Subjects/Methods: Twelve MS patients (BMI 27.5±4.5 kg·m⁻²; age 51.3±9.5 years) attended the laboratory at baseline and post-intervention. Six subjects participated in a ten week exercise intervention (1 physiotherapist led exercise class in addition to prescribed independent aerobic exercise per week; exercise group; (EXE): n = 6). Six others were instructed to maintain their habitual lifestyle over the same time period (control group (CTL); n = 6). GE was assessed by ¹³C octanoic acid breath test, HRV in the frequency domain from resting electrocardiogram, subjective appetite sensations by visual analogue scales and food intake by food diary.

Results: There were no significant correlations between any variables. Exercise intervention did not change GE or HRV. Mean daily energy intake increased in the EXE group compared to a decrease in the CTL group (p = .007). Area under the curve for 4hr postprandial hunger sensations was significantly suppressed in the EXE group following intervention compared to the CTL group (p=.047). A large inter and intra individual variability was evident in all parameters which may be attributable to the large variability in individual characteristics.

Conclusion: Findings indicate that GE rate is not significantly associated with cardiac autonomic function in MS. A ten week exercise intervention did not improve autonomic function as reflected by GE and HRV parameters but increased daily energy intake and decreased postprandial hunger sensations. The ¹³C-OBT is a promising method for future studies examining the effects of therapeutic interventions on GE in MS patients. Future studies with larger sample sizes are needed.

Key Words: Multiple Sclerosis, Exercise, Gastric Emptying, Appetite, Heart Rate Variability.
4.1 Introduction

Multiple Sclerosis (MS) is a chronic and often progressive disease of the central nervous system (CNS), characterised by disseminated patches of demyelination in the brain and spinal cord (Umphred 2001). Autonomic nervous system (ANS) dysfunction is a common phenomenon (Haensch et al 2006) and gastrointestinal (GI) disturbances are one of the most frequent (Haensch et al 2006), yet often overlooked (Hennessey et al 1999) autonomic symptoms in MS.

Gastric Emptying (GE) refers to the rate at which solids and/or liquids empty from the stomach into the small intestine. Impaired GE is a relatively frequent complication in various diseases (Chaudhrui & Fink 1991), yet little research has investigated GE in MS. One study has indicated delayed GE to be more prevalent in MS (El-Maghraby 2005), which may have many health implications. Both obesity and malnutrition are common with detrimental consequences to functional abilities (Payne 2000). The high incidence of overweight and obesity in MS has been attributed to fatigue causing a decrease in physical activity while energy intake is not down regulated (Hewson et al 1984). Persuasive evidence suggests that GE can affect appetite and energy intake (EI) (Sepple & Read 1989). Further understanding the physiological mechanisms mediating changes in EI such as GE in MS is important for preventing and treating obesity and malnutrition. Furthermore, MS patients often take many medications and therapeutic agents, and altered GE can affect the absorption of some drugs (Symonds et al 2003).

Although for many years patients with MS have been advised to avoid exercise in order to minimise exacerbations and fatigue (LaRocca & Kalb 1992), prescribed exercise does not appear to increase the rate or severity of exacerbations in MS (Petajan et al 1996). In fact many benefits of exercise have been demonstrated including increased quality of life, improved cardiorespiratory fitness, muscle function (Petajan et al 1996), fatigue, cognitive ability and energy (Paty 1993) and reductions in depression, anxiety and secondary complications associated with MS (Petajan et al 1996).

It is conceivable that exercise may also have a beneficial effect on GE, appetite and energy intake in individuals with MS. Prior cross-sectional research has demonstrated better appetite regulation (Long et al 2000) and a faster rate of GE (Carrio et al 1989, Shimamoto et al 2002) in active compared to sedentary individuals. A more
predominant parasympathetic tone as a result of athletic training has been proposed as an explanation for the latter finding (Carrio et al 1989).

The parasympathetic (vagal) nervous system can exert great influence on gastric motility (Lu et al 2000). Using heart rate variability (HRV) analysis to investigate autonomic function in MS patients, Diamond et al (1995) found significantly lower vagal power at rest compared to healthy controls. Therefore, parasympathetic nervous system dysfunction may be implicated in the delayed GE that has been previously observed in MS patients. As increased physical activity has been shown to increase vagal power in healthy adults (Yamamoto et al 2000), it is plausible that exercise may increase parasympathetic nervous system activity in MS patients and concomitantly accelerate GE.

Using a ten week exercise intervention involving physiotherapist led exercise classes run by the MS Society of Ireland, the hypotheses of this study were that 1) the exercise intervention would lead to an acceleration in GE 2) this would be associated with changes in HRV as an indicator of the interaction between sympathetic and parasympathetic activity in autonomic functioning, and 3) an accelerated GE would alter appetite sensations and energy intake in MS patients. Optimal dietary intake and physical activity are important to promote the health of all people, including those with a chronic illness such as Multiple Sclerosis (Goodman & Gullick 2008). An increased understanding of the pathophysiological mechanisms in autonomic dysfunction in MS, along with the effects of therapeutic interventions such as physical activity will assist in the understanding and implementation of more effective strategies to enhance wellbeing in individuals with MS.
4.2 Methods

The exercise intervention presented in this chapter was conducted by chartered physiotherapist Maria Garrett as part of the 10 week ‘Getting the Balance Right Programme’ offered by the MS Society of Ireland to MS Patients. The exercise programme was designed for MS patients specifically by Dr. Susan Coote and her research team in the Physiotherapy Department at the University of Limerick. Compliance with the intervention reported in this chapter was monitored by the chartered physiotherapist. All other data collected and reported was undertaken solely by the author and is presented solely in this thesis.

4.2.1 Subjects

Following ethical approval from the Health Service Executive Mid-Western Area Research Ethics Committee (Appendix E), fifteen subjects were recruited through the MS Society of Ireland. Patients with Relapsing Remitting, Primary Progressive and Secondary Progressive MS were included. All subjects’ general practitioners were informed of their patient’s involvement in the study. At this stage one subject was excluded from participating as they did not have a confirmed diagnosis of MS. Other exclusion criteria were those with other possible causes of dysautonomia such as diabetes mellitus, a Guy’s Neurological Disability Scale (GNDS) (Paltamaa et al 2005) > 2, those who were experiencing an exacerbation of symptoms due to relapse or had had steroid treatment within 3 months of baseline, those under 18 years of age and individuals with a history of gastrointestinal surgery.

Subjects were assigned to either an exercise group (EXE; n = 7) or control group (CTL; n = 8). The study was not randomised as the exercise interventions were being run by the MS Society of Ireland independently of the present research study and the opportunity to participate in the exercise interventions was offered by the MS Society to all MS patients. Recruitment for the present study was a separate process. Patients who had decided to participate in the exercise intervention were given the option of also participating in the present research study and were automatically assigned to the EXE group. Patients who volunteered for the present study but had decided not to participate in an exercise intervention were assigned to the CTL group. These patients were instructed to maintain their normal lifestyle and were considered controls on the assumption that they were maintaining their habitual physical activity levels between baseline and post-intervention. Those in the CTL group were given the option of
undertaking the exercise programme at a later date. Each participant gave informed consent to participate in the study.

4.2.2 General Study Protocol

Testing was completed during 2 visits to the laboratory, approximately eleven weeks apart. One or sometimes 2 subjects were tested per day. The first testing session took place prior to the exercise intervention (baseline) and the second within 2 weeks of intervention completion (post-intervention). Both testing sessions followed an identical protocol. To reduce inherent variability, subjects were instructed to refrain from moderate to heavy exercise and consuming alcohol for 24 hours beforehand. In addition, all subjects used the same mode of transport (car) to get to the laboratory on the morning of testing and arrived following a standard 12 hour overnight fast.

4.2.3 Anthropometry

Height was measured without shoes, to the nearest 0.1cm using a freestanding stadiometer (Holtain Ltd, Great Britain). Weight was measured in light clothing without shoes on platform scales (SECA, Germany) to the nearest 0.01kg. BMI was calculated as weight in kilograms divided by the square of height in meters.

4.2.4 Heart Rate Variability

Following anthropometric measurements, a three lead ECG was recorded using Powerlab (ADInstruments, Hastings, UK) for 20 minutes in the relaxed sitting position. The room was temperature controlled (23°C), quiet and darkened to minimize arousal stimuli. A naturalistic breathing pattern was accepted as enforced respiratory rate introduces an independent effect on HRV (Guzik et al 2007). HRV data was analysed from t10min to t15min from the 20 min ECG trace to ensure subjects had sufficient time to attain baseline autonomic function (5–10min). HRV data was derived using Chart 5 HRV module software (AD Instruments, Hastings, UK). Intervals between adjacent r waves were detected using a threshold detection from 0.5 to 1.0 mV and classified as artifact (<250ms and >2000ms), ectopic (250ms to 500ms and 1600ms to 2000ms), and normal (500 to 1600ms). In addition, each ECG trace was manually scanned for the identification of artifacts which were excluded. The frequency domain parameters measured were: total power (TP), high frequency power (HF) (0.15–0.4 Hz), low frequency power (LF) (0.04–0.15 Hz), the LF/HF ratio, and HF and LF expressed in normalised units (HFnu and LFnu, respectively). The power in the low frequency (LF) band represents sympathetic activity and part of parasympathetic activity, whereas HF
power is almost entirely mediated by vagal or parasympathetic activity. The LF/HF power ratio indicates sympatho-vagal balance. High values suggest a sympathetic predominance.

4.2.5 Gastric Emptying Measurement

Gastric emptying parameters were calculated using the $^{13}$C-octanoic acid breath test ($^{13}$C-OBT), a method which has been validated and described in detail previously (Maes et al 1998). Briefly, the egg yolk of a standardized pancake breakfast meal (250 kcal; 26g CHO, 13g PRO, 11g Fat) was labeled with 100mg $^{13}$C-octanoic acid (Eurisotop, Saint-Aubain, France). The meal (identical to that described in section 3.2.4.1) was ingested within 10 minutes together with 150ml of water. Where subjects usually took daily medication with breakfast, medication was taken as normal at this time. Breath samples were obtained in duplicate at baseline, every 5 minutes for the first 30 minutes and subsequently every 15 minutes until 4 hours after ingestion of the test meal. The study period was spent in sedentary activities such as reading and watching television in the metabolic suite. Room temperature was controlled at 23°C on all study days.

4.2.5.1 Stable isotope $^{13}$C breath test data analysis

$^{13}$C-isotopic enrichment of breath samples was measured using continuous flow isotope ratio mass spectrometry (ABCA; Europa Scientific, Crewe, UK). The results were expressed relative to Pee Dee Belemnite, the internationally agreed standard of known $^{13}$C concentration. Breath $^{13}$CO$_2$ enrichment data were fitted to the established gastric emptying model to derive the outcome measures lag time ($t_{lag}$) and half time ($t_{1/2}$) corrected to scintigraphy equivalent values using the regression equations defined by Ghoos et al. (1993). For all data $r^2$ coefficient between the modelled and raw data was calculated and $r^2 > 0.90$. The parameters latency time ($t_{lat}$) and ascension time ($t_{asc}$), defined by Schommartz et al (1998) were also calculated.

4.2.6 Physical Activity

The short form of the International Physical Activity Questionnaire (IPAQ) (Craig et al 2003, Appendix F) was used to assess change in PA level. The IPAQ contains six items that measure the frequency and duration of walking, moderate and vigorous activities over the previous 7 day period. As used in previous PA research in MS patients (Motl et al 2009, Gosney et al 2007) frequencies were multiplied by 8 (vigorous), 4 (moderate) and 3.3 (walking) metabolic equivalents and then summed to get a continuous measure of PA.
4.2.7 Appetite
Measures of hunger, desire to eat and fullness were assessed with 100mm visual analogue scales (VAS) (Flint et al 2000) before (t -10min) and at 30 minute intervals after (t 0, 30, 60, 90, 120, 150, 180, 210, 240) the pancake test meal on gastric emptying study days. The area under the time curve (AUC) for each of the feelings was calculated using the trapezoidal rule. Variables thirst, coldness and tiredness were used to distract subjects from their satiety status.

4.2.8 Food Intake
Habitual food intake was assessed by 4 day food diary. Participants were instructed to record using a weighing scales all food and drinks consumed and supplements and medications taken for the rest of the day after the gastric emptying study day and for the following 3 days. 24 hour energy intake was measured from the gastric emptying study day and 3 day intake averaged for the following 3 days to detect changes in energy intake. Total amount (g and kcal) and macronutrient content of food/drink consumed was also calculated. Food diaries were analysed using WinDiets (Robert Gordons University, Aberdeen, UK).

4.2.9 Exercise Intervention
Exercise classes were delivered by the same chartered physiotherapist over 10 weeks. Classes followed the Strand A aerobic exercise and strength training intervention as part of the ‘Getting the Balance Right Programme’ described in detail elsewhere (Coote et al 2009). Briefly, participants attended an hour long circuit class once a week for 10 weeks. Classes included a circuit consisting of sit to stand/squat, bridging, resisted shoulder flexion, walking/bike, resisted elbow flexion, lunges or resisted knee extension, hip extension and calf raises. All strength exercises were completed at 50-80% one repetition maximum. Each participant was given a target heart rate calculated using the Karvonen formula (Target Heart Rate = Resting Heart Rate (RHR) + 65% Maximum Heart Rate (MHR). MHR was calculated as age predicted maximum heart rate (220-age). Participants were instructed to take their own RHR by feeling their carotid pulse for 30 seconds on waking. If unable to detect a pulse, participants were instructed to exercise at a rate of perceived exertion level of 11-14. Participants were instructed to complete 2 independent sessions of aerobic activity per week for twenty-thirty minutes, and at week 5 this was increased to three times per week. To ensure correct exercise intensity, participants were instructed to check their pulse every ten
minutes during aerobic exercise. Compliance was monitored by the chartered physiotherapist.

4.2.10 Statistical Analysis
Statistical analysis was carried out using SPSS for Windows (version 16.0, SPSS Inc., Chicago, IL, U.S.A.). Values are reported as mean +/- standard deviation. All variables were checked regarding their normal distribution using the Shapiro-Wilk test. Independent Student’s t tests were used to compare baseline descriptive characteristics and percentage change over the intervention between EXE and CTL groups. Fisher’s Exact Test was used to compare discrete baseline variables between groups. Mixed ANOVA was used to examine main effects [group (EXE vs. CTL), time (baseline vs. post exercise)] and their interaction comparing baseline and post exercise variables for PA level, body composition, GE, HRV, appetite and food intake. If the model assumptions were violated (non-normality of the residuals), a log-transformation was applied before the statistical analysis. Pearson’s correlations were used to test for correlations between variables. Retrospective power analysis was carried out to determine the suitability of the sample size employed and minimum effect sizes required to detect a hypothetical treatment effect (with 80% power) were determined. Statistical significance was set at P ≤ 0.05.
4.3 Results

All subjects completed the test meal within 10 minutes. Two subjects reported feeling nauseous after the meal. All other subjects reported the meal to be palatable and tolerated the tests well.

4.3.1 Sample Characteristics

Two subjects in the CTL group did not return for post-intervention testing, one due to time commitments and the other due to injury (unrelated to the study). There were no significant differences in their baseline characteristics compared with those who completed the study. Data is reported for the twelve subjects (n = 12; (EXE n = 6, CTL n = 6)) who completed all elements of the study. Descriptive demographic and clinical characteristics for each subject at enrollment into the study are outlined in Table 4.1.

Table 4.1 Individual Demographic and Clinical Characteristics at enrollment

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Type of MS</th>
<th>Time Since Diagnosis (yrs)</th>
<th>GNDS</th>
<th>Other condition (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>RRMS</td>
<td>18</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>RRMS</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>SP</td>
<td>14</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>RRMS</td>
<td>7</td>
<td>1</td>
<td>IBS</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>RRMS</td>
<td>8</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>RRMS</td>
<td>19</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>CTL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>SP</td>
<td>15</td>
<td>0</td>
<td>High BP</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>SP</td>
<td>9</td>
<td>1</td>
<td>High BP</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>SP</td>
<td>7</td>
<td>0</td>
<td>High BP</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>RRMS</td>
<td>9</td>
<td>1</td>
<td>IBS</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>SP</td>
<td>5</td>
<td>2</td>
<td>Reflux</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>RRMS</td>
<td>5</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>

RRMS = Relapsing Remitting Multiple Sclerosis, SP = Secondary Progressive, GNDS = Guys’ Neurological Disability Scale, BP = Blood Pressure, IBS = Irritable Bowel Syndrome, Other condition (s) = Any other diagnosed medical condition in addition to MS.

4.3.1.2 Concurrent Treatment in MS patients

2 patients in the CTL group were taking antibiotics (not known to influence GI motility) for urinary tract infection at post-intervention, but not at baseline. Considerable supplementation to food intake including multivitamins and minerals was reported as
has previously been observed in MS patients (Goodman & Sullick 2007). For MS treatment 2 patients were receiving Tysabri treatment, four patients were taking Avonex injections and 2 patients Copaxone injections.

One CTL and one EXE subject were smokers. Two subjects who had previously been diagnosed with IBS (Table 4.1) were not taking any medication known to influence GI motility. Three subjects with high BP assigned to the CTL group (Table 1) maintained the same medication routine throughout the study period. Similarly, one subject in the CTL group previously diagnosed with reflux remained on the same medication (known to influence GI motility) routine throughout the study period. Mean demographic, clinical and anthropometric characteristics of participants who completed the study were not significantly different between CTL and EXE groups at baseline (Table 4.2).

**Table 4.2 Group baseline characteristics**

<table>
<thead>
<tr>
<th></th>
<th>CTL (n = 6)</th>
<th>EXE (n = 6)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>48.0 ± 10.6</td>
<td>54.6 ± 7.8</td>
<td>.243</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.1 ± 7.9</td>
<td>168.3 ± 10.2</td>
<td>.875</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.8 ± 15.6</td>
<td>77.3 ± 8.9</td>
<td>.845</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>27.3 ± 6.6</td>
<td>27.6 ± 1.3</td>
<td>.892</td>
</tr>
<tr>
<td>Time since diagnosis (yrs)</td>
<td>8.3 ± 4.4</td>
<td>11.2 ± 7.0</td>
<td>.403</td>
</tr>
<tr>
<td>GNDS</td>
<td>1.0 ± 0.9</td>
<td>1.3 ± 0.8</td>
<td>.515</td>
</tr>
<tr>
<td>Type of MS</td>
<td></td>
<td></td>
<td>.242</td>
</tr>
<tr>
<td>SP</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>RRMS</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD. All p values refer to Independent Student’s two-tailed t tests apart from Type of MS which was compared using Fisher’s exact test.

**4.3.2 Effect of Exercise Intervention**

**4.3.2.1 Compliance**

Participants undertook walking, ergometer rowing, cycling and aqua aerobics to fulfill the independent aerobic exercise component of the intervention. Mean attendance at exercise classes was 68%. One subject attended 3 exercise classes as transport to the classes was a problem. However, the subject self reported to the physiotherapist to follow the exercise programme independently weekly. All others completed between 6 and 9 of the 10 exercise classes.
4.3.2.2 Physical Activity
Baseline physical activity levels were not significantly different between groups (p = .527, Table 4.3). The EXE group increased physical activity levels significantly compared to the control group between baseline and post-intervention (significant time x group interaction (F (1, 10) = 8.18, p = .017, Table 4.3)).

4.3.2.3 Anthropometric Characteristics
BMI was significantly reduced in the whole group at post-intervention compared to baseline (main effect of time F (1, 10) = 8.57, p = .015)). There were no significant main effects of group or time and no significant time x group interaction for weight (Table 4.3).
Table 4.3 Anthropometric, Physical Activity and Gastric Emptying Results at baseline and post-intervention in EXE (n = 6) and CTL (n = 6) groups.

<table>
<thead>
<tr>
<th></th>
<th>CTL (n = 6) Baseline</th>
<th>Post</th>
<th>% change</th>
<th>EXE (n = 6) Baseline</th>
<th>Post</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>75.8 ± 15.6</td>
<td>74.3 ± 14.9</td>
<td>-1.9 ± 2.8 (4.84, 1.04)</td>
<td>77.3 ± 8.9</td>
<td>76.7 ± 8.6</td>
<td>-7 ± 1.6 (-2.43, 1)</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>27.3 ± 6.6</td>
<td>26.6 ± 6.4</td>
<td>-2.5 ± 2.6 (-5.28, 0.49)</td>
<td>27.6 ± 1.3</td>
<td>27.3 ± 1.2</td>
<td>-1.3 ± 1.7 (-3.04, 0.48)</td>
</tr>
<tr>
<td>PA (mets/wk)</td>
<td>2336 ± 2311</td>
<td>1812 ± 1397</td>
<td>2.8 ± 102 (-104.7, 110.28)</td>
<td>1634 ± 1248</td>
<td>2470 ± 1330</td>
<td>99 ± 111 (-16.8, 216.61)</td>
</tr>
<tr>
<td>t₁/2 (mins)</td>
<td>98.3 ± 54.5</td>
<td>99.6 ± 86.7</td>
<td>-19 ± 58 (-80.85, 41.8)</td>
<td>87.3 ± 49.2</td>
<td>84.3 ± 32.9</td>
<td>-4.1 ± 34 (-40.50, 68.15)</td>
</tr>
<tr>
<td>tᵦ (mins)</td>
<td>33.8 ± 18.2</td>
<td>32.3 ± 27.7</td>
<td>-3 ± 45 (-50.6, 44.47)</td>
<td>29.9 ± 18.3</td>
<td>29.5 ± 14.1</td>
<td>12 ± 53 (-43.5, 68.15)</td>
</tr>
<tr>
<td>tᵯ (mins)</td>
<td>149 ± 58.3</td>
<td>146.4 ± 80.7</td>
<td>-9 ± 29 (-39.15, 21.1)</td>
<td>136.2 ± 49</td>
<td>133.9 ± 34.3</td>
<td>-2 ± 24 (-27.08, 22.97)</td>
</tr>
<tr>
<td>tᵲ (mins)</td>
<td>27 ± 6.3</td>
<td>31.2 ± 18</td>
<td>10 ± 42 (-34.21, 54.15)</td>
<td>27.5 ± 8</td>
<td>26.5 ± 6.3</td>
<td>2.8 ± 34 (-33.39, 39.03)</td>
</tr>
</tbody>
</table>

Data are means ± SD. 95% confidence intervals for mean % change are indicated in brackets in mean % change column. †Significant time by group interaction p < .05. ††Main effect of time p < .05.
4.3.2.4 Gastric Emptying

There were no significant differences between CTL and EXE groups in any gastric emptying parameters at baseline ($t_{1/2}$ $p = .704$, $t_{asc}$ $p = .689$, $t_{lag}$ $p = .704$, $t_{lat}$ $p = .994$; analysis based on log transformed data). There were no statistically significant changes in GE. There were no significant main effects of group or time and no significant time x group interaction for any gastric emptying parameters ($p > .05$) (Table 4.3) (analysis based on log transformed data). Figure 4.1 illustrates individual changes in GE half time from baseline to post-intervention in A) EXE and B) CTL groups. Dashed lines indicate an increase (i.e. delay) in $t_{1/2}$ and solid lines a decrease (i.e. acceleration).

![Figure 4.1 Individual changes in gastric emptying half time ($t_{1/2}$) in A) EXE ($n = 6$) and B) CTL ($n = 6$) groups from baseline to post-intervention. Legend indicates subject number. — indicates group mean.](image)

To assess the possibility of a type 2 error, a retrospective power analysis was performed using the data of control and exercise subjects. 48 subjects were required in each group to detect a treatment difference of $1/3$ gastric emptying half time (29 min) at
a two sided 5% significance level, based on the assumption that the standard deviation of the response variable was 54.5 min (Motulsky, 1995).

4.3.2.5 Heart Rate Variability

Resting heart rate (RHR) derived from the 5 min ECG recording decreased non-significantly from 67 ± 8 (95% CI: 59-75) beats·min at baseline to 65 ± 8 (95% CI: 57-74) beats·min at post-intervention in the EXE group. In the CTL group, RHR was 70 ± 15 (95% CI: 54-86) beats·min and 72 ± 17 (95% CI: 54-90) beats·min at baseline and post-intervention respectively. The change in RHR from baseline to post-intervention did not reach statistical significance as there were no significant main effects or time x group interaction for resting heart rate (p > .05).

Table 4.4 shows the raw data for HRV in the frequency domain for the CTL and EXE groups at baseline and post-intervention. Statistical analyses of HRV data carried out following transformation using a natural logarithmic function indicated no significant main effects or interactions for any HRV parameters.

**Table 4.4 Mean ± SD heart rate variability in the frequency domain at baseline and at post-intervention in the EXE and CTL groups. LF: 0.04-0.15 Hz, HF: 0.15-0.4Hz.**

<table>
<thead>
<tr>
<th></th>
<th>Total power (ms²)</th>
<th>LF (ms²)</th>
<th>HF (ms²)</th>
<th>LF (N.U.)</th>
<th>HF (N.U.)</th>
<th>LF/HF ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EXE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2218 ± 1548</td>
<td>369 ± 235</td>
<td>150 ± 138</td>
<td>66 ± 14</td>
<td>28 ± 11</td>
<td>3.7 ± 4.2</td>
</tr>
<tr>
<td>Post</td>
<td>2065 ± 2043</td>
<td>400 ± 217</td>
<td>166 ± 144</td>
<td>62 ± 27</td>
<td>32 ± 23</td>
<td>2.9 ± 2.0</td>
</tr>
<tr>
<td><strong>CTL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1408 ± 1026</td>
<td>377 ± 289</td>
<td>226 ± 113</td>
<td>57 ± 13</td>
<td>38 ± 15</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>Post</td>
<td>1388 ± 886</td>
<td>470 ± 264</td>
<td>183 ± 126</td>
<td>69 ± 16</td>
<td>28 ± 15</td>
<td>3.7 ± 3.2</td>
</tr>
</tbody>
</table>

Data are means ± SD.

4.3.2.6 Food Intake

One subject in the CTL group did not complete full food diary records citing being too tired to fill it in as a reason. Therefore food intake data as assessed by food diary is reported for all other subjects (CTL, n =5; EXE, n = 6) in Table 4.5. There were no significant main effects or time x group interaction for 24 hour EI (energy intake for the remainder of the gastric emptying study day) (Table 4.5). There was a significant time x
group interaction for mean energy intake from 3 day food diary records \((F (1, 9) = 11.89, p= .007)\) (Table 4.4) indicating the EXE group responded differently to the CTL group over the intervention. As shown in Table 4.5, the EXE group showed an increase in total EI compared to a decrease in the CTL group. There were no significant main effects or time x group interactions for macronutrient preference from 3 day food diary diaries \((p > .05)\) (Table 4.5).

**Table 4.5 24 hour energy intake (24 hours starting with gastric emptying test breakfast), average daily energy intake and macronutrient distribution over the following 3 days at baseline and at post-intervention assessed by 4 day food diary.**

<table>
<thead>
<tr>
<th></th>
<th>CTL (n = 5)</th>
<th>EXE (n = 6)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>24 hour</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EI (kcal)</td>
<td>1126 ± 525</td>
<td>1266 ± 420</td>
<td>-11.55 ± 32.59</td>
<td>1373 ± 431</td>
<td>1322 ± 691</td>
</tr>
<tr>
<td></td>
<td>(-63.41, 40.33)</td>
<td></td>
<td></td>
<td>(47.05, 52.15)</td>
<td></td>
</tr>
<tr>
<td><strong>Average 3 day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EI (kcal)†</td>
<td>1777 ± 369</td>
<td>1237 ± 230</td>
<td>-26.91 ± 24.72</td>
<td>1324 ± 148</td>
<td>1459 ± 207</td>
</tr>
<tr>
<td></td>
<td>(-57.6, 3.7)</td>
<td></td>
<td></td>
<td>(3.63, 16.39)</td>
<td></td>
</tr>
<tr>
<td>Fat (%)</td>
<td>15.1 ± 7.4</td>
<td>15.1 ± 8.6</td>
<td>-19.5 ± 30.7</td>
<td>20 ± 5.2</td>
<td>19.8 ± 5</td>
</tr>
<tr>
<td></td>
<td>(-57.63, 18.63)</td>
<td></td>
<td></td>
<td>(-13.75, 29.83)</td>
<td></td>
</tr>
<tr>
<td>CHO (%)</td>
<td>64.1 ± 13.2</td>
<td>65.6 ± 14.3</td>
<td>-17.5 ± 25.55</td>
<td>56.5 ± 10</td>
<td>56.9 ± 11.4</td>
</tr>
<tr>
<td></td>
<td>(-49.23, 14.22)</td>
<td></td>
<td></td>
<td>(-8.13, 24.41)</td>
<td></td>
</tr>
<tr>
<td>PRO (%)</td>
<td>20.7 ± 6.5</td>
<td>19.3 ± 7.3</td>
<td>-23.95 ± 31.2</td>
<td>23.5 ± 6.4</td>
<td>23.3 ± 7.4</td>
</tr>
<tr>
<td></td>
<td>(-62.7, 14.8)</td>
<td></td>
<td></td>
<td>(-13.07, 26.34)</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD. 95% confidence intervals for mean % change are indicated in brackets in mean % change column.
† Significant time x group interaction \((p < .01)\).
* Percentage change significantly different to CTL \((p < .05)\).

**4.3.2.7 Subjective Appetite Sensations**

Changes in subjective appetite sensations from baseline to post-intervention are illustrated in Figure 4.2. There were no significant effects or interactions for changes in baseline hunger and fullness \((p > .05)\). However, there was a significant time x group interaction for baseline desire to eat \((F (1, 10) = 5.25, p = .046)\). As illustrated in Figure 4.2, the EXE group had a lower desire to eat at post-intervention compared to an increase from baseline to post-intervention in the CTL group.
There was a significant time x group interaction for hunger AUC (F (1, 10) = 5.1, p = .047). As evident in Figure 4.2 hunger AUC was lower at post-intervention indicating reduced postprandial hunger sensations in the EXE group compared to an increase in hunger AUC in the CTL group from baseline to post-intervention. Mean hunger AUC decreased in the EXE group from 11012 ± 3911 (95% CI: 6908-15117) mm·min at baseline to 8137 ± 3884 (95% CI: 4061-12213) mm·min at post-intervention compared to an increase in the CTL group from 7569 ± 3759 (95% CI: 3623-11514) mm·min at baseline to 9754 ± 5631 (95% CI: 3844, 15633) at post-intervention. The time x group interaction for desire to eat AUC was approaching significance (F (1, 10) = 3.7, p = .082) and followed a similar pattern to changes in hunger AUC in both groups. There were no time x group interactions for fullness AUC.
Figure 4.2 Mean ± SD (error bars) absolute ratings of subjective hunger at baseline (unfilled markers) and post-intervention (filled markers) in A: exercise (EXE (n = 6)) and B: control (CTL (n = 6)) groups. Breakfast was consumed between t = -10 and t = 0 min. † Significant time x group interaction for hunger ratings area under the curve (p = .046). * Significant time x group interaction for baseline desire to eat (p = .047).
4.3.2.8 Correlations

4.3.2.8.1 Associations between variables at baseline
There were no significant correlations between GE lag or half times with other variables at baseline (Table 4.6). In addition there were no significant correlations or approaching significance between GE ascension or latency times and other variables (p > .05).

**Table 4.6 Correlations between GE lag and half times and age, BMI, PA, energy intake and heart rate variability parameters at baseline.**

<table>
<thead>
<tr>
<th></th>
<th>GE $t_{lag}$</th>
<th>GE $t_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>$r = .33$, $p = .32$</td>
<td>$r = -.24$, $p = .48$</td>
</tr>
<tr>
<td>BMI (kg·m$^{-2}$)</td>
<td>$r = .03$, $p = .92$</td>
<td>$r = .13$, $p = .70$</td>
</tr>
<tr>
<td>PA (mets·wk)</td>
<td>$r = -.01$, $p = .98$</td>
<td>$r = -.10$, $p = .77$</td>
</tr>
<tr>
<td>24hr EI (kcal)</td>
<td>$r = .03$, $p = .94$</td>
<td>$r = .18$, $p = .62$</td>
</tr>
<tr>
<td>3dayEI (kcal)</td>
<td>$r = .10$, $p = .76$</td>
<td>$r = .02$, $p = .96$</td>
</tr>
<tr>
<td>LF/HF ratio</td>
<td>$r = -.15$, $p = .65$</td>
<td>$r = .18$, $p = .59$</td>
</tr>
<tr>
<td>Total power (ms$^{-2}$)</td>
<td>$r = -.48$, $p = .13$</td>
<td>$r = -.47$, $p = .26$</td>
</tr>
<tr>
<td>LF (nu)</td>
<td>$r = -.10$, $p = .77$</td>
<td>$r = .08$, $p = .82$</td>
</tr>
<tr>
<td>HF (nu)</td>
<td>$r = .06$, $p = .86$</td>
<td>$r = .12$, $p = .72$</td>
</tr>
</tbody>
</table>

BMI: Body Mass Index, EI: Energy Intake, LF: Low Frequency, HF: High Frequency

4.3.2.8.2 Associations between changes in variables from baseline to post-intervention
There was no significant association between changes in LF/HF ratio and GE parameters (% change GE $t_{1/2}$ and HF/LF ratio illustrated in Figure 4.3), indicating changes in gastric emptying were not significantly associated with changes in parasympathetic cardiac autonomic activity.

![Figure 4.3](image-url) % Change in GE half time ($t_{1/2}$) plotted against % change in HF/LF ratio from baseline to post-intervention for the whole group (n = 9 (excluding n = 3 taking blood pressure medication)); $r = .236$, $p = .485$. 82
Figure 4.4 % Change in GE half time ($t_{1/2}$) plotted against % change in physical activity for the whole group ($n = 12$); $r = .100$, $p = .770$. 
4.4 Discussion

Autonomic dysfunction is common in MS patients and gastrointestinal autonomic disturbances are often encountered (El-Maghraby et al 2005). In healthy populations regular physical activity has been implicated as a factor which accelerates gastric emptying (Carrio et al 1989, Shimamoto et al 2002) and which beneficially affects heart rate variability (HRV) (Swenne et al 1999), suggesting exercise may be a potentially effective therapeutic strategy for improving autonomic function in MS. HRV measurement is an indicator of autonomic balance which has been directly related to gastrointestinal autonomic activity (Mazur et al 2007). The primary aim of the present study was to determine the effect of a ten week exercise intervention on autonomic function and the association between changes in GE and cardiac autonomic function in MS patients, using the $^{13}$C-octanoic acid breath test to assess gastric emptying and HRV analysis to assess cardiac autonomic function.

No statistically significant effect of exercise on either parameter was found when compared to a control group of MS patients suggesting that exercise does not improve autonomic function in MS. In addition, there was no significant association between GE and HRV and between changes in both parameters from baseline to post-intervention. Weekly physical activity levels increased in the exercise group between baseline and post-intervention compared to the control group indicating the intervention was effective in increasing physical activity levels. The finding of no change in GE with increasing PA therefore contrasts with previous findings of a faster rate of GE in more active individuals (Shimamoto et al 2002, Carrio et al 1989). However, these studies were cross sectional and therefore adaptations in GE may only have occurred after a much longer time period of physical activity or may be related to other associated lifestyle or cognitive factors in chronic exercisers such as diet. It is possible that intervention studies with an increased number and intensity of sessions may have more significant effects on altering GE.

Similar to the finding of no change in GE, the finding of no change in resting HR or any HRV parameters contrasts with previous findings of reduced heart rate and increased parasympathetic activity with endurance training (Swenne et al 1999, Yamamoto et al 2001). Using an endurance training program of cycle ergometer exercise at 80% VO$_{2}$max for 40 minutes 4 days per week over 6 weeks, Yamamoto et al (2000) found a mean decrease in resting HR of 15 bpm as a result of the endurance training. An enhanced parasympathetic modulation was suggested by the authors to
contribute in part to the decreased resting HR observed. In the present study a non-significant mean decrease of 2bpm and non-significant increase in HF power (indicating parasympathetic activity) in the exercise group was observed at post-intervention compared to baseline. As increased parasympathetic tone has been proposed as one explanation for an accelerated GE in more active individuals (Carrio et al 1989), the non-significant change in parasympathetic activity in the present study may be one explanation for the lack of significant change in gastric emptying with exercise.

An additional factor which may account for the non-significant change in GE in the present study is the small sample size. As retrospective power analysis indicated that to detect a treatment effect of 1/3 GE half time 48 subjects would be required in both exercise and control groups, the sample size in the present study of 6 individuals per group suggests that type II error is highly likely. Therefore, it is not possible to clearly conclude that exercise does not improve GE based on the present study. However this study provides an idea for future studies of sample sizes required to detect an effect of therapeutic intervention on GE in MS patients.

To further examine the association between HRV and gastrointestinal autonomic activity that has been suggested by others (Mazur et al 2007) and to examine if autonomic dysfunction was evident in GE and HRV parameters in the population tested in the present study, baseline results can be interpreted. Previous cross sectional research has indicated a slower rate of GE in MS patients compared to healthy controls. Using scintigraphy to measure GE in 44 MS patients, El-Maghraby et al (2005) reported a mean (SD) half time of 98.6 ± 22.4 minutes in MS patients compared to 41.3 ± 18.7 minutes in healthy controls. In the present study at baseline mean (SD) GE half time was 92.8 ± 49.9 minutes for the whole group of MS patients (n = 12). Although, the mean half time observed in in the present study is comparable to that observed previously in MS patients (El-Maghraby et al 2005), it is not possible to conclude that GE was delayed in the subject population in the present study as a different method of GE measurement and a different test meal were used. In addition, a healthy control group were not tested. However, in a previous study of healthy adolescent females (chapter 3) using the same test meal and method as this study, a mean half time of 79.7 ± 17.2min was observed suggesting that GE is not significantly slow in the population of MS patients tested in the present study. Ghoos et al (1993) using similar methods to the present study found a normal range of GE $t_{1/2}$ to be between 20 and 118 minutes in healthy adults further suggesting that mean GE $t_{1/2}$ was not abnormally slow in the MS
patients tested in the present study. At baseline two out of all patients (n = 12) had GE half times greater than 118 minutes.

In contrast, results of HRV analysis at baseline indicate impairment in cardiac autonomic function. The ratio of low frequency to high frequency power represents overall cardiac sympathovagal balance, and a reference range of 1.5-2.0 is cited for healthy adults (Odemuyiwa et al 1991). In the present study a mean (SD) LF:HF ratio of 3.7 ± 4.2 in the exercise group and 1.8 ± 0.8 in the control group was observed at baseline. Excluding the 3 subjects in the control group taking antihypertensive medication, a factor known to influence HRV (Schroeder et al 2003), mean (SD) LF:HF ratio was 3.2 ± 3.3 for the rest of the patients (n = 9) in the present study at baseline. A higher LF:HF ratio outside the normal reference range is consistent with a predominance of cardiac sympathetic activity in this population (Diamond et al 1995).

In addition to no significant correlations between GE and HRV parameters, a HF:LF ratio outside the reference range but a relatively normal GE indicates no significant association between cardiovascular and gastrointestinal autonomic neuropathy in the population of MS patients tested in the present study. This is consistent with previous findings of no association between cardiac and gastrointestinal neuropathy in diabetic patients using similar study methodologies (Zahn et al 2003) and suggests that it is not possible to predict disordered GE on the basis of other autonomic function disorders in MS. However, these results need to be interpreted with caution because the sample size was small with large individual variation.

A secondary aim of the present study was to investigate the effects of the exercise intervention on appetite and energy intake. It was hypothesized that changes in gastric emptying as a result of the exercise intervention would alter appetite sensations and energy intake. In contrast to this hypothesis, despite observing no significant change in GE as a result of the intervention there was a significant change in appetite sensations. Postprandial hunger sensations decreased in the exercise group following exercise intervention compared to an increase in the control group. This would suggest that mechanisms other than gastric emptying may have been mediating changes in appetite in the present study. The neuroinflammatory disease process in MS involving the release of pro-inflammatory cytokines may be one factor influencing appetite sensations (Ziemssen 2009). However, as no patients experienced a relapse throughout their participation in the study, it is likely that appetite was not affected by an acute
inflammatory phase. Furthermore, it would be expected that any disease associated processes influencing appetite would influence control and exercise groups similarly. It is therefore plausible that the increased physical activity levels as a result of the exercise intervention contributed to the reduced postprandial hunger sensations observed in the exercise group at post-intervention. A recent study in obese individuals demonstrated an increased satiating efficiency of a fixed meal following 12 weeks of exercise intervention (King et al 2009), and changes in peptides associated with appetite were proposed as a possible explanation. An increased sensitivity to these peptides may be one mechanistic explanation for the significant change in appetite observed in our study. Another mechanism that has been proposed by which activity induced improvements in appetite regulation occur is insulin sensitivity (Long et al 2000) as insulin sensitivity is known to be involved in satiety (Holt et al 1992) and to increase with exercise (Nassis et al 2005, Andersson et al 1991). As these mechanisms were not measured in the present study, further research is necessary to determine the underlying processes mediating the changes in appetite observed.

Unexpectedly, fasting (pre-breakfast) hunger and desire to eat ratings showed contrasting responses in the exercise group. Hunger ratings increased non-significantly and desire to eat ratings decreased significantly at post-intervention compared to an increase in both ratings in the control group. An additional unexpected finding was that fullness postprandial ratings showed no significant change at post-intervention despite a significant change in hunger and change in desire to eat approaching significance. This is unusual as subjects usually rate fullness as the opposite of hunger and desire to eat (Stubbs et al 2000). However, the age of participants in the present study may also have influenced this finding as a weakening of the inverse association between hunger and fullness present in young adults has been demonstrated with increasing age (Wurtman et al 1988, Rolls et al 1995, Clarkston et al 1997). It may also have been that the distention stimulus (250kcal meal) was insufficient for a population with a mean BMI of approximately 27 kg·m⁻². Another possible explanation is that mechanoreceptor stimulation may not be affected to the same degree as other appetite parameters (hunger and desire to eat) by exercise in an MS population. Gastric distention with eating contributes to the feeling of fullness. Although the mechanisms are unclear, distending the stomach stimulates gastric stretch receptors, which trigger vagal discharges that activate hypothalamic neurons (Anand & Pillai 1967) and induce the feeling of fullness (Deutsch et al 1978). It has been previously suggested that in some MS patients the
demyelinating disease could affect the vagal nerve nuclei (Reddymasu et al 2007). Disturbance of the vagal pathways associated with the nature of the disease may therefore be one explanation for the lack of change in fullness sensations with exercise.

Changes in appetite sensations were not associated with changes in either 24 hour food intake for the remainder of the gastric emptying study day or 3 day food intake as assessed by food diary. The exercise group experienced an increase in 3 day energy intake compared to a decrease in the control group. Previous research indicates that as exercise continues day to day energy intake begins to track energy expenditure over the course of 1-2 weeks (Whybrow et al 2008). Therefore, the increase in energy intake in the exercise group may be a compensatory response to the increased energy expenditure as a result of the intervention. Although not evident in the small sample size, weight loss, malnutrition and cachexia are well recognized features of patients with MS (Kamalian et al 1975, Cook et al 1978, Fantelli et al 1978, Williams et al 1988, Wozniak-Wowk 1993), and therefore an increase in food intake with exercise may be beneficial for these patients.

Several limitations of the present study must be recognised including the non-randomised design of the study. The subject population may have been biased from self-selection. Although differences in physical activity levels were not significantly different between groups at baseline, the large standard deviation in the control group indicates that there were large differences in habitual physical activity levels between individuals. Future studies involving more homogenous subject populations randomly assigned to groups would be desirable. Mean energy intake assessed by food diary was low for all subjects (n = 12) which is paradoxical when ten subjects were classified as overweight or obese (BMI > 25). This has previously been observed in MS patients (Goodman & Sullick 2008) and suggests an underreporting of food intake. The limitations of self report physical activity and food intake measures may be enhanced in MS patients as cognitive impairment is a common symptom (Hewson et al 1984) and therefore may impact recall. Nevertheless, the short form of the IPAQ used in this study to assess physical activity has evidence of validity in MS patients (Motl et al 2009, Gosney et al 2007).

Another explanation for the inconsistency in patients’ low energy intakes and high BMI is that the ‘window’ of weight gain was missed in the present study. For example, oral glucocorticoids, medication often prescribed during MS relapses can
increase appetite and lead to weight gain (Cavagnini et al 2000). Drugs can affect, threaten and compromise nutritional status in chronic disabling conditions, particularly if patients are on complex regimes (White & Ashworth 2000). Many patients in the present study were taking multiple medications. Although, only one patient was taking medication individually known to influence GE, the complex interactions of many patients’ drug regimens may indirectly influence nutritional status and cause a large intra and inter individual variability in appetite and food intake. On the other hand as many MS patients take medication, the findings of this study may be considered a more accurate reflection of the effects of chronic exercise intervention on these parameters in everyday life in this population.

Severe exclusion criteria for this type of study are necessary to eliminate the impact of other diseases and medications on gastric emptying, appetite and heart rate variability. However, as many of these complications are common in MS, it was not possible to exclude for all criteria in the present study. For example, one CTL and one EXE subject had previously been diagnosed with IBS, and although not taking medication known to influence GI motility, fullness sensations may be heightened and gastric emptying slowed in patients with IBS (Portincasa et al 2003). This may have been an overriding factor influencing changes in gastric emptying and appetite in these patients. Future studies employing even more severe exclusion criteria may yield more significant findings of the effects of exercise on autonomic function in this population.

Up to now this discussion has focused on statistically significant changes observed in the results. Although not statistically significant, some individual changes might be clinically important. Ideally, each case of MS should be considered individually because of the wide variety of clinical presentations. Large standard deviations and 95% confidence intervals in all results are evident and may be explained by the large variability in individual characteristics (age, BMI, gender, disease duration, other medical complications). This is particularly evident in Figure 4.1 illustrating individual changes in GE in both CTL and EXE groups from baseline to post-intervention. Despite a non-significant change in mean \( t_{1/2} \), it is clear that some individuals had large changes in GE \( t_{1/2} \) between baseline and post-intervention. This is in contrast to other findings of reproducible patterns of gastric emptying of the same test meal in the laboratory setting in healthy individuals (Nair et al 2008). A limitation of intervention studies in MS patients is that other factors such as change in medication or health complications associated with the disease such as fatigue, drug nutrient
interactions, dysphagia and depression may cause a larger day to day variability in nutrient intake and indirectly alter gastric emptying. Therefore changes in food intake and gastric emptying may be attributable to factors other than exercise (as is highlighted by the individual changes in the CTL group). These findings document the vital importance of including a control group in intervention studies assessing changes in parameters such as gastric emptying in MS patients, in order to determine whether changes lie outside the normal variation seen in this population.

The small sample size in the present study limits the characterization of individual responses to determine common factors which may explain a delay in GE in some individuals but an acceleration in others. The small sample size also led to the possibility of type II statistical errors and therefore lack of significant associations should be interpreted with caution.

This is the first documented $^{13}$C-OBT study in MS patients known to the authors. Although, the validity of the test to assess GE in this population may be questioned as the method has not been validated against the widely considered ‘gold standard’ scintigraphy, the $^{13}$C-OBT has been validated in other conditions of autonomic dysfunction and disordered GE such as diabetes (Ziegler et al 1996, Zahn et al 2003). Apart from 2 subjects who reported feeling nauseous after the test meal, all subjects reported the pancake meal to be palatable and tolerated the tests well. This provides encouragement for future studies investigating the effects of therapeutic interventions on GE in this population, as the $^{13}$C-OBT does not expose subjects to radioactivity, and therefore it makes repeated measures studies more feasible.

Overall management of MS calls for principles that will affect maximal health in general (Umphred 2001). Exercise is universally available and can be readily designed for virtually any level of neurological disability (Johnson 1996), yet remains under-utilised as an intervention strategy in the MS population (Paty 1993). We observed a significant suppression of postprandial hunger after a 10 week exercise intervention compared to a control group. In addition, an increase in average energy intake over 3 days in individuals after a 10 week exercise intervention compared to a control group may reflect an increased ability to match energy intake to energy expenditure in this population. These data suggest a beneficial effect of exercise on the appetite regulatory system in MS patients. Further studies are required to understand the mechanisms behind these changes. Although, no significant change in autonomic function was observed as reflected in HRV or GE parameters, a large variability in individual responses was evident and individual
characteristics may be influencing these parameters. Data from the present study suggests no association between gastrointestinal and cardiac autonomic function in MS. Further studies that include larger sample sizes, more stringent inclusion criteria and an exercise program that induces a significant reduction in resting heart rate would be ideal to fully understand the effects of exercise on autonomic function and the possible association between gastrointestinal and cardiac autonomic neuropathy in MS patients.
Chapter 5 Summary and conclusions

The role of exercise in increasing energy expenditure is well established. Exercise may also influence energy balance indirectly by altering many aspects of the integrated regulatory process of appetite control including hormonal, neural and behavioural factors and consequently alter energy intake. Gastrointestinal signals play an important role in the physiological regulation of appetite. Gastric emptying (GE; the delivery of nutrients from the stomach to the small intestine) influences appetite by modulating both gastric distension and the presence of nutrients in the small intestine. A considerable body of research has linked altered GE in obese individuals to an increased energy intake. Limited cross sectional research has indicated chronic exercisers have an accelerated GE (Carrio et al 1989, Shimamoto et al 2002), implicating physical activity as a modifiable lifestyle factor which may accelerate the delivery of nutrients to the small intestine. Therefore, it was postulated that GE may be one mechanism mediating changes in appetite and energy intake with exercise. However, a limitation of cross sectional studies is that they provide no indication of causality and therefore intervention studies are needed.

This thesis aimed to increase understanding regarding the effects of chronic exercise intervention on gastric emptying. In addition, food intake and appetite sensations were assessed with the aim of determining if GE plays a role in mediating changes in appetite sensations and energy intake with exercise. Two different populations were investigated, both prone to physical inactivity. The first study examined the effects of exercise on GE in healthy adolescent schoolgirls (Chapter 3). The focal population of the second study was Multiple Sclerosis (MS) patients; a population in which delayed GE has been previously reported (Chapter 4). In both studies changes between baseline and post-intervention were compared to changes in a control group over the same time period. GE of the same pancake test meal was assessed in both studies using the $^{13}$C octanoic acid breath test ($^{13}$C-OBT). Breath samples were collected before the meal and for 4 hours postprandially, and subsequently analysed using isotope ratio mass spectrometry.

In Chapter 3, increasing BMI was found to be associated with slower gastric emptying in nineteen adolescent schoolgirls. The schoolgirls were divided into 2 groups: an exercise and a control group. The 7 week exercise intervention did not significantly alter GE compared to changes in the control group. There were no changes
in appetite sensations except for hunger which was significantly reduced at 30 minutes after a standardised test meal in the exercise group after intervention compared to changes in the control group. As GE did not change, this suggests that mechanisms other than GE were mediating the change in hunger ratings observed at this time point. Prior research that has demonstrated increased glucagon-like peptide 1 (GLP-1) levels at 30 minutes postprandially following exercise intervention (Chanoine et al 2007) suggest that GLP-1 may be one potential mechanism involved. However, gut peptides were not measured in this thesis.

In Chapter 4 along with GE, appetite sensations and food intake, cardiac autonomic function was assessed using Heart Rate Variability (HRV) analysis. Multiple Sclerosis is a progressive autoimmune disease in which both delayed GE and autonomic dysfunction have been previously reported. It has been previously suggested that an accelerated GE in athletes is the result of a more predominant parasympathetic tone. The additional measurement of HRV therefore provided an indication of changes in autonomic balance and in cardiac autonomic function with the exercise intervention. Measurements taken at baseline in this study do not support previous findings of delayed GE in MS patients, or an association between gastrointestinal and cardiac neuropathy. However, the lack of significant association between GE and HRV should be interpreted with caution, as a small sample size introduced the possibility of type II error. There were no statistically significant changes in GE or HRV following the exercise intervention. Average daily food intake as assessed by 3 day food diary was increased following the exercise intervention and postprandial hunger ratings for 4 hours following the standardised test meal were significantly reduced. There was a large inter and intra individual variability in all parameters which may be attributed to a multitude of differing individual characteristics such as age, BMI, gender, disease characteristics and drug nutrient interactions.

Collectively, chapters 3 and 4 highlight a number of methodological issues that should be considered in future investigations assessing change in GE with chronic exercise intervention. In chapter 3, the control group had an unexpected minor but significant weight loss, while there was no change in weight in the exercise group. This highlights a fundamental issue in studies investigating energy balance. Is the weight change due to change in energy intake? change in energy expenditure? or a combination of both factors? As exercise was supervised in the intervention in chapter 3, it is assumed that energy expenditure is increased in the exercise group. However the lack of
significant weight loss in this group suggests either compensation in energy intake or a reduction in activity throughout the rest of the day or a combination of both occurred. The methods used in this thesis of assessing EE and EI by self report may not have been sensitive enough to detect important changes that may have occurred. This is an important issue to consider as changes in EI and EE may have different influences on GE (Andersson et al 2001, Shimamoto et al 2002). A strength of this thesis is the inclusion of a control group for both studies, without which different conclusions may have been drawn. Despite no mean change in GE in MS patients in chapter 4 for example, it is clear from examining individual changes that there were some large changes in GE within individuals between baseline and post-intervention. However, as this was also the case in the control group, it was possible to conclude that the 10 week exercise intervention did not significantly alter GE. A number of limitations must also be considered in interpreting the findings of both studies. In the adolescent study (chapter 3), subjects were randomly pre-assigned to exercise and control groups. However in Chapter 4, MS patients were not randomly allocated to groups. Self selection may therefore have biased the subject population in each group and may have been a confounding factor influencing results. It must be recognised that neither study was originally designed with gastric emptying, energy intake or appetite as a primary outcome measure. Both studies were collaborations of which the primary outcome measures in this thesis were subsequently added. Both exercise interventions had been previously designed with the aim of increasing physical activity levels in populations prone to physical inactivity. Future studies involving more homogenous subject populations, objective measures of physical fitness and activity and randomised designs would be desirable to yield further understanding on the effects of chronic exercise intervention on GE.

Collectively, findings suggest that GE may not play a role in mediating changes in appetite sensations with exercise interventions as changes in hunger ratings were observed in both studies following intervention, despite no changes in GE. Both Chapters 3 and 4 showed exercise interventions of 7 weeks and 10 weeks did not change gastric emptying in female adolescents and MS patients respectively. Both interventions involved a relatively modest increase in weekly energy expenditure. It may be that longer duration and more intensive exercise interventions are needed to cause the significantly accelerated GE that has been previously observed in regular exercisers. As previous studies showing accelerated GE in more active individuals were
cross sectional, it is also possible that other lifestyle factors associated with regular exercise such as diet may be responsible for this adaptation. Furthermore, as neither intervention induced a significant weight loss, it may be that GE only adapts to exercise indirectly as a result of lower fat mass. Therefore, future studies investigating the effects of an exercise induced significant weight loss on GE may yield further understanding of what could be an important interactive relationship between BMI, physical activity and GE.

Furthermore, the changes in hunger sensations observed in both studies provide an interesting avenue of investigation for future study into exploring the mechanisms behind these changes. The findings of this thesis suggest GE is not responsible. However, a multitude of factors influence appetite and despite no mean changes in GE, it was clear that some individuals experienced large changes in GE. It is possible that GE may have a role in changes in appetite with exercise not as an isolated mechanism but as part of a larger integrative process controlling appetite. In particular, long term adiposity related signals such as the hormones leptin and insulin and short term episodic signals such as the peptides CCK and GLP-1 have been highlighted in the control of appetite (Crowell et al 2006).

Although many different theories have attempted to explain the control of food intake by single factors such as Mayer’s (1953) glucostatic theory and Kennedy’s (1953) lipostatic theory, it is now evident that appetite involves the integrated response of many different factors (Schwartz et al 1999). The focus of this thesis concerned the effects of exercise on gastric emptying, just one aspect of the complex integrative process controlling food intake.

5.1 Key Findings

- Increasing BMI was associated with slower GE in adolescent girls.
- A 7 week exercise intervention did not change GE or food intake but reduced 30 min postprandial hunger sensations in adolescent girls.
- GE was not significantly associated with cardiac autonomic function in MS patients. Larger sample size studies are needed.
- A ten week exercise intervention did not improve autonomic function reflected by GE and HRV parameters but increased daily energy intake and decreased postprandial hunger sensations in MS patients.
The $^{13}$COBT provides potential as a method for assessing GE in MS patients.

Changes in control groups illustrate the importance of using control groups in future intervention studies assessing changes in parameters measured in this thesis.

5.2 Perspectives
The finding of no change in GE with chronic exercise intervention suggests that a longer duration or more intensive intervention may be needed for an adaptation to occur. Furthermore, it is clear that changes in hunger ratings occur with chronic exercise intervention without change in GE, and therefore other mechanisms must contribute to this change.

The finding of a slower GE with increasing BMI in adolescent girls is a novel one in this population but is consistent with previous findings in adults (Lavigne et al 1978, Brogna et al 1988). Interestingly, no studies have reported the opposite correlation of faster GE with increasing BMI in non-obese individuals. This is in contrast to the highly inconsistent findings of studies comparing GE between lean and obese groups. Based on the findings in this thesis and findings of others, it is hypothesised that GE becomes deregulated at extremes of the body mass spectrum and this may be one explanation for the contrasting findings of studies comparing GE in lean and obese individuals.

5.3 Future Work
Chapter 3 illustrates a significant association of increasing BMI with slower GE in adolescent girls. Future intervention studies inducing significant changes in BMI are needed to investigate the causality of this association. Does GE adapt to BMI? or does slower GE play a role in increasing BMI?

Both chapters 3 and 4 in this thesis measured changes in GE in response to a single standardised test meal before and after a long-term exercise intervention. Others have shown that exercise improves appetite control through an ability to better regulate energy intake in response to preloads of different energy content (Martins et al 2007, Van Walleghan et al 2007). Therefore, although no change in GE was observed in response to the same test meal, it is possible that exercise may lead to a more sensitive GE whereby GE better adapts to prior nutrient intake following exercise intervention.
This was not possible to determine in the studies in this thesis but represents an area worthy of future investigation.

The issue of gender differences must also be considered in future study. Chapter 4 involved both males and females, and although no gender difference in GE was observed between males and females, the multitude of characteristics differing between individuals with MS including disease type, duration and medication routine may have an overriding influence on GE compared to healthy adults where gender differences have been observed. The focal population of chapter 3 on the other hand was adolescent schoolgirls. Therefore future studies investigating adolescent boys are needed.

Future studies of larger sample sizes are needed. This would a) reduce the likelihood of any type II error and b) allow for the characterisation of common factors by which some individuals change and others don’t in the parameters of interest in response to exercise interventions. Simply focusing on group changes may be masking important individual responses.

Both studies utilised the $^{13}$C octanoic acid breath test as the method of measurement for GE. In people with MS, this is the first documented study using this method. Tests were well tolerated in both studies, providing encouragement for the use of this method in future longitudinal studies in both adolescents and people with MS.

Ideally, future studies should utilise the gold standard methods of measuring energy intake and energy expenditure in carefully controlled laboratory settings to deduce the exact roles of changes in energy intake and energy expenditure in altering GE and effects on appetite ratings and energy intake.

5.4 Conclusions
This thesis aimed to determine the effect of exercise interventions on GE, appetite and food intake in two different populations, which share the common factor of being prone to physical inactivity. It is concluded that 7 and 10 week moderate intensity exercise interventions did not significantly alter GE in female adolescents and Multiple Sclerosis patients respectively. However hunger sensations were influenced by exercise intervention. More integrative studies of the mechanisms mediating changes in appetite with exercise are needed.
References


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APPENDICES

Appendix A: Ethics Application 90/08

University of Limerick Research Ethics Committee (ULREC) Application Form

1 Title of Research Project
Changes In Gastrointestinal Function Following A Physical Activity And Lifestyle Education Programme

2 Period for which approval is sought
2 years from date of approval

3 Project Investigators

3a Principal Investigator
Name: Dr. Amir Shafat
Department: PESS Department
Position: Lecturer
Qualifications: BSc, PhD
Telephone Number: 061 234228
e-mail address: amir.shafat@ul.ie

3b Other Investigators

Name Qualifications & Affiliation
Katy Horner BSc Sport & Exercise Sciences, MSc candidate
Deirdre Harrington BSc Sport & Exercise Sciences, PhD candidate
Alan Donnelly PhD, Professor of Exercise Science

4 Head of Department(s)

I have read through this application and am aware of the possible risks to subjects involved in this study. I hereby authorise the Principal Investigator named above to conduct this research project.

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Date</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drew Harrison</td>
<td>PESS Department</td>
<td>21/01/09</td>
<td></td>
</tr>
</tbody>
</table>
5 Study Descriptors

Please indicate the terms that apply to this research project

<table>
<thead>
<tr>
<th>Healthy Adults</th>
<th>Healthy Children (&lt; 18 yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Adults</td>
<td>Patient Children (&lt; 18 yrs)</td>
</tr>
<tr>
<td>Potentially Vulnerable Adults</td>
<td>Potentially Vulnerable Children</td>
</tr>
<tr>
<td>Physical Activity</td>
<td>Questionnaire/Interview</td>
</tr>
<tr>
<td>Medical Devices / Drugs</td>
<td>Video Recording/Photography</td>
</tr>
<tr>
<td>Food/Drink Supplementation</td>
<td>Collection of Personal Details</td>
</tr>
<tr>
<td>Measure Physical in Nature</td>
<td>Measure Psychological in Nature</td>
</tr>
<tr>
<td>Body Tissue Samples</td>
<td>Observational</td>
</tr>
<tr>
<td>Body Fluids Samples (e.g. blood)</td>
<td>Record Based</td>
</tr>
</tbody>
</table>

6 Project Design

6a Justification for Research Project (Include reference to published work)

Low physical activity (PA) levels are a rapidly growing problem in children and understanding the regulation of energy homeostasis in children will assist in the understanding and implementation of countermeasures and strategies for the prevention of obesity in adolescents and children (Jurimae et al. 2007).

In addition to dietary factors and physical inactivity, changes in gastrointestinal motility may contribute to the ever-expanding problem of obesity (Hellmig et al., 2006). Rate of gastric emptying (GER) refers to the rate at which the stomach emptys of solids and/or liquids. Hunt et al. (1975) drew a link between GER, satety and obesity, postulating that rapid GE and hence a shortened satety period may contribute to the development of obesity.

Various studies have shown PA may play a role in altering GER, suggesting a link between PA and gastrointestinal motility in the pathogenesis of obesity. Carrio et al. (1989), for example, found a faster GER in marathon runners when compared to sedentary individuals. Jackson et al. (2004) found delayed GE in obese compared to non-obese individuals. Regulatory hormones such as ghrelin which play a role in regulating GER have been found to be altered by regular PA in a schoolgirl population suggesting GER may too be altered by regular PA (Jurimae et al. 2007).

However, the majority of research with regard to GER and PA is limited to acute exercise studies. As a result, a knowledge gap exists in investigating how an individual's GER is altered as they progress from a sedentary to a physically active lifestyle. The aim of the present study is to investigate this knowledge gap by measuring GER before and after the "UL Fitness" exercise intervention to assess the effect of increasing PA on GER in an adolescent female population.

Harris et al. (1991) investigated the effects of chronic exercise on orocecal transit time, concluding that the hyperphagia of chronic exercise in humans may be linked with significant gastrointestinal adaptations. Therefore, a secondary aim of the current study is to investigate the effect of increasing PA levels on food intake and subjective ratings of satifity and hunger of the schoolgirls participating in the exercise intervention study.

6b Hypotheses or questions to be answered
The first null hypothesis is that completion of the exercise intervention will have no effect on the rate of gastric emptying in healthy schoolgirls. The second null hypothesis is that completion of the exercise intervention will have no effect on the dietary intake of healthy schoolgirls.

6c Plan of Investigation
A subject population of up to 30 schoolgirls who are taking part in the study titled "The Health, Fitness And Physical Activity Levels Of Adolescent Females Following A Physical Activity And Lifestyle Education Programme" in Laurel Hill Secondary School will be recruited from for the current study. The aforementioned study has already received ethical approval from this committee (PESSEC 43/08). The students will be recruited from those who have already been recruited for PESSEC 43/08. However, the students will be made aware that participating in the additional measures involved in the current study is completely voluntary and under no circumstances will their decision to volunteer or not for the additional measures involved in the current study affect their participation in the "UL Fitness" classes.

Students will be informed of the study by word of mouth during an assigned UL fitness class. Those who are interested in participating will be given information sheets and parent consent forms. Those students who consent to participate and whose parents consent to their daughter's participation in the study will attend the physiology laboratory in UL on 2 occasions, a pre-test prior to the exercise intervention commencing in February and a post-test upon completion. Those who choose not to participate will attend school as normal. Both testing sessions will follow an identical protocol.

Students will be given a food diary to record their pre-intake for 3 days before coming into the lab. On arrival in the laboratory, participants' height and weight will be taken. Participants will then be given a physical activity (PA) questionnaire to assess their current PA levels. GE will be measured using the 13C octanoic acid breath test.

CONTINUED ON ADDITIONAL SHEET

6d Research procedures
SS028 Anthropometry measurements
SS029 Assessment of physical activity

6e Associated risks to subjects
13C breath tests have been used in research for over 30 years. The 13C-octanoic acid breath test has been widely used in healthy and patient infants, children and adults. There are no known risks associated with the method. It is ideally suited for measuring GE in children (Hauser et al. 2006). There is risk to the student that they will not like the breakfast meal, if they are not used to eating it. This will be minimised by using a pancake meal found palatable by children in previous GE studies. Students may also choose not to finish the

6f Statistical approach to be used and source of any statistical advice
Data will be tested for parametric assumptions and compared by paired two tailed t test using SPSS v15.0. Katy Horner will consult with the Principal Investigator, Dr. Amir Shafat, for statistical advice.
Location(s) of Project
Physiology laboratory (PG050, PG051), PESS Building, University of Limerick.

7 Subjects

7a How will potential research participants be sourced and identified?
Collaboration with the exercise intervention coordinators and Laurel Hill Secondary School has been established and ethical approval for the exercise intervention has already been granted (PESSREC 43/08). Participants will be sourced from those who have already been recruited for PESSREC 43/08 and will be recruited by word of mouth. They will be informed about the current study during a "UL Fitness" class. All pupils who volunteer must also provide parental consent.

7b Will research participants be recruited via advertisement (poster, e-mail, letter)?

YES  [ ]  NO

If YES, please provide details below, or attach the recruitment advertisement if written.

7c How many subjects will be recruited?

Male  0  30  Female

Provide further information if necessary
A maximum of 30 subjects will be recruited. As this study will take place in an all girls secondary school, females only will be recruited for this study.

7d What are the principal inclusion criteria? (Please justify)
The principal inclusion criteria is that the participants are transition year students in Laurel Hill Secondary School who are going to participate in the "UL Fitness" exercise intervention as part of their transition year programme. Students who are participating as controls in the exercise intervention study will also be recruited for this study as controls.

7e What are the principal exclusion criteria? (Please justify)
Any individual who is not a transition year student in Laurel Hill Secondary School will be excluded from this study. Anyone who is not deemed fit to participate in this research by PESS pre-test questionnaire. Any individuals who are not participating in any aspect of the exercise intervention (either in the exercise classes or as controls) will be excluded from this study.
For Office Use Only: ULREC No: /

<table>
<thead>
<tr>
<th>7f</th>
<th>What is the expected duration of participation for each subject?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The expected duration of participation for each subject is 15 hours consisting of a 1 hour familiarisation session, 2 separate 6 hour testing sessions (pre, post) and filling in a weighed food diary for 3 days prior to each session, which will take approximately 20 minutes per day.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>7g</th>
<th>What is the potential for pain, discomfort, embarrassment, changes to lifestyle for the research participants?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The potential for pain, discomfort, embarrassment, changes to lifestyle for the research participants is very minimal. Participants are free to withdraw from the study at any time. Embarrassment will be minimised by ensuring confidentiality and anonymity with regard to participants results and personal details.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>7h</th>
<th>What arrangements have been made for subjects who might not adequately understand verbal explanations or written information in English?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All participants will have an understanding of the English language and if necessary demonstration will be used.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>7i</th>
<th>Will subjects receive any payments or incentives, or reimbursement of expenses for taking part in this research project?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YES ☐ ☐ NO</td>
</tr>
<tr>
<td></td>
<td>If YES, please provide details below, and indicate source of funding:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>8</th>
<th>Confidentiality of collected data</th>
</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td>What measures will be put in place to ensure confidentiality of collected data?</td>
</tr>
<tr>
<td></td>
<td>Collected data will be stored in a format whereby specific subject information cannot be identified. Subject names will be assigned codes, and only the data linked to these codes will be stored electronically. During the study period, all named investigators may hold the codes and names of subjects, but appropriate measures will be taken by investigators to ensure that the stored data (electronic and hard copy) is secure at all times.</td>
</tr>
</tbody>
</table>

| 8b | Where will it be stored? Information will be stored in the PESS building in the project investigators' (listed in section 3) office computers, which are password protected, and data sheets will be kept in a locked cupboard in the principal investigator's office (P1-020, Pess Building). |

<table>
<thead>
<tr>
<th>8c</th>
<th>Who will have custody and access to the data?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Investigators named in Section 3 of the application.</td>
</tr>
</tbody>
</table>
Data to be stored for 7-10 years after publication (Please provide details of storage of data during this period)
Data is stored with the Principal Investigator during this period.

9 Drugs or Medical Devices
Are Drugs or Medical Devices to be used?
YES ☐ ☑ NO
If YES please complete 9a to 9c

9a Details of the Drugs or Devices (including name, strength, dosage, route of administration)

9b Details of Clinical Trial Certificate, Exemption Certificate or Product Licence (The Product Licence must cover the proposed use in the Project – see Guidelines No. 11)

9c Details of any Risks (Both to subjects and staff; indicate current experience with the drug or device)

10 Professional Indemnity
Does this application conform to the University’s professional indemnity policy?
YES ☐ ☑ NO
If NO please indicate the professional indemnity arrangements in place for this application (attach policy if necessary):
### Information Documents

Please note: failure to provide the necessary documentation will delay the consideration of the application. Please complete the checklist below:

<table>
<thead>
<tr>
<th>Documents</th>
<th>Included?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject Information Sheet</td>
<td>YES</td>
</tr>
<tr>
<td>Parent/Carer Information Sheet</td>
<td>YES</td>
</tr>
<tr>
<td>Subject Informed Consent Form</td>
<td>YES</td>
</tr>
<tr>
<td>Parent/Carer Informed Consent Form</td>
<td>YES</td>
</tr>
<tr>
<td>Questionnaire</td>
<td>YES</td>
</tr>
<tr>
<td>Interview/Survey Questions</td>
<td>YES</td>
</tr>
<tr>
<td>Recruitment Letters/Advertisement/e-mails, etc</td>
<td>YES</td>
</tr>
<tr>
<td>Risk Assessment Form(s)</td>
<td>YES</td>
</tr>
<tr>
<td>Acceptance of UL Child Protection Form</td>
<td>YES</td>
</tr>
</tbody>
</table>

*Please ensure any additional documents are included with this application. These should be attached as a single document and included in the e-mail submission.*

### Declaration

The information in this application form is accurate to the best of my knowledge and belief, and I take full responsibility for it.

I undertake to abide by the ethical principles outlined in the UL Research Ethics Committee guidelines.

If the research project is approved, I undertake to adhere to the study protocol without unagreed deviation, and to comply with any conditions sent out in the letter sent by the UL Research Ethics Committee notifying me of this.

I undertake to inform the UL Research Ethics Committee of any changes in the protocol, and to submit a Report Form upon completion of the research project.

<table>
<thead>
<tr>
<th>Name of Principal Investigator</th>
<th>Dr. Amir Shafat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signature of Principal Investigator</td>
<td>(or Head of Department*)</td>
</tr>
<tr>
<td>Date</td>
<td>21/01/09</td>
</tr>
</tbody>
</table>

*Please note: where the Principal Investigator is not a permanent employee of the University of Limerick, the relevant Head of Department should sign this declaration.*

1. Once completed, this form along with a single document containing and additional documentation should be submitted electronically to the Vice President Academic and Registrar's Office at vpae@gstaffmail.ul.ie.

2. In addition, 10 copies of the fully signed application and any attachments should be submitted to: The Secretary, University of Limerick Research Ethics Committee, Vice President Academic and Registrar's Office.
Ethical Approval Email: 90/08

PESSREC 90/08 Changes In Gastrointestinal Function Following A Physical Activity And Lifestyle Education Programme

Dear All

The above application was application was approved by PESSREC on the 29th January 2009.

Regards

Rhoda

Rhoda Sohun
Experimental Officer
Department of Physical Education & Sport Sciences
University of Limerick

Tel: +353 61 234185
Fax: +353 61 202814
Web: www.ul.ie/pess
Appendix B: Physical Activity Questionnaire for Adolescents (PAQ-A)

*Physical Activity Questionnaire*

Name:_________________________ Age:___________

We are trying to find out about your level of physical activity from the last 7 days (in the last week). This includes sports or dance that make you sweat or make your legs feel tired, or games that make you breathe hard, like tag, skipping, running, climbing, and others.

**Remember:**
3. There are no right and wrong answers — this is not a test.
4. Please answer all the questions as honestly and accurately as you can — this is very important.

1. Physical activity in your spare time: Have you done any of the following activities in the past 7 days (last week)? If yes, how many times? (Mark only one circle per row.)

<table>
<thead>
<tr>
<th>Activity</th>
<th>No</th>
<th>1-2</th>
<th>3-4</th>
<th>5-6</th>
<th>7 times or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skipping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rowing/canoeing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>In-line skating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tag</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking for exercise</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bicycling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jogging or running</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Aerobics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swimming</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseball, softball</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Football</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Badminton</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Skateboarding</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Soccer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Street hockey</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volleyball</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Floor hockey</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basketball</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice skating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross-country skiing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice hockey/ringette</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

139
2. In the last 7 days, during your physical education (PE) classes, how often were you very active (playing hard, running, jumping, throwing)? (Check one only.)

Hardly ever ..............................................................
Sometimes ..............................................................
Quite often ..............................................................
Always .................................................................

3. In the last 7 days, what did you normally do at lunch (besides eating lunch)? (Check one only.)

Sat down (talking, reading, doing schoolwork)......
Stood around or walked around .............................
Ran or played a little bit ..........................................
Ran around and played quite a bit ............................
Ran and played hard most of the time .....................

4. In the last 7 days, on how many days right after school, did you do sports, dance, or play games in which you were very active? (Check one only.)

None .................................................................
1 time last week .....................................................
2 or 3 times last week ............................................
4 times last week ...................................................
5 times last week ...................................................

5. In the last 7 days, on how many evenings did you do sports, dance, or play games in which you were very active? (Check one only.)

None .................................................................
1 time last week .....................................................
2 or 3 times last week ............................................
4 or 5 last week ...................................................
6 or 7 last week ...................................................

6. On the last weekend, how many times did you do sports, dance, or play games in which you were very active? (Check one only.)

None .................................................................
1 time .................................................................
2 — 3 times ...........................................................
4 — 5 times ..........................................................
6 or more times ...................................................
7. Which one of the following describes you best for the last 7 days? Read all five statements before deciding on the one answer that describes you.

F. All or most of my free time was spent doing things that involve little physical effort .................................................................  🙋.

G. I sometimes (1 — 2 times last week) did physical things in my free time (e.g. played sports, went running, swimming, bike riding, did aerobics) ............... 🙋.

H. I often (3 — 4 times last week) did physical things in my free time ........... 🙋.

I. I quite often (5 — 6 times last week) did physical things in my free time .... 🙋.

J. I very often (7 or more times last week) did physical things in my free time ...... 🙋.

8. Mark how often you did physical activity (like playing sports, games, doing dance, or any other physical activity) for each day last week.

<table>
<thead>
<tr>
<th>Day</th>
<th>None</th>
<th>Little bit</th>
<th>Medium</th>
<th>Very Often</th>
<th>Often</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
</tr>
<tr>
<td>Tuesday</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
</tr>
<tr>
<td>Wednesday</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
</tr>
<tr>
<td>Thursday</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
</tr>
<tr>
<td>Friday</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
</tr>
<tr>
<td>Saturday</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
</tr>
<tr>
<td>Sunday</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
<td>🙋</td>
</tr>
</tbody>
</table>

9. Were you sick last week, or did anything prevent you from doing your normal physical activities? (Check one.)

   Yes ................................................. 🙋
   No ...................................................... 🙋

If Yes, what prevented you? ________________________________
**Appendix C** Chapter 3 Figures illustrating changes in subjective sensations of desire to eat and fullness.

**Figure A 3.1** Mean ± SD absolute ratings of subjective desire to eat and fullness at pre (unfilled markers) and post (filled markers) test in the exercise and control groups. Breakfast was consumed between t=-10 and t=0. An ad libitum buffet lunch meal was consumed between t=240 and t=270.
### Appendix D: Chapter 3 Pearson Correlations

#### Baseline BMI and GE

**BMI and half time**

<table>
<thead>
<tr>
<th>Descriptive Statistics</th>
<th></th>
<th></th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td></td>
</tr>
<tr>
<td>BMI Pre</td>
<td>22.1300</td>
<td>2.15864</td>
<td>19</td>
</tr>
<tr>
<td>GEhtscintpre</td>
<td>79.7226</td>
<td>17.23263</td>
<td>19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Correlations</th>
<th>BMI Pre</th>
<th>GEhtscintpre</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI Pre Pearson Correlation</td>
<td>1.000</td>
<td>.615**</td>
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**: Correlation is significant at the 0.01 level (2-tailed).

**BMI and lag time**

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BMI and ascension time

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***. Correlation is significant at the 0.01 level (2-tailed).
BMI and latency time

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Post-intervention BMI and GE

**BMI and half time**

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### BMI and latency time

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### BMI and ascension time

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### Changes between baseline and post-intervention

**Change in lag time versus change in self report PA**
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**Change in half time versus change in self report PA**

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**Change in BMI versus change in lag time**

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*Change in BMI versus change in ascension time*

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Appendix E: HSE Ethics Application (Chapter 4)

Mid-Western Regional Hospital Complex

SCIENTIFIC RESEARCH ETHICS COMMITTEE
PROTOCOL

THIS FORM MUST BE TYPE WRITTEN AND SUBMITTED TO THE
SCIENTIFIC RESEARCH ETHICS COMMITTEE TOGETHER WITH
14 COPIES OF BOTH THE FORM AND SUPPORTING
INFORMATION. YOU MUST ALSO SUBMIT ONE ADDITIONAL
COPY ELECTRONICALLY, TYPED IN MS WORD (WORD '97 OR
LATER), VIA EMAIL TO bernadettef.ryan@mailh.hse.ie

1 GENERAL

Title of Study: A pilot study to assess the effect of a 10-week exercise
program on rate of gastric emptying in people with Multiple Sclerosis

Date of submission:

Name of principal investigator and department:
Dr. Susan Coote BSc(Physio), PhD,
Physiotherapy Department, University of Limerick
Dr. Amir Shafat BSc, PhD
Physical Education and Sport Sciences Department, University of
Limerick

Telephone No: 061-234278 (Dr. Susan Coote)
061-234228 (Dr. Amir Shafat)

Signature of principal investigator: ____________________________

2 INVESTIGATING PERSONNEL (If the Principal Investigator is not
a Consultant, the project must be supervised by a Consultant)
Is each investigator a registered medical practitioner?
Dr. Susan Coote, Neasa Hogan and Maria Garrett are chartered physiotherapists. Dr. Amir Shafat is a lecturer in physiology. Katy Horner is a qualified sport and exercise scientist undertaking postgraduate research.

Have the investigators any vested interest in the outcome of the study?
NO

What payments, monetary or otherwise, if any are to be made to any of the investigators, either directly or indirectly?
NONE

What payments, monetary or otherwise, if any, are to be made to any person or institution providing facilities to be used for the purpose of the project?
NONE

3. ENCLOSURES WHICH MUST ACCOMPANY THIS APPLICATION:

(i) A draft Patient Information Sheet, giving information under the following headings:

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</tr>
<tr>
<td>Alternative treatment</td>
<td>Further information</td>
</tr>
</tbody>
</table>
The Patient Information Leaflet should be brief and in language which is easily understood. See Attached

(ii) A copy of the draft Consent Form, which should provide for signature by the Investigator, and an independent witness, as well as the Patient and, if the Patient is not in a position to sign, by a consentor, parent or guardian acting on behalf of the patient. The draft Consent Form should cover (a) the fact that the Patient has read and understands the Patient Information Sheet, (b) the voluntary nature of participation by the Patient including the freedom to withdraw, (c) access to the Patient’s medical notes if relevant, (d) permission to inform the Patient’s GP, (e) agreement by the Patient to notify the Investigator of any side effects arising during the study, and (f) agreement by the Patient to take part in the study. See Attached

(iii) An appropriate letter of indemnity. See Attached

(iv) Medicines Board approval covering the relevant drugs, the investigator and the participating institution. N/A as this study is not using drugs.

Please send all these papers, with the Protocol, to:

The Chairman
Scientific Research Ethics Committee
Limerick Regional Hospital, Dooradoyle, Limerick
4 TITLE OF PROJECT

Please indicate the Project Title: A pilot study to investigate the effect of a 10-week exercise program on rate of gastric emptying in people with Multiple Sclerosis

Proposed Start Date: January 2009

5 SUPPORT

Is there any financial or other support by a drug company or other outside commercial organisation? NO

If so, which organisation and does it agree to abide by the IPHA guidelines? N/A

What funding arrangements have been made for the conduct of additional diagnostic tests being carried out in the study? N/A

6 OBJECTIVE

(a) What hypothesis is it intended to test?
There is positive evidence as to the benefits of exercise in patients with MS in terms of improvements in strength, balance, quality of life and fatigue, however the effect on secondary risk factors is not clear. Rate of gastric emptying (GER) refers to the rate at which the stomach empties of solids and/or liquids. Haensch et al. (2006) lists gastrointestinal disturbances as one of the most common autonomic symptoms in people with MS. MS patients have been shown to have slow gastric emptying rates at rest (El-Maghraby et al. 2005), which may correlate with autonomic dysfunction. Gastric emptying, in turn, may determine lipaemia, glycaemia and other established risk factors for obesity and diabetes. Exercise can reduce the risk of these metabolic disorders and also has been shown to play a role in altering GER in normal subjects (e.g. Carrio et al. 1989). We hypothesise that exercise may have a beneficial effect on GER in MS patients. However, the effect of an exercise intervention on GER in MS patients has not been investigated. This study aims to investigate the effect of a ten-week exercise program on patients’ gastric emptying and heart rate variability. The exercise program patients’ will receive is part of the
“Getting the Balance Right” project, which was granted ethical approval by this committee.

(b) What is the value of the study to the patient(s) or volunteers? Participants will be informed of their own results in the study. As part of the study, they will also fill out a diary recording their food intake before and after the exercise program. Participants will be given a full dietary analysis and breakdown of their results. The study has an indirect value as it will help contribute to human digestive research specific to multiple sclerosis. 48% MS patients were shown to have delayed gastric emptying in a study by El-Maghraby (2005). Gastric emptying in turn may determine levels of fat and sugar in the blood and other established risk factors for obesity and diabetes. These are all major determinants of cardiovascular disease, a disease which people with MS are particularly vulnerable to (Paty et al. 1993). Exercise has been shown to accelerate rate of gastric emptying in a normal population (Carrio et al. 1989). However, it is unknown whether exercise can improve rate of GE in a population with MS. This study will provide more information on more underlying physiological responses to exercise in MS, which may play a part in contributing to greater understanding of disease progression and treatment of MS in the future.

7 CONDUCT OF PROJECT

Will the conduct of the project conform to the principles of the Declaration of Helsinki (the latest version of which can be found on the World Medical Association website, at: www.wma.net)?

YES

8 DESIGN OF THE STUDY

Describe briefly, including proposed methods for the analysis of the results.

- The study will consist of 2 identical testing sessions, one pre exercise intervention and one post exercise intervention.
- The exercise intervention that patients will undertake is one which is part of the ‘Getting the Balance Right Project’.
- Each testing session will last approximately 4 – 6 hours.
- Testing will take place either in the physiology laboratory, PESS Department, University of Limerick or in the patient’s local training centre.
- On each testing day, subjects will arrive following a standard 12 hour overnight fast (only water may be consumed). Food intake will be measured by means of a 7 day weighed food diary which will be given to subjects on arrival at the laboratory.
• On arrival, patients will consume a standard breakfast meal which is supplemented with $^{13}$C-octanoic acid. Gastric Emptying Rate (GER) will be measured non-invasively using the $^{13}$C-octanoic acid breath test, a method which has been frequently used for GE studies in our laboratory (e.g. Clegg et al. 2007). The test involves the subject blowing through a straw into a small glass tube at regular intervals for up to 6 hours after meal ingestion. Visual Analogue Scales will be given to the subject at half hour intervals during this time to assess satiety levels. Heart rate variability (HRV) will be measured by electrocardiogram (ECG), a consensus approved method (Kleiger & Stein, 2005). ECG will be taken on arrival at the laboratory and while the subject is seated in the laboratory for GE measures.

• GER and parameters of HRV will be compared using paired, two-tailed t-test.

9 SCIENTIFIC BACKGROUND

If this investigation has been done previously with human subjects, why repeat it? Previous research (El-Maghrawy et al. 1995) has shown gastric emptying to be delayed in people with MS. However, no previous studies have investigated the effect of regular exercise on GER in MS.

If it has not been done with humans before, has the problem been worked out as fully as possible in animals, analytically, technically and to assess possible toxic effects? N/A

10 ETHICAL PROBLEMS

Please itemise here any ethical problems which you perceive to be associated with the research project:

There is perceived to be no ethical problems associated with this research project.

11 SUBJECTS AND CONTROLS

How will the subjects and controls (if any) be selected and what wider population will they be representative of?

Recruitment for this study will take place at the same time as recruitment for the “Getting the Balance Right” study. Participants will be asked to volunteer for measurements of GE and HRV before and after their exercise program. Participants may elect to be part of the exercise component of the GTBR exercise program without volunteering for GE and HRV measures. Information leaflets will be distributed by the MS
Society by post along with the MS Society newsletter before the start of the “Getting the Balance Right” exercise program. Participants will be given the information sheet at least 8 days in advance of the first testing session in order for them to thoroughly consider their involvement. Controls will be recruited from people with MS on a waiting list to participate in an exercise program as part of the “Getting the Balance Right” study.

Will participants or controls undergo independent medical examination, before, during or after the project? As part of the “Getting the Balance Right” study, all patients will have been assessed by a Chartered Physiotherapist prior to participating in the exercise program.

What is the nature and extent of the medical examination that participants and controls are to undergo before participating in this project? N/A

How will the health of the participants and controls be monitored during and after the project? N/A

If a placebo group is to be used, will the group receive the best standard of therapy? N/A

Will pregnancy be excluded? Yes- it was excluded under the exclusion criteria of the “Getting the Balance Right” study.

How many subjects and controls will be involved, and in what age groups? Participants will all be over age 18. 16 subjects will be recruited who are going to participate in the exercise program. 8 controls that are not participating in a current exercise program but are on a waiting list to participate in one will be recruited.

Have sample size calculations been checked with an expert statistician? Yes, sample size calculations have been checked with statistician Dr. Jean Saunders, University of Limerick.
12 DRUGS

(i) If drugs are to be used, do the drugs that are the subject of the investigation have Medicines Board approval for use in a clinical trial? N/A

(ii) Drugs

(a) Please state all drugs involved in the study: N/A

(b) Are these drugs being supplied by a drug company? N/A

(c) Are the drugs used in the normal course of medical treatment? N/A

(iii) Pharmacy Support

(a) Has the Hospital Pharmacist been informed? N/A

(b) Where will supplies of drugs be kept? N/A

(c) It is recommended that a copy of the Trial Codes be kept in Pharmacy. Do you object? If so, why? N/A
(iv) What efforts will be made to exclude unknown drugs or other unknown medication in patients or volunteers? N/A

(v) Substances to be given to subjects

(a) Describe any special diet, isotopic tracers, or other information related to this study.
13C octanoic acid will be given to the subject as part of a standard breakfast meal (e.g. pancakes). This will allow gastric emptying to be measured from the amount of 13C subsequently present in exhaled breath, a non-invasive method frequently used to measure gastric emptying rate in our laboratory.

(b) State routes of administration, amount and effect expected.
100µg 13C octanoic acid will be administered orally as part of a breakfast meal.

13 RADIOACTIVE SUBSTANCES

If radio isotopes are to be used, you are required to register the project with the Radiation Protection Adviser (St James’s Hospital). Are radio isotopes to be used in this study? N/A

If radio isotopes are to be used, please indicate that approval has been obtained from the IRPP (and please provide a copy of the Authority Certificate). N/A

14 SAMPLES TO BE TAKEN FROM THE SUBJECT
(Venepuncture, arterial, urine, biopsy etc)

(a) State type of sample, frequency and amount
Breath samples will be taken from the subject before they consume their breakfast meal, they will blow into 2 separate test tubes to get baseline samples. Breath samples will also be given at 15 minute intervals for up to 6 hours after meal consumption.

(b) Would the samples(s) be taken especially for this investigation or as part of normal patient care?
Breath samples will be taken especially for this investigation as it is the non-invasive method of measuring gastric emptying that will be used in this study.

15 PROCEDURES

Describe the exact procedures which will be applied to each subject. Participants (up to 4 at a time) will be welcomed at the door and guided to the testing area in the room. They will have fasted for 12 hours overnight. Participants will be asked to sign an informed consent form and to fill out a standardized pre-test questionnaire. Body weight and height measurements will be taken by SECA scales and stadiometer respectively. 3 electrodes will then be placed on the subject when they are seated to measure heart rate variability (HRV) by ECG. ECG will be taken throughout the testing session. The subject will be seated at a desk with computer, internet and reading materials they may use during the testing session. Two baseline breath samples will be taken, whereby the subject blows through a straw into a test tube and puts the lid on the tube. The subject will then be given a breakfast meal (e.g. pancakes) containing 13C octanoic acid. Gastric emptying will be measured non-invasively using the 13C-octanoic acid breath test. After consumption of the meal, subjects will give breath samples at 15 minute intervals for up to 6 hours. Participants will also fill out Visual Analogue Scales (VAS), describing their feelings of hunger and thirst at 30minute intervals throughout testing. Participants will record their dietary intake for the 7 days prior to each test day using a food diary and a set of scales that will be provided by the investigator. Both testing days will follow the same protocol.

16 DISCOMFORT AND ADVERSE EVENTS

What discomfort or interference, however slight, with their activities may be suffered by all or any of the subjects?

There is perceived to be no significant adverse affects to the patient participating in the testing session. However there is a possibility that patients will get bored by remaining seated for up to 6 hours. They may also become embarrassed by having the electrodes placed on them for ECG measurement.

Indicate how adverse events are to be notified and evaluated.

Boredom will be minimized by providing various reading, DVD, internet and computer materials to keep the subject entertained. Subjects may also undertake their own work, provided they remain seated. Care will be taken when placing the electrodes on the subject for ECG measurement to put subjects at ease as much as possible. It will be indicated at the start of the testing session to the subject to mention to the researcher whenever they are experiencing feelings of discomfort.
17 SAFETY AND RISKS

Please give details of any potential hazards or side-effects, or other risks to subjects or to controls from investigative or therapeutic procedures or from withholding of therapy (this information must also be included in the Patient Information Sheet - see 3 (i)). There is minimal risk to subjects associated with the procedures involved in this study.

18 INFORMATION TO PATIENTS’ GENERAL PRACTITIONERS

(a) Please indicate briefly the information that will be given to GPs about the involvement of their patients in the research project (for drug studies, this should include the name of the active drug, the possible mode of action, and known side effects). All patients GPs will be notified about their patient’s participation in the “Getting the Balance Right” Study and what the study involves. They will also receive a second information leaflet about this study.

(b) GPs may know of reasons why patients should not participate in the Study and a letter should be sent to the GP to ask whether he/she knows of any such reasons. Please confirm that such a letter will be sent. The patients GPs will be notified that their patients are taking part in the “Getting the Balance Right Study” of which this research project is an extension. An additional letter will be sent to the GP if their patient volunteers for the measures in this study.

19 VOLUNTEERS

(a) Are any payments to be made to volunteers? If so, please give details. NO

(b) Where it is proposed to recruit medical students or student nurses as volunteers, the supervising authorities must be informed. If applicable, please confirm that the supervising authorities have been so informed. NO

20 CONSENT
21  PATIENT INFORMATION SHEET

(a) A written Information Sheet about the Trial should be given to all participants before they are asked to give written consent (see 3(i)). This Information Sheet should be submitted to the Scientific Research Ethics Committee with this Protocol before approval can be given to the Study.

Is the draft Information Sheet attached?
I confirm that the patient sheet is attached
The Patient should be given the opportunity to take away and consider the Information Sheet and sign the consent form later. If this is not practical, the Patient should at least be given sufficient time to read and discuss it with relatives if he/she wishes to do so. Any discussion about the Trial between Patient and Investigator should be in person and not by telephone.

(c) Where Patients entering a Trial are under 16 years of age, you will be required to obtain the consent of both the Patient and the Patient’s parents or guardian(s). If applicable, please indicate that such consent will be obtained: N/A

22  THE PRINCIPAL INVESTIGATOR IS RESPONSIBLE FOR INFORMING COLLEAGUES AND OTHER GROUPS WHO MAY BE INVOLVED OR AFFECTED BY THE RESEARCH.

Yes- The “Getting the Balance Right” study is being run in collaboration with the MS society, and the MS Society is aware of the added measures of GE and HRV being taken as part of this study also.
Appendix F

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ days per week

   □ No vigorous physical activities  ➞ Skip to question 3

2. How much time did you usually spend doing vigorous physical activities on one of those days?

   _____ hours per day

   _____ minutes per day

   □ Don’t know/Not sure

Think about all the moderate activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and
make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ days per week

   [ ] No moderate physical activities  ➔ **Skip to question 5**

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

   _____ hours per day
   _____ minutes per day

   [ ] Don’t know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

   _____ days per week

   [ ] No walking  ➔ **Skip to question 7**
6. How much time did you usually spend walking on one of those days?

______ hours per day

______ minutes per day

☐ Don’t know/Not sure

The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the last 7 days, how much time did you spend sitting on a week day?

______ hours per day

______ minutes per day

☐ Don’t know/Not sure

This is the end of the questionnaire, thank you for participating.