Development of an eating topography protocol and an investigation of its effects on body composition, appetite and mindfulness

By

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Abstract

Eating rate (ER) is part of the microstructure of meal ingestion and has been of increasing scientific interest due to manipulations being implicated in energy intake, appetite control and mindfulness. The current thesis aims to develop and test the slow eating rate (SER) protocol for use in overweight-free living adults and to investigate the protocol’s effects on body weight, hormones, metabolites and mindfulness.

The developmental part spanned over 5 studies. Studies A and B the SER was refined and finalised through volunteer feedback and in Study C successfully transformed into a 2-minute, online-friendly video which was then incorporated into an online (website and application) weight loss tool. In Study D software (AlexNet) which could identify chewing rate through ER video play back was developed. In Study E, the Mindful Eating Questionnaire -under development (MEQ-UD) was not found as a valid proxy for measuring ER.

The final study was a 10-week parallel, open label randomised controlled trial (control group: n = 7 intervention: n = 8) testing the SER protocol in a 6-week community intervention. Significant changes in body composition were seen in the intervention group, with reductions in weight (p= 0.006), BMI (p=0.006), body fat % (p= 0.026) and visceral fat (p= 0.007) and a trend towards a reduced energy intake (p=0.086) as compared to stable anthropometrics in the control group (n=7). The SER protocol resulted in significantly increased mindful eating in the intervention group.

The online monitoring (web and app) proved effective, with duration of intervention (days), total online session duration (minutes) and average online session duration/visit (mins) shown to be the most influential parameters correlated with BMI change. Combined, these data provide novel insights into the effects of a SER protocol in controlled environments and the community. Replication and evaluation in larger and diverse population groups is warranted.
Published works

Research articles:


Conference Abstracts:


Declaration

This thesis and the work to which it refers are the results of my own efforts. Any ideas, data, images or text resulting from the work of others (whether published or unpublished) are fully identified as such within the work and attributed to their originator in the text, bibliography or in footnotes. This thesis has not been submitted in whole or in part for any other academic degree or professional qualification. I agree that the University has the right to submit my work to the plagiarism detection service TurnitinUK for originality checks. Whether or not drafts have been so-assessed, the University reserves the right to require an electronic version of the final document (as submitted) for assessment as above. The thesis is available for Library use on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement or consent.

September 2018

Filip Koidis
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List of Abbreviations

AAL       active and assisted living
Ag        Unlabelled antigen
Ag⁺       Labelled antigen
AgRP      agouti-related peptide
AIM       Automatic Ingestion Monitor
ANNs      Artificial neural networks
ANOVA     Analysis of variance
ANS       Autonomic Nervous System
App       application
AUC       Area under the Curve
BMI       Body Mass Index
°C        Celsius
CAALYX    Complete Ambient Assisted Living Experiment
CCK       cholecystokinin
CE        cholesterol fatty acid ester
CHO       Carbohydrates
CIU       Clinical Investigation Unit
cpm       Counts per minute
CV        Coefficient of variation
CVD       Cardiovascular Disease
DASH      Dietary Approaches to Stop Hypertension
DEBQ      Dutch Eating Behaviour Questionnaire
df        degrees of freedom
EARS      electronic appetite rating systems
EDTA      Ethylenediamine tetraacetic acid
ENS       Enteric Nervous System
ER        Eating Rate
(F)       Free
FC Augmented fork feedback condition
FHMS Faculty of Health and Medical Sciences
FI Food Intake
g grams
GI Gastrointestinal Tract
GLP1 glucagon-like-peptide1
GOAT Ghrelin O-acyl-transferase
HAR Human Activity Recognition
HDL high density lipoprotein cholesterol
HOMA-IR homeostasis model assessment of insulin resistance
HRI human–robot interaction
HRI human–robot interaction
HtM Hand to mouth
iAUC Incremental Area under the Curve
IL-1β Interleukin 1 beta
K Constant
Kcal Calories
Kg Kilograms
kJ Kilo joule
L Litre
LDL Low density lipoprotein cholesterol
LOD limit of detection
MEQ Mindful Eating Questionnaire
MEWUD Mindful Eating Questionnaire Under Development
min minutes
ml Milligrams
mmol Millimole
MP3 Metroid Prime 3
NEFA’s Non-Esterified Fatty Acids
NER Normal eating rate
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<td>NFC</td>
<td>No feedback (augmented fork)</td>
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<td>ng</td>
<td>Nanograms</td>
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<td>NHS</td>
<td>National Health System</td>
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<td>NICE</td>
<td>National Institute for Health and Care Excellence</td>
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<td>NPY</td>
<td>Neuropeptide Y</td>
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<td>NSB</td>
<td>Non-specific binding</td>
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<td>p</td>
<td>Level of significance</td>
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<td>PC</td>
<td>Personal Computer</td>
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<td>PEG</td>
<td>Polyethylene glycol</td>
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<td>pH</td>
<td>Potential Hydrogen</td>
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<td>POD</td>
<td>Peroxidase enzyme solution</td>
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<td>PPI</td>
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<td>QC</td>
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<td>Qualisys Track Manager</td>
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<td>smart assisted living</td>
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<td>SD</td>
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<td>Sussex Ingestion Pattern Monitors</td>
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<td>SMS</td>
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<td>SVM</td>
<td>Support Vector System</td>
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<td>(TI)</td>
<td>tele-immersion</td>
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<td>TAGs</td>
<td>Triglycerides</td>
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<td>TAUC</td>
<td>Total Area Under the Curve</td>
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<td>Acronym</td>
<td>Description</td>
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<td>TFEQ</td>
<td>three Factor Eating questionnaire</td>
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<td>UEM</td>
<td>Universal Eating Monitor</td>
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<td>VAS</td>
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<td>Web</td>
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<td>μg</td>
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Chapter 1

Introduction
1.1 Obesity

Obesity has been a growing problem for the UK for many years now. In the 2016 Health Survey for England (Health Survey for England 2016) 26% of men and 27% per cent of women were reported as obese, a 15% increase since 1993, but a similar level since 2010 (see Figure 1.1). Furthermore 2% of men and 4% of women were reported to be morbidly obese (BMI $\geq$of 40kg/m²), with males leading the overweight category (40% versus 30% of females).

Figure 1.1: Adult overweight and obesity, Health Survey for England 2016.

According to the National Institute for Health and Care Excellence (NICE 2018), the measurement of waist circumference should be used for people with a body mass index (BMI) less than 35kg/m². For adults with a BMI of 35kg/m² or more, risks are assumed to be very high with any waist circumference (Figure 1.2).

![Waist Circumference Chart]

Figure 1.2: Adult Obesity: Health risks associated with body mass index (BMI) and waist circumference, Health Survey for England, 2016.

Pietrobelli (2005) defines obesity as an accumulation of excess body fat and recommends a diagnosis should be grounded on an accurate measure of body fat since BMI does not take into account the body composition (lean and fat tissues) or the distribution of adiposity in the body.
Abdominal fat produces more inflammatory cytokines and is more pathogenic than peripheral fat stores (Borgman 2006).

On a global scale 1 in 3 adults are now overweight or obese and more than 3 million deaths are attributed to the condition every year (Ng et al., 2014). As well as being associated with increased mortality (Whitlock et al., 2009), being overweight or obese is linked to a wide range of chronic health conditions such as type 2 diabetes, hypertension, coronary heart disease, stroke, cancer, metabolic syndrome and osteoarthritis (Kopelman, 2007). As such, it affects not only quality of life, but also the wider economy. Overweight and obesity, are estimated to have a global cost of $2.0 trillion annually (Dobbs et al., 2014), and to cost the National Health System (NHS, England) a staggering £5.1 billion (Scarborough et al., 2006/7).

1.1.1 Obesity and cardiometabolic risk factors
Obesity is classically associated with an amalgamation of metabolic risks including insulin resistance, dyslipidaemia, hypertension and low-grade inflammation, often termed cardio-metabolic risk factors (Boursier et al., 2018). These factors predict an increased risk of cardiovascular disease (CVD) and diabetes, and when grouped together are called metabolic syndrome (O’Neil and O’Driscoll 2015). Insulin resistance is thought to be the common denominator in development of both CVD and Type 2 Diabetes. There is a strong association between increased visceral fat, hypertension, dyslipidaemia and insulin resistance (Borgman et al., 2006). The impaired action of insulin leads to slow uptake of glucose by cells. To compensate for this more glucose is produced from the liver raising blood glucose levels. This increase can cause higher than normal (but below the diagnostic range for diabetes) blood glucose levels (6.1-6.9 mmol/L). The impaired insulin action is also known as pre-diabetes. Borgman et al (2006) states that within 10 years individuals with pre-diabetes will develop diabetes. There are no symptoms of pre-diabetes/insulin resistance so it can be hard to identify without a blood test. Additional factors that can contribute to metabolic syndrome include a pro-inflammatory state – which can be caused by excess adipose tissue producing inflammatory cytokines – and a pro-thrombotic state which refers to the tendency for blood to clot. In order to treat metabolic syndrome a focus on obesity and inactivity is important (O’Neil and O’ Driscoll 2015).
1.1.2 Obesity and lifestyle management
The core of obesity care is assisting patients in making healthier dietary, physical activity and behavioural choices that will result in a net negative energy balance (Kushner 2018). Weight loss is mainly dependent on a reduced energy intake and not the proportions of macronutrients (i.e. proportion of energy from fat, carbohydrates and protein) in the diet (Saks et al., 2009) as these will be determined by the patient’s culture, taste preferences and cooking style. However, tailoring the dietary macronutrient composition to the patient’s underlying medical condition and disease risk factors is also important (Saks et al., 2001, Elmer et al., 2006, Blumenthal et al., 2010, Nordman et al., 2011). For example, a patient suffering from high blood pressure should follow a calorie-reduced Dietary Approaches to Stop Hypertension (DASH) diet (Saks et al., 2001) whereas a patient with metabolic syndrome would benefit more from a Mediterranean diet (Shai et al., 2008).

In addition to reducing energy intake, patients are often encouraged to increase their energy expenditure by increasing their physical activity and incorporating exercise into their daily lifestyle (American College of Sports Medicine 2006). Amongst the most effective treatments for obesity and weight loss maintenance is behaviour therapy, major components of which are the aforementioned energy deficit and expenditure as well as effective self-monitoring (Jacobs et al., 2017) which can yield 2-5kg of weight loss over 6 months (Kim et al., 2013). Higher adherence to self-monitoring is more effective for weight loss (Boutelle and Kirschenbaum et al., 1998, Wadden et al., 2002, Cobain and Foreyt 2005, Burke et al., 2011) however adherence to paper-based forms of self-monitoring decreases overtime (Acharya et a., 2011) and is associated with participant burden (Burke and Wang 2011). Self-monitoring by web or mobile devices has shown increased rates of adherence over paper and pencil methods (Tate et al., 2006, Beasley et al., 2008, Wang et al., 2012) suggesting that smartphone applications could be used to facilitate self-monitoring. Recently, a study Jacobs et al (2017) demonstrated that a smartphone app (in this case Noom) could be used as a stand-alone intervention to facilitate short-term weight loss with participants (7,633 overweight men and women, age 35.5 ±5.6 years) having higher adherence attaining a significantly lower BMI after 3 months [-1.9 BMI points, and for every 10% increase in adherence there was a decrease of 2.59 BMI points, (-1.36kg, SE =0.24, p<0.001)].

1.1.3 Obesity and use of technology in healthcare
The use of technology in healthcare, also commonly referred to as eHealth, has been described by Eysenbach (2001) as “an emerging field in the intersection of medical informatics, public health and business, referring to health services and information delivered or enhanced through the use of internet and related technologies”. As the technical capacity of the internet grows, it becomes better
able to offer a feasible medium for health behaviour interventions and research (Atkinson & Gold, 2002). Approximately 4.5% of all searches on the web might be health-related (Eysenbach & Kohler, 2004). The use of eHealth or technology-based interventions has several potential advantages. For example, it gives the opportunity to tailor information to the specific needs of individuals, such as lifetime monitoring where patients can receive comprehensive additive data on their past, present and future health (Farahani et al., 2018). Another benefit is ease of use, as eHealth can be easily adopted by users, since they only require clicks or simple operations on smartphone apps and/or wearable devices (Farahani et al., 2018). Furthermore, it improves the capability of combining a variety of media to address the particular purposes of an intervention and it increases the possibility for users to remain anonymous and receive support from peers or experts about sensitive health issues (Atkinson & Gold, 2002). In its e-health Action plan 2012-2020, the European Commission (European Commission 2012) explicitly mentions that, “e-health – when applied effectively—delivers more personalised ‘citizen-centric’ healthcare, which is more targeted, effective and efficient and helps reduce errors, as well as the length of hospitalisation. It facilitates socio-economic inclusion and equality, quality of life and patient empowerment through greater transparency, access to services and information and the use of social media for health.” Bol et al (2018), when investigating the differences in mobile health app use, found that age and level of education were significantly related to mobile health app use (younger and more educated using more apps than older and less educated) however they also suggested that users of fitness apps and reproductive health apps are generally younger whereas users of self-care and vitals apps are typically older.

Technology-based clinical trials, investigating the use of computers and/or mobile applications, focusing on weight change in the overweight or obese, have demonstrated beneficial effects on weight loss (Bacigalupo et al., 2013; Thomas & Bond, 2014; Tufano & Karras, 2005; Wieland et al., 2012). Furthermore, a number of systematic reviews and meta-analyses have examined the potential of e-health interventions in the treatment of overweight and obese adults. Only in the last 3 years, (up to 2017) at least five e-health meta-analyses on weight management in adults have been published indicating that behavioural e-health interventions are indeed feasible for weight management (e.g., Hutchesson et al., 2015; Raaijmakers, Pouwels, Berghuis, & Nienhuijs, 2015; Seo & Niu, 2015; Sherrington et al., 2016; Tang, Abraham, Greaves, & Nikolaou, 2016).

In the United Kingdom, overall 39% of overweight adults reported using at least one weight management aid (Figure 1.3) with the use of websites or mobile phone apps coming second only to attending the gym or participating in other exercise (Health Survey for England 2016). Furthermore,
as of April 2017, the google Play Store (Google Inc) reported 50-100 million installs of MyFitnessPal (MyFitnessPal) globally; a nutrition and physical activity self-monitoring app (Lieffers et al., 2018).

Figure 1.3: Use of weight management aids, Health Survey for England 2016.

1.2 Eating rate
Eating rate is part of the microstructure of meal ingestion (which also includes the behavioural components associated with eating, bite size and meal duration) which has been of increasing scientific interest in an attempt to identify targets for interventions to constrain energy intake (Almiron-Roig et al., 2015). Slow eating rate is also mentioned as part of the latest guidelines by National Institute for Health and Care Excellence (NICE 2018) recommended lifestyle changes for adults who are overweight or obese.

Eating rate and aspects of meal microstructure were first studied in a laboratory setting in the early 70’s following Schachter’s theory of “externality”, namely that internal state is irrelevant to eating by obese individuals, and that external, food-relevant cues (such as food palatability) trigger eating (Schachter 1968), and the set-point theories of Nisbett, Sclafani and Kluge (1972) according to which body weight is determined by a set-point, which regulates weight in the manner that the thermostat in a central heating system regulates temperature (Stroebe et al., 2008). A note-worthy study was carried out by Schachter and Rodin (1974) where obese and nonobese male undergraduates where videotaped whilst consuming meals of high and low preference under high vs low hunger conditions. The meal microstructure parameters measured were: meal length, number of bites and the amount of food consumed (g). The study showed that obese subjects ate more grams
per second than the nonobese ones as well as more high preference foods and less low preference foods.

In the paediatric literature, where self-presentation may be less important, obese children eat faster during the course of a meal than healthy-weight children (Drabman et al., 1979, Argas et al., 1987, Barkeling et al., 1992). Furthermore, eating rate in early infancy predicts adiposity at 1,2 and 3 years of age (Argas et al., 1990) and differentiates between higher and lower risk of obesity (according to parental Body Mass Index (kg/m²) at 3 months of age (Stunkard et al., 2004). Due to the aforementioned associations of eating rate and obesity in children, the Children’s Eating Behaviour Questionnaire that came out in 2001, was the first one to have a question on speed of food consumption (Wardle et al., 2001). Finally, in a twin study of 10-12y old children and the first of its kind (Llwellyn et al., 2008), faster eating was found to be a heritable behavioural phenotype related to higher weight.

Westertep-Plantenga (2000) reviewed the use of cumulative intake curves in characterising eating behaviours in humans. He concluded they are a good tool to use and demonstrated distinct differences in the intake curves of normal weight, unrestrained women and overweight, restrained women, with normal weight women having a more decelerated eating curve and overweight women eating at a linear rate. Following this Zandian (2009) began to classify eaters as linear and decelerated. Linear (also referred to as restrained) eaters eat at a constant rate whereas decelerated eaters have an initial higher eating rate which slows down as the meal progresses giving a biological satiation curve. The initial rate of the curve reflects both palatability of food and hunger and the change in rate over time shows the progression of satiation.

1.2.1 Eating rate and obesity

Eating rate (ER) is commonly referred to as the speed at which food and drink is consumed (Martin et al., 2007, De Graaf & Kok 2010, Viskaal-Van Dongen, Kok, & de Graaf 2011, Robinson et al., 2014, Bolhuis and Keast 2016). It is found to play a role in health outcomes including cardiovascular disease (CVD), obesity and diabetes (Otsuka et al., 2008) and has also shown to contribute to feelings of satiety (Privitera, Cooper & Cosco 2012, Andrade, Greene & Melanson 2008). Eating rate has also been found to influence food intake, with fast eaters consuming more energy than slow eaters (Viskaal-van Dongen, Kok & de Graaf 2011, De Graaf & Kok 2010, Robinson et al., 2014). As eating rate has been found to influence the obesity, reducing eating rate may be beneficial for tackling obesity (Ekuni et al., 2012).

Modern lifestyles have changed our pattern of eating behaviour (Bisogni et al., 2007); from being
seated while eating to a more rapid food consumption pattern-on the go, in conjunction with the switch from minimally processed foods, to highly processed, energy dense, and low fibre foods (Fraser et al., 2011). This change in eating behaviour has been strongly implicated in the increased prevalence of overweight and obesity in the last three decades (Ng et al., 2014). Foods and beverages that can be consumed quickly are associated with overconsumption since the speed of eating bypasses the usual “oral metering” which is necessary for the full expression of satiation and satiety (de Graaf 2011). This is attributed to insufficient mastication and/or to reduced levels of orosensory signalling during eating, leading to limited cephalic-phase responses and delayed onset of satiety.

1.2.2 Eating rate, obesity and energy intake

Over a quarter of adults remain obese (BMI>30kg/m^2), (Health Survey for England, 2016) and with many studies suggesting eating rate is a contributing factor, it is vital to investigate the relationship between eating rate and energy intake (Laessle, Lehrke & Dückers 2007, Viskaal-van Dongen, Kok & de Graaf 2011).

Since the 70’s reducing eating rate has been advocated as a simple and effective method for control of food intake and thus body weight (Bellack, 1975). More recently several cross-sectional studies, relying on self-reported ER, suggested that eating quickly was associated with the increased prevalence of obesity, primarily in Asian populations (Sassaki et al., 2003, Otsuka et al., 2006, Mayurama et al., 2008, Tanihara et al., 2011, Sakurai et al., 2012, Ohkuma et al., 2013). However, a systematic review and meta-analysis by Ohkuma et al (2015) concluded that the evidence remains inconclusive. The meta-analysis included 20 cross-sectional studies, two longitudinal studies and only one study where ER was measured using a monitor. It excluded interventional studies and those targeting children. An earlier systematic review and meta-analysis by Robinson (2014) showed that slower eating rate was associated with lower energy intake, regardless of the type of manipulation used to change the eating rate (instructions, different food textures and manner of consumption). Robinson’s meta-analysis, which focused on 22 studies (including experimental ones), also highlighted the need for effective interventions that can reduce ER and can be adopted in everyday life to help limit excess consumption.

Laboratory studies have shown that obese individuals consume a meal with larger bites (or spoonfuls) and consume it at a higher eating rate, both of which contribute to greater food intake (Hill & McCutcheon, 1984; Laessle, Lehrke, & Duckers, 2007). Laessle et al (2007) highlighted the relationship between eating rate and obesity, finding that obese subjects had a significantly faster rate of eating and greater total intake than their normal weight counterparts. Obese
participants also consumed larger proportions of food throughout the duration of a meal such as larger spoonfuls. Laessle therefore concluded that the findings under laboratory conditions of increased eating rate and larger spoonfuls are predictive of the development of obesity should these behaviours be extrapolated to the free-living state. A study by Spiegel (2000), had conflicting findings as they demonstrated that obese female participants (n=9) had the same ER as the lean female participants (n=9), however only females were investigated in this particular experiment and detection of chews was performed via an external electromyographic recording from the masseter muscle (jaw), which might have affected the participants natural chewing.

Andrade et al (2008) performed a study in 30 women (mostly healthy weight, only 4 had a BMI>25) where an ad libitum pasta meal (870kcal) was consumed under slow eating rate instructions (small bites, small tea spoon and put it down between bites, chew each bite 20-30 times) and fast eating rate instructions (large soup spoon, eat as quick as possible, no pauses between bites). During the slow ER condition the meal duration was extended by approximately 21 minutes and the total energy intake was significantly reduced (by an average of 67kcal) as a result of the reduced eating rate, which was expressed in kcal/minute (66kcal/minute less than under the fast eating rate instructions). Interestingly, Andrade repeated his study with the same protocol with the addition of controlling for water intake (300ml to be consumed by both groups). Although the slow eating rate group reported increased feelings of satiety and reduced hunger, there were no statistically significant differences in total energy intake. The authors did point out the thirst ratings at meal completion suggest that the slow eating rate group might have consumed less water if that option was possible.

1.2.3 Eating rate and cardiometabolic risk factors
Cardiovascular disease (CVD) encompasses health problems such as ischemic heart disease and hypertension and is the leading cause of death in developed countries (Lee et al., 2012). Early identification and management of cardio-metabolic risk factors has become an area of interest in order to reduce the onset and burden of CVD.

A study by Lee et al (2012) found a steady increase of weight, BMI, blood pressure and fasting blood glucose as well as levels of cholesterol and triglycerides amongst individuals of increasing eating rate categories. High density lipoprotein (HDL) cholesterol was the only factor found to be inversely related to eating rate. The fastest (<5min/meal) ER group had significantly increased odds ratios for cardiometabolic risk factors such as high glucose and low HDL-cholesterol levels in
males, even after adjusting for BMI. Risk factors of CVD therefore increased with eating rate. However, eating rate was subjectively reported in a nutritionist-administered survey, asking ‘What eating rate corresponds to your ordinary meals?’ Furthermore the study involved only Korean subjects and therefore is potentially not generalisable to other populations. Cassady et al (2009) have shown that eating rate can increase lipid bio-accessibility in a study of healthy adults where eating rate was manipulated when chewing almonds.

1.2.4 Eating rate and diabetes risk
The association of rapid eating and the development of diabetes have been investigated, mostly in the Asian population (Zhu et al., 2015, Otsuka et al., 2015, Saito et al., 2018). One more novel area of focus has been the influence of eating rate on levels of circulating inflammatory markers and cytokines such as interleukin (IL) 1β, thought to be important indicators of diabetes onset (Misaki et al., 2010, Mochizuki et al., 2012). IL-1β is responsible for the impairment of insulin secretion, by inducing apoptosis of the β-cells within the pancreas (Loweth et al., 1998). In relation to eating rate, during a cross-sectional study of middle aged male subjects in Japan, circulating levels of IL-1β were found to be positively associated with an increased eating rate (Mochizuki et al., 2012) indicating that eating rate may be related to the onset and development of insulin resistance.

Studies directly measuring insulin resistance have found the homeostasis model assessment of insulin resistance (HOMA-IR) to increase significantly with the increase of eating rate in both men ($P<0.001$) and women ($P<0.01$). However, this relationship did not retain significance in female subjects when adjusted for BMI (Otsuka et al., 2008). Two more recent studies by Zhu et al (2015) (older Japanese adults, mean age 63.7±6.9), and Saito et al., (2018) (aged Japanese population, 75-80yrs), suggested that eating quickly (assessed via a self-reported questionnaire, corrected for Age, BMI and denture health) was correlated independently and significantly with higher odds of metabolic syndrome in the Japanese population.

As a result of these findings, it can be suggested that eating at a slower rate may help prevent insulin resistance in old age amongst normal weight Eastern individuals. However, once again the generalisability of the findings to other populations, particularly those in the West or those with a high BMI, may be limited.
1.3 Measuring ER and monitoring techniques

1.3.1 Tools for monitoring and measuring Eating Rate

Originally, in order to measure eating rate, food was liquidised and provided through a flexible tube or a straw (Hashim and Van Itallie 1964), though this methodology did not mimic a natural way of eating (Chapelot, 2013). Later on, Bellsile’s team (Bellsile and Le Magnen, 1980) designed the Edogram, an oscillograph where a participant’s chewing and swallowing movements were continuously recorded, allowing precise temporal analysis of eating sequences. In the same period, a revolutionary device called the Universal Eating Monitor (UEM) was created by Kissileff’s research group (Kissileff et al., 1980). The UEM is a coupling between an electronic balance and a computer that allows covert continuous weighing of a subject’s plate or other food. Measurements can be taken precisely every 3 seconds throughout a single-course meal consisting of a relatively homogenous mixture of foods. The monitor has the capacity to compare meal duration, total intake, initial rate of intake and the deceleration of intake in subjects ingesting either a liquid or solid version of the same food after a 3 or 6 hour fast (Kissileff et al., 1980). The UEM monitor became widely used and its methodology was adopted by other laboratories (Westertrep-Plantenga et al., 1990) such as VIKTOR (Barkeling et al., 1995), the Sussex Ingestion Pattern Monitors (SIPM) (Yeomans, 2000) and the Mandometer® (Ioakimides et al., 2009, Ford et al., 2010). All adaptations of the UEM followed the same basic concept; that of a set of hidden scales connected to a digital computer where the weight of the plate is measured at regular intervals as the participant consumes their meal, for within-meal eating rate comparisons.

Barkeling’s adaptation of the UEM (Barkeling et al., 1995) consisted of a hidden scale placed under the test-subject’s table, connected to a microcomputer whereas the SIPM added a custom software allowing data detection of both continuous (uninterrupted) meal and as a series of defined eating bouts as well as ratings (visual analogue or general labelled magnitude scale) of appetite and well-being. (Yeomans, 2000). The Mandometer was developed at the Section of Applied Neuroendocrinology and Mandometer Clinic, Karolinska Institutet, Stockholm, Sweden (figure 1.4) and was the first UEM adaptation that incorporated visual and auditory feedback via a computer screen placed in front of the volunteers, instructing them at what rate to eat by following the cumulative eating rate curves on display (Ioakimides et al., 2009) (Please see Appendix 1 for more details on the Mandometer function).
Figure 1.4: Mandometer method for assessing eating rate (Ioakimides et al., 2009) depicting digital computer screen, connected to a set of electronic scales.

The Mandometer method was also used by Ford (Ford et al., 2010) in the first eating rate study in a community setting, specifically as an eating behaviour retraining tool in adolescent obese young people aged 9-17 years. In this study, the intervention group (children and family members) underwent training on how to use the Mandometer and were then asked to consume one meal/day (evening meal) at home, using the Mandometer. Follow up (in the children’s hospital) was by a nurse trained-in the Mandometer method (once a week for the first six weeks, then every second week for 6 weeks and every 6th week thereafter, as well as phone support every second week from the 12th week onward). Both groups received follow ups by a dietitian every four months, following standard healthy eating guidelines. Both groups lowered their BMI by 12 months with the Mandometer group achieving a significantly lower BMI, an effect which was maintained at 18 months. The intervention group also significantly reduced their mean meal size at 12 months and had a greater improvement in their concentration of high density lipoprotein cholesterol (HDL). This study showed that an intervention aimed at slowing down the speed of eating and reducing portion size through retraining eating behaviour is a useful adjunctive therapy to standard lifestyle modification in obese adolescents. However, as the authors of the study note, the difference in outcomes might reflect the higher intensity of contact in the intervention group receiving Mandometer training. Furthermore, the intervention group had a 15% higher attendance at the follow up clinic. Practical limitations to the Manometer approach have also been raised, not least it might be too cumbersome or artificial for use in real-life eating contexts (Hermans et al., 2017) and the need to train participants and clinicians in the Mandometer method makes the intervention more time consuming and more challenging to roll out in a community setting. Although the UEM-type devices have shown good reproducibility between investigators (Westerterp-Plantenga et al., 1990, Barkeling et al., 1995, Martin et al., 2005) the use of specialised equipment (digital scales, computer hardware) and the need for staff and user training and specialised data analysis suggests
they may also be too ‘artificial’ (Allirot et al., 2012) or too cumbersome to be adopted in the community (Hermans et al., 2017).

1.3.2 Measuring ER through Video Playback
Video recording was first used in the 1970’s to compare eating behaviour in healthy and obese subjects (Hill and McCutcheon 1975, Rogers and Blundell 1979) with the total amount of food eaten, meal length, and number of bites all being measured by video replay. More recently Llewellyn (Llewellyn et al., 2008) suggested that simple video recording can provide an alternative solution to measuring the eating rate of children. In her study, 10-12 year old twin children eating a standard meal at home were video recorded and eating rate was measured in bites/minute over the 4 quarters of the meal. Several specific rules were used to analyse the video such as “Fruit bites were counted when the child bit half a piece of fruit or more but were not counted if he or she ate less than half a piece of fruit or merely sucked the fruit” and that “A series of tiny rapid nibbles (e.g., taking several nibbles to eat the corner of a sandwich quarter) was counted as one bite, but an isolated nibble was not counted” which would be challenging to be adopted by other eating rate analysis studies due to their vague, potentially subjective nature and the labour intensive coding/ analysis required. Allirote et al (2012) assessed the reproducibility of intakes and meal mechanics (cumulative energy intake, number of bites, bite rate and mean energy content per bite) by video-recording participants through cameras in the ceiling of a restaurant setting. He concluded that although video recording didn’t allow for an in-depth analysis of eating microstructure (cumulative intake assessed by bite analysis instead of grams/minute as it would normally be assessed via a UEM) it was quite informative for assessing eating rates in a normal eating rate environment.

1.3.3 Automatic Methods of measuring ER in a clinical setting
Thompson (Thompson et al., 2010), in their overview of dietary assessment methods, highlighted the need for new technological approaches for the objective and accurate assessment of free-living food intake (FI) patterns to monitor eating behaviours. Food intake (FI) was described as the use of ‘hand to mouth (HtM) gestures, chews and swallows’. Advances in the area of FI monitoring have focused on replacing self-reporting methods (Sazonov et al., 2008, Junker et al., 2008, Melanson et al., 2009, Sazonov et al., 2012, Dong et al., 2012, Liu et al., 2012) with studies focusing on the use of chewing sounds captured through an in-ear microphone to characterise and detect FI activity (Troster et al., 2009, Psssl er and Fischer 2011). The aforementioned studies, developed specialised algorithms that could process the chewing signal and produce acceptable results for single meal
experiments in laboratory studies, where the number of food items was restricted. Dong et al (2012), in a study focusing on recognition of gestures via the use of wearable sensors, developed a watch-like device that contained a miniature gyroscope, measuring intake via an automatic tracking of wrist motions during hand-to-mouth movements (bites). This study showed a high sensitivity for bite counting though does rely on the subject to switch it ON and OFF at every meal, in order to avoid spontaneous hand gestures registering as bites. Liu et al (2012) presented a wearable sensor platform, which consisted of a microphone and a camera-detecting and characterising FI with a high recognition (80% and above) rate (see figure 1.5). Although these technologies presented satisfactory performances in clinical settings, their accuracy for detecting unrestricted food intake in free-living environments is yet to be tested.

![Wearable sensor](image)

**Figure 1.5:** wearable sensor by Liu et al (2012): Left: a subject wearing the sensor during food intake. Right: the profile of the sensor.

### 1.3.4 Automatic Methods of measuring ER in a free-living population

A further innovation came later on courtesy of Fontana and colleagues (Fontana et al., 2014), specifically a non-invasive wearable device (see Figure 1.6) named the Automatic Ingestion Monitor (AIM). AIM wirelessly integrates three different sensors with a smartphone: a jaw motion sensor to monitor chewing, a hand gesture sensor to monitor hand to mouth (HtM) gestures, and an accelerometer to monitor body motion. A novel approach to sensor information fusion and pattern recognition based on artificial neural networks (ANNs) was used in an attempt to achieve robust and accurate detection of FI in free-living conditions that present a substantially more challenging environment than the laboratory. This FI detection technology was developed against the gold standard, which was measured by food journals, and by pushing a button during the chewing process in order to inform the software’s timing intervals. In a clinical trial, AIM showed an average accuracy of 89.8% for automatic and objective monitoring of eating behaviour in 12 participants (6 males, 6 females) who wore it for 24 hours with no activity restrictions (except for showers). As the authors note, the main limitation of the study was the use of self-report as the
gold-standard for developing the FI detection methodology as subjects may provide inaccurate (Black and Cole 2001) information about their intake and thus affect the results.

![Figure 1.6: AIM. Left: wearable sensor system: (a) jaw motion sensor, (b) wireless module, (c) RF transmitter, and (d) smartphone. Right: subject wearing AIM. Fontana et al (2014).]

A recent study by Hermans et al (2017) involving 114 participants, was carried out in a naturalistic setting (laboratory furnished as a small restaurant), where the effect of real-time vibrotactile feedback delivered through an augmented fork (Figure 1.7) on eating rate, satiation and food intake was tested. Participants in the ‘augmented fork feedback condition’ (FC) received vibrotactile feedback from their fork when eating faster than 1 bite/10 seconds [as pre-tests showed that 10s bites speed slows down fast eaters, without making it too difficult for them to finish their meal (Hermsen et al., 2016)] whilst a second group received no feedback (NFC). The fork automatically measured eating rate (as total number of bites/minute) and success ratio by measuring the number of bites outside of the 10second time interval/total bites. The study found that the use of the augmented fork successfully decelerated participant’s eating rate by reducing significantly (p=0.011) the number of bites/minute (FC: 4.55 bites/min NFC 5.28 bites/min) and increasing the success ratio in the FC group. Although this is a novel approach, the long term effectiveness of this form of feedback and the feasibility of this approach across multiple meals requires further investigation. Furthermore, participant’s baseline ER was self-reported as part of their screening questionnaires and not measured, which might affect the results produced from the vibrating-fork as the researchers would be unable to tell how much each participant’s ER changed compared to their habitual ER.
1.4 ER and mindfulness

Fester et al (1962) first introduced the idea that obese people take larger bites and eat faster than lean people. He argued that obese people would eat less if they took smaller bites and ate slower. Subsequent behaviour therapists; Stuart and Davis (1972), Jordan (1976) and Brownell (1990), have recommended that obese people try to eat more slowly because it would help them to eat less and to be satisfied with less food. Eating rate has also become the basis for research investigating the effect of behavioural therapy on weight loss (Andrade et al., 2008, Keranen et al., 2009). Interestingly, Kristeller and Wolever (2010) have proposed a theory, whereby mindfulness enhances awareness of and responsiveness to satiety cues, and therefore functions adaptively to reduce energy intake. Therefore, the effect of reducing ER on weight outcomes may also be mediated by psychological (increased mindfulness) and not only biological reasons.

1.4.1 Mindfulness

The term ‘mindfulness’ can be used to refer to a range of different practices and different authors have conceptualized mindfulness in slightly different ways (Tapper et al., 2017). Mindful eating has been found to be a specific subset of mindfulness and distinguishable from it (Clementi et al., 2017).

Kabat-Zinn (2003) defines mindfulness as ‘awareness that emerges through paying attention on purpose, in the present moment, and nonjudgmentally to the unfolding of experience moment by moment’. This definition arguably encompasses two key ideas; that of paying attention to present
moment experience, and also of taking a non-judgmental attitude towards this experience. Paying attention to present moment experience requires attention regulation, and this is highlighted in most definitions of mindfulness (e.g. Bishop and Shapiro 2004). Indeed, most mindfulness practices include exercises in which the individual attempts to maintain their attention on a particular aspect of their present experience. For example, they may attend to their breath, shifting attention back to the breath whenever it wanders. This practice involves several different attentional processes; monitoring the focus of attention, disengaging from distractions, and re-orienting attention back to the original focus (Lutz, Slagter, Dunne, & Davidson, 2008).

1.4.2 Mindful eating and weight management

One approach to weight management that is becoming increasingly popular is the use of mindfulness-based interventions. These are currently being employed by a number of healthcare organizations, as well as being promoted as a strategy for weight management and eating regulation amongst the general public. However, the strength of the evidence for such an approach is unclear. For example, Olson and Emery (2015) conducted a systematic review of 19 mindfulness-based interventions for weight loss. Whilst 13 of these showed significant reductions in weight, it was not certain that these changes were brought about by increases in mindfulness; a conclusion reached by the authors themselves. They cite a need for further research to isolate mindfulness as an active component of treatment, for example by measuring changes in mindfulness alongside more traditional weight loss outcomes.

However, assessing change in mindfulness itself seems to be challenging. In particular, questionnaires designed to assess mindfulness tend to show poor convergent validity and their items may be interpreted in different ways by those with and without experience of mindfulness. They may also be subject to significant desirability bias especially where one group has received training in mindfulness practice and thus subsequently becomes aware of what they are ‘meant’ to be answering (Grossman, 2011).

An alternative means of identifying a relationship between mindfulness and a particular outcome is simply to restrict the experimental manipulation to mindfulness techniques only. This means that any change in the outcome variable can be more confidently attributed to the mindfulness component. However, because weight loss is difficult to achieve, and because experts recommend that interventions contain multiple elements (NICE, 2018), in practice such an approach is rare in research examining the effects of mindfulness on weight loss. Nevertheless, there are studies that have examined the independent effects of mindfulness, or mindfulness-related strategies, on what can be regarded as surrogate measures of weight loss, for example energy intake or food choice.
Such outcomes are also relevant for weight maintenance.Whilst there is no guarantee that changes in such outcomes will necessarily translate into weight loss or weight maintenance, they enable us to more confidently conclude that the change was, indeed, due to the mindfulness-related strategy or strategies employed.

1.4.3 Mindfulness and weight loss
As Tapper et al (2017) noted, most studies that have examined the use of mindfulness for weight loss have employed a combination of mindfulness and non-mindfulness techniques, making it difficult to establish the independent effects of the mindfulness components. However, Tapper et al (2017) identified three studies that have looked at the effects of mindfulness-only components on weight loss. Mantzios and Wilson (2014) examined the effects of increasing present moment awareness of the sensory properties of food as a way of assisting weight loss. They did this by asking undergraduate students to answer a series of questions every time they ate for a 5-week period. These questions were provided in the form of a diary that participants were asked to complete either whilst they were eating or immediately afterwards. In the mindfulness condition the diary included questions relating to how the meal tasted and smelled as well as its colour and texture. Participants were encouraged to answer these in as much detail as they could and to revisit the questions every 2 to 3 min. In this way they were prompted to repeatedly return their attention towards their present moment experience of eating. In the control condition the diary consisted of questions that encouraged them to think about their meal in a way that was not related to their present moment experience. Although this study suffered from a high level of attrition (64 of the 136 participants failed to return for follow up measures and/or did not adhere to instructions), the results showed that those in the mindfulness condition lost significantly more weight than those in the control condition.

Alberts et al (2010) examined the effects of present moment awareness and acceptance amongst overweight and obese adults. Although this study was primarily aimed at reducing food cravings, it also assessed weight loss. All participants attended a series of 10 weekly meetings that consisted of information on healthy food choices and an hour-long session of physical activity. Those assigned to the mindfulness group received an additional instruction manual designed to develop present moment awareness and acceptance skills, together with audio instructions on an MP3 player and daily emails containing quotes about acceptance-based craving regulation. The manual contained eight chapters that were designed to be read over seven weeks and included exercises aimed at developing present moment awareness of bodily sensations, eating behaviours, and craving related
thoughts, as well as acceptance of craving related bodily sensations and thoughts. Although those in
the mindfulness group lost more weight than those in the control group (mean weight loss: mindfulness: 3.51kg vs control 2.42, within group significant weight loss p<0.05, p value not reported), with just 19 participants in total the study was likely underpowered and this difference was not significant.

Finally, Mantzios and Wilson (2015) examined the effects of two different types of mindfulness interventions on weight loss over a 12-month period with military employees. All participants initially attended a presentation of information relating to eating behaviours and weight loss and received corresponding written materials. Participants assigned to the control group were then simply asked to watch their weight and food consumption with the help of these materials. By contrast, those assigned to the two mindfulness conditions (mindfulness meditation versus mindful self-compassion) attended a 2-day workshop on mindfulness meditation and were asked to practice three times a day with a meditation teacher, for a period of 5 weeks.

The workshops included exercises that promoted present moment awareness of bodily sensations, thoughts, emotions, environmental cues and the sensory properties of food. Those assigned to the mindfulness with self-compassion condition attended an additional day's workshop that included exercises that emphasized kindness to the self. Two of their three daily practice sessions were also devoted to meditation practice that was designed to promote kindness to the self. Weight was assessed 5 weeks post-baseline (i.e. immediately following the end of the intervention period), at 6 months and at 12 months. The results showed significantly greater weight loss in the two mindfulness groups compared to the control group at 5 weeks and 6 months, but no difference between groups at 12 months. However, attrition in this study was both high and biased. Of the 88 individuals who were randomized, 25 dropped out and all of these were from the two mindfulness conditions. In other words, in the mindfulness meditation and mindfulness self-compassion groups there were attrition rates of 34 and 52% respectively whereas there was no attrition in the control group. This means that participants remaining in the mindfulness groups at follow-up were likely to have been relatively more motivated to lose weight and/or have higher self-regulatory skills. As such it is difficult to attribute the differences in weight loss to the mindfulness interventions alone.

Thus, whilst average levels of weight loss in the above studies were all higher in the mindfulness conditions, methodological weaknesses (high attrition and small sample sizes) limit the conclusions that can be drawn from these studies regarding the use of mindfulness for weight loss (Tapper et al., 2017).
1.5 Eating rate and appetite
Appetite is a term that is applied to a number of dimensions of eating behaviour including preference, selection, and motivation to eat (de Graaf, 2012). It can be considered as being the “desire for food” (Forde et al., 2013). It is experienced as the sensation which motivates intake and can be present even in the absence of a physiological need. For example, the sight or smell of food can promote salivation and food intake. Conversely, satiation can be considered as the “process that leads to the termination of eating” (de Graaf, 2012). Satiation controls meal size and is influenced by a number of feedback mechanisms, such as declining food preference (sensory specific satiety) and gastric fullness. Satiety is the “process in which further eating is inhibited” and occurs as a consequence of having eaten (Miquel-Kergoat et al., 2015). The intensity of the satiety response is measured by the duration between meals and/or the amount of food consumed at the next meal (Ferriday et al., 2013). Satiety is influenced by a number of pre-absorptive and post-absorptive feedback mechanisms. Together, satiation and satiety are integral processes controlling food intake and feeding behaviour. Appetite, satiation and satiety are regulated by a number of internal factors that include chronobiology (Leong et al., 2012), the size and composition of the previous meal (Maruyama et al., 2002), an individual’s activity level (Otsuka et al., 2006), and genotype (Llwellyn et al., 2008). Through repeated experience of these factors over time, appetite control becomes influenced by learning and expectations. The role of learning is important since this is a modifiable component in the control of eating and counters the idea that appetite is entirely determined by biological factors. Evidence demonstrating the complex integration of internal and external cues that control eating behaviour is growing (Murakami et al., 2012, Ford et al., 2010, Karl et al., 2007, Kokkinos et al., 2010, Li et al., 2011).

Appetite, satiation and satiety are primed, in part, by cognitive and gastrointestinal processes even before food enters the mouth. Once food enters the mouth it is processed through mastication to increase its surface area to volume ratio to facilitate swallowing and aid in digestion efficiency. Chewing provides motor feedback to the brain related to mechanical effort reflecting food texture and it also exposes food particles to sensory receptors for the detection of flavour (taste and smell). Kegroat et al (2015), in their systematic review and meta-analysis of the effects of chewing on appetite, food intake and gut hormones, suggest that although chewing of food is an integral element of ingestion and digestion of food, it is unclear to what extent chewing and orosensory feedback influence satiation or satiety and therefore impact food intake. She also further noted that the effect of chewing on food intake could be potentially relevant in the fight against the increasing burden of overweight and obesity worldwide.
Satiety and its relation to eating rate is of particular importance as research has conveyed that a slower eating rate enhances satiety, reducing food intake (Privitera, Cooper & Cosco 2012, Andrade, Greene & Melanson 2008). Brownell et al (2000) have also suggested that eating slowly prolongs the pleasure sensations and thereby decreases the feelings of deprivation (cited in Martin et al., 2007).

Kissileff (2001) mentions that obese people usually fit a linear eating curve and lean people fit a decelerated eating curve. Furthermore, he also reported is that for obese people the lack of the satiation curve indicates a misleading perception of satiety which could cause over eating.

Krop et al (2018) in their recent comprehensive systematic review and meta-analysis, focused on the impact of oral processing (including chewing and lubrication) on appetite and food intake. A total of 42 original research reports were analysed, where a significant effect of oral processing aspects related to chewing on both self-reported hunger (-0.20 effect size, 95% confidence interval (CI): -0.30, -0.11) and food intake (-0.28 effect size, 95% CI -0.36, -0.19) were confirmed.

**Table 1.1:** Oral processing parameters as compared across studies (adapted from Krop et al., 2018).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comparison factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bite size (5-15g)</td>
<td>Large</td>
</tr>
<tr>
<td>Eating rate</td>
<td>Fast</td>
</tr>
<tr>
<td>Number of chews (10-40 chews)</td>
<td>Low</td>
</tr>
<tr>
<td>Oral residence time (3e30s)</td>
<td>Short</td>
</tr>
<tr>
<td>Texture</td>
<td>Liquid</td>
</tr>
<tr>
<td>Texture complexity</td>
<td>Low</td>
</tr>
</tbody>
</table>

*In brackets: the lowest and highest values of the different oral processing parameters that were used in the different studies. For instance in the study by Cassady et al., 2009, the lowest number of chews was 10, whereas the lowest number of chews by Li et al., 2011 was 15 number of chews (for both the highest number of chews was 40 per mouthful).

In all studies, the researchers intended to vary only one characteristic of oral processing. However, manipulating one characteristic inevitably had an effect on other characteristics (i.e. a higher eating rate might directly shorten the oral residence time). In 16 studies a test food was given with manipulated texture, such as liquid versus semi-solid food, and in two studies a texture complexity component was added. In six studies the number of chews per bite was manipulated, in three studies the oral residence time was directly influenced, and in five studies participants were instructed to
eat at a specific chewing rate. Another three studies were included where the bite size was changed. Interestingly, the minimum and maximum oral processing characteristic were compared to one another (Table 1.1) with the maximum values set as the commonly recommended values for reducing food intake and controlling appetite, such as small bites, high number of chews and long oral residence time (Smit et al., 2011).

1.5.1 Appetite regulation
Appetite is responsible for regulating food intake via the release of hormones such as ghrelin, cholecystokinin and glucagon-like-peptide1 (GLP1) pre- and post-prandially, controlling subsequent energy intake. These hormone peptides stimulate appetite centres in the brain resulting in hunger and satiety sensations (Karl, Young & Montain 2011). Perceived hunger relates to satiety hormones and perceived fullness relates to gastric emptying and stomach expansion. Some studies have proposed the mechanisms by which increased eating rate influences satiety, suggesting that increased chewing and a slower eating speed influences appetite hormones such as CCK, PYY (Peptide tyrosine tyrosine and cholecystokinin) and GLP-1. Karl et al (2011) investigated the combined effect of energy density and eating rate on measures of satiety and gut hormones (PYY and CCK concentrations). Although there was a change in gut hormones there was no significant effect on perceived satiety and there was no compensation during a subsequent ad libitum meal. However, this study has many limitations such as the test meal used (corned beef hash) which is not a commonly consumed meal. Furthermore, participants were asked to consume 360ml of water during the postprandial period to ensure adequate blood samples could be obtained, which may have affected ratings of satiety (Rolls and Roe et al., 2002). Therefore, although significant effects of eating rate on satiety were not found, results can be questioned due to methodology. Kokkinos et al (2010) demonstrated a significant increase in PYY and GLP-1 with a slow eating rate compared to a fast eating rate (consuming 300ml of ice cream in 5 vs 30 minutes). Furthermore, they showed a trend towards higher fullness rating after the slow meal compared to the fast. Cassady et al (2009), showed that healthy participants had lower post-prandial GLP-1 concentrations with a faster chewing rate (25 chews vs 40 chews of 55g of almonds, p<0.05) suggesting an association between faster ER and higher feelings of hunger.
1.5.2 Appetite and the gut-brain axis

The brain and the gastrointestinal tract (GI) interact in a complex, bidirectional communicational system known as the gut-brain axis (Weltens et al., 2018). The gut-brain axis is constituted of the core neurobiological substrate that regulates digestive processes in health and integrates these with the overall emotional and physical state of the body (Mayer 2011, Craig 2002). In particular, the sympathetic and parasympathetic efferent branches of the autonomic nervous (ANS) connect directly the central autonomic brain circuits and emotional-arousal with the enteric nervous system (ENS), which in turn innervates end-organ structures that affect the GI secretory, immune, motor, endocrine and sensory functions (Van Oudenhove et al., 2013 and 2016). Contrarily, vagal and spinal afferent nerves transmit signals from the GI tract to the brain stem and sensorimotor brain circuits, where they both modulate and are modulated by affective and cognitive networks (Mayer et al., 2011, Van Oudenhove 2013, Craig 2003). Furthermore, gut-brain signalling mechanisms also include several peptide hormones secreted by enteroendocrine cells along the gut. These peptides regulate food intake and energy balance according to the body’s nutritional needs and energy resources, by signalling the short-term metabolic state of the organism via humoral and neural routes to homeostatic brain circuits located in the brain stem and hypothalamus (Figure 1.8) (Steinert et al., 2017). Furthermore, apart from the homeostatic mechanisms, food intake and

Figure 1.8: Gut-brain axis and food intake regulation schematic.
appetite are also driven by hedonic processes regulated by the reward system (Alonso-Alonso et al., 2015). More specifically, the majority of orexigenic and anorexigenic hormones are released in response to the presence or absence of nutrients within the GI tract, depending on the energy content and type of macronutrient ingested (Weltens et al., 2018). Ghrelin, the only known orexigenic/appetite stimulating gut hormone is modified post-prandially by ghrelin O-acyl-transferase (GOAT) into its biologically active format which allows it to pass through the blood-brain barrier (Steinert et al., 2017, Muller et al., 2015, Karra et al., 2010). Ghrelin plasma concentrations fluctuate dependant on the energy and macronutrient content of a meal; increasing before a meal and decreasing rapidly after food intake (Muller et al., 2015).

The anorexigenic gut hormones (GLP-1, PYY and CCK) and adipocyte-derived leptin signal to the brain to decrease hunger and promote meal termination, which is why they are also considered satiety-stimulating hormones. The main site of action for gut hormones in the brain is the arcuate nucleus (ARC) in the ventral hypothalamus, within which a subset of neurons expresses agouti-related peptide (AgRP) and neuropeptide Y (NPY). AgRP/NPY neurons are rapidly inhibited by food ingestion and activated by food deprivation and regulate feeding by incorporating excitatory and inhibitory inputs (Heisler and Lam, 2017). Leptin is secreted by white adipose tissue, and its function is considered to be at least in part mediated by the excitation of POMC neurons and suppression of AgRP/NPY neurons (Sohn, 2015).

Apart from their effect on the homeostatic-metabolic control of food intake in the hypothalamus, gut hormones also affect hedonic brain regions. For example, ghrelin not only plays a role in homeostatic feeding regulation by activating hypothalamic AgRP/NPY orexigenic pathway, but also influences striatal food-cue responses.
1.5.3 Psychological effects on appetite and satiety
The effect of psychological stimuli such as stress and anxiety on food intake has been investigated. Schulz & Laessle (2012) found stress to induce an increase rate of eating amongst obese individuals with binge eating disorder. Participants also showed reduced signs of control towards the end of the meal, with poor deceleration exhibited. However, results were only found to be significant amongst obese participants with binge eating disorder and therefore the results of stress-induction on eating rate in normal weight individuals as well as those without disordered eating are unknown. Furthermore, the study took place in a laboratory setting and consequently the ecological validity of the findings can be questioned.

Using a stress-eating survey, additional research has found females to experience an increase in appetite when stressed (Kandiah et al., 2006). Furthermore, stressed individuals chose to eat a larger amount of sweet foods and the continuity of a healthy diet declined from 80% of subjects to 33% during stress conditions. However, stress was also found to reduce appetite in 37% of subjects, illustrating that the effects of stress on appetite are varied. Different results may be found if carried out on elderly patients where taste perception is reduced.

1.5.4 Mastication
One of the first steps in ingesting food is to chew it in order to reduce particle size before swallowing. This act of mastication elicits an array of effects that impact digestive and absorptive processes, including physical signalling and chemical signalling that play important roles in appetite and food intake regulation. Among these effects is the increased bio-accessibility of nutrients for absorption and subsequent utilisation impacting post-absorptive energy control mechanisms. Studies reporting increased blood glucose (Laboure et al., 2002, Mattes and Considine 2013) and insulin (Zhu et al., 2012, Zhu et al., 2014) with increased chewing are likely explained by the greater accessibility of nutrients (e.g., carbohydrates) for absorption. The increased bioaccessibility imparted by chewing is also important for stimulation of pre-absorptive mechanisms, such as those involved in neuro-hormonal regulation of food intake, including CCK, GLP-1, PPY and ghrelin (Miquel-Kergoat 2015). Specifically, Miquel-Kergoat in their systematic review and meta-analysis (17 studies, only two were in a controlled laboratory environment) on the effects of chewing on appetite, food intake and gut hormones, highlighted that : 1) enhancing oral processing by prolonged chewing influences appetite and food intake, 2) chewing significantly reduced self-reported hunger, 3) increasing the number of chews/bite increased gut hormone release and that 4) mastication promotes satiety by influencing appetite, food intake and hormone release.
Chewing has been found to be a determinant of eating rate. Ekuni et al (2012) reported a significant relationship between eating rate and the number of chews per mouthful of food. Both male and female participants reporting a fast eating rate conveyed a significantly reduced amount of chews and shorter duration of chewing compared to other subjects. The study took place in Okayama, Japan and the test food used was part of local cuisine, strengthening the study; however results cannot be generalised to other countries. Subjects were also asked to rate their preference of the food provided as this may affect eating rate. Eating rate was not directly measured and instead a questionnaire containing four qualitative categories for eating rate was provided. The reported speed of eating was therefore subjective.

1.5.5 Methods of measuring appetite and satiety in ER studies

Human behaviour can be measured by questioning feelings of motivation, sensations and attitudes (Stubbs et al., 2000). Appetite and satiety are measured using individual responses to questions pre- and post-prandially. Appetite is assessed by asking questions surrounding feelings of prospective intake and satiety can be measured by questioning feelings of fullness and hunger.

1.5.5.1 Visual analogue scales

The food visual analogue scale (VAS) are linear questionnaires with two extreme cases of varied questions adapted from the Hill and Blundell (1982) model (Stubbs et al., 2000). In the current studies, questions were programmed into an electronic patient reported outcome diary (PRO-Diary®, CamNtech Ltd, Cambridge, UK) which was then used to complete the questionnaires. The PRO-Diary is a wrist-worn electronic device for participant self-report of feeding behaviour. It has an integrated monitor activity with a touch sensitive screen to allow a participant to slide the on-screen icon either left, which signifies low level of hunger or to the right which signifies high level of hunger on a scale of 0 to 10. It also recorded the exact time at which the questionnaire was completed. The questions were presented in a randomised order, which meant each time point has a different question order. The data was then exported to a computer for analysis. Using the PRO-Diary is a more convenient and accurate technique compared to pen and paper VAS method, as it reduces errors from manual measurement and entry onto electronic spreadsheets. Questions used in these studies included “How hungry do you feel?” to assess hunger, “How full do you feel?” to assess fullness or satiety after a standard meal and “How much do you think you can eat?” to assess prospective food consumption (Stubbs et al., 2000; Kral, 2006). Additional questions were included such as: "How thirsty do you feel?" and “Would you like to eat something 'sweet', 'salty', 'fatty' and, 'savoury’?” (Appendix 2).
1.5.5.2 Electronic devices
Electronic devices used to measure aspects of appetite, satiety and prospective intake such as Pro-diaries and Pocket PC’s have been evaluated against paper VAS for their accurateness and reproducibility. It has been recommended in various research that electronic appetite rating systems (EARS) and paper methods are not used interchangeably as participants are found to be more precise when using EARS compared to paper VAS (Stubbs et al., 2000 & 2001, Almiron-Roig et al., 2009).

EARS are less error-prone and more efficient than paper methods with improved accuracy (Almiron-Roig et al., 2009). During repeated measure designs, participants used either a paper VAS or an EARS initially to rate appetite sensations and then used the alternative device. Research concluded that there were no significant differences between the two methods (Almiron-Roig et al., 2009). It has however been identified that subjects have a tendency to avoid the two extremes on the EARS than when using a paper VAS, occurring in both laboratory and free-living conditions (Stubbs et al., 2001). Although EARS have been shown to be accurate and comparable to paper VAS in both laboratory and free-living conditions, their limitations must be considered such as their use with the elderly population to ensure validity within the research.

The use of PRO-diaries to measure appetite via VAS has been validated, identifying no differences in the scoring of appetite VAS between PRO-diaries and paper VAS methods except the question ‘how full do you feel?’ after 60 minutes (Hampton and Middleton, 2011).

1.5.5.3. Three factor eating questionnaire
The three factor eating questionnaire (TFEQ) (Appendix 3) is a common method for measuring three aspects of eating behaviour that influence food intake; dietary restraint, disinhibition and hunger (Stunkard and Messick 1985). Dietary restraint refers to an individual limiting their food intake seen in disordered eating such as anorexia nervosa. Disinhibition refers to a loss of control over intake and an inability to detach intake from emotional cues. Lastly, hunger refers to the awareness of hunger sensations and the ability to govern these feelings in relation to food intake (Langlois et al., 2011). The TFEQ consists of 51 questions covering areas of the three topics. A revised 18 question version has since been developed which measures cognitive restraint, uncontrolled eating and emotional eating, which is easier to use when studying population groups where individuals are required to complete a number of questionnaires (de Lauzon et al., 2004).
1.6. Factors effecting eating rate

1.6.1. Eating rate and intrinsic factors

1.6.1.1. Slow eating rate and gender

Studies of eating behaviour have concluded that males have a faster rate of eating than females, though these were based on self-reported ER and specific to Asian populations (Laessle, Lehrke & Dückers 2007, Martin et al., 2007, Otsuka et al., 2008). Laessle et al (2007) found a significant decrease in food intake when eating rate was reduced amongst both genders. However, participants were instructed to eat a piece of chicken every time the computer provided an auditory prompt (beep sound) therefore this study shows how specific ER instructions effect gender. Furthermore, additional studies have found this result was restricted to males (Martin et al., 2007). A decline in mean food intake was reported amongst female participants although this was not significant (Martin et al., 2007). Participants in this study were instructed to complete a visual analogue scale (VAS) during every minute of the test meal which would have affected eating rate and overall food intake as cognitive tasks have been shown to effect food intake (Hetherington et al., 2006), flawing results.

Gender differences in eating rate of overweight participants (BMI >25mg/m²) have also been reported, with overweight males eating at a faster rate than overweight females (Laessle, Lehrke & Dückers 2007). The study by Laessle et al., (2007) was a ‘covert’ design, where subjects were informed they were taking part in a taste test and were not aware their eating rate was being monitored, avoiding changes in eating habits. However, it only included one food (chocolate pudding) which doesn’t represent everyday eating habits.

1.6.1.2. Age

Research shows that the number of family meals consumed for adolescents per week has reduced from 4.0 (in 1990) to 3.6 (in 2010) with a greater reduction being seen in low socioeconomic backgrounds (Neumark-Sztainer et al., 2013). The social change seen may cause a difference in eating rate across ages, as eating with reduced company such as family members has been shown to increase eating rate (Marshal and Bell 2003).

Furthermore, increasing age may affect eating rate due to elderly individuals becoming edentulous (lacking teeth), effecting chewing ability. A lack of functioning teeth has been associated with a poor chewing ability in a study looking at hospitalised elderly individuals (mean age 83.3 +/- 8.1) (Vehkalahti 2005). However Lee et al., (2012) found that male subjects who were categorised into a
fast eating rate group were older than those in the slower eating category (49.6 yr vs 47.6yrs, statistically significant; p>0.001). The study is weakened as eating rate was self-reported and the difference between the two groups, although statistically significant was only 2 years, therefore this questions if the results are practically significant. Consequently, to the best of the author’s knowledge, there is insufficient evidence to support any effect of gender and/or age on ER.

1.6.1.3. Ethnicity

When investigating differences in eating behaviour amongst ethnicities, it has been recognised that Caucasian individuals report more disordered eating than non-Caucasians (Wildes et al., 2001). The reasons for cultural differences in eating behaviours are unknown and a meta-analysis carried out to study these differences by Wildes consisted of >90% females and therefore it cannot be concluded whether ethnic differences in eating behaviors are also present in males. Differences can be seen in cultural eating behavior; however, its relation to eating rate is an area in which more research is required. Section 1.3.2 reports on several ER cross-sectional studies which have been carried out in predominantly Asian populations and rely on self-reported ER.

1.6.1.4. Disordered eating and eating rate

The relationship between eating rate and eating disorders has been investigated, with research conveying that individuals with disordered eating often eat at a linear rate (Ioakimidis et al., 2009, Zandian et al., 2009). It has therefore been questioned whether manipulating the rate at which food is consumed could play a role in the treatment of eating disorders (Ioakimidis et al., 2009). An alternative is a decelerated style of eating whereby the rate of consumption declines as a meal progresses. This eating style is often a characteristic of healthy weight individuals showing no restrained eating behaviours, whereas a linear eating style has been found in restrained women across a range of body weights (Westerterp-Plantenga 2000). Women with signs of binge eating disorder also convey signs of accelerated cumulative food intake curves, whereby eating rate increases during the course of an eating episode (Westerterp-Plantenga 2000)

When eating rate is controlled using a computer informing subjects of the rate to eat, linear eaters are found to under eat when eating rate is reduced and over eat when eating rate is increased (Ioakimidis et al., 2009, Zandian et al., 2009). Linear eaters are therefore more susceptible to changes in eating behaviours, exhibiting less control of their food intake and may benefit from interventions which focus on altering the rate at which food is consumed. This has led to the development of new devices such as the Mandometer, consisting of a computer screen and built in
scales which help individuals develop a desired pattern of eating (Ioakimidis et al., 2009). Restrained eating refers to an individual consciously limiting food intake to monitor body weight (Burger, Stice 2011). It influences eating rate, with restrained eaters displaying a linear cumulative food intake, eating at a constant rate throughout a meal Westertep-Plantenga (2000).

1.6.2 Eating rate and extrinsic factors

1.6.2.1 Location of eating

The amount an individual eats may alter depending on the location in which the food is consumed. Burke et al., (2007) highlighted individual’s total energy intake from fat was higher when food was consumed outside the home (for example at another individuals home or at a restaurant), despite the quantity eaten being less. It was also identified that those who ate the majority of their meals outside their home had a significant reduction in the overall micronutrient content of their meals, (Burke et al., 2007). Although the findings provide insight into the effects of location on food consumption, only children aged 7-12 years were included and thus results cannot be generalised. Furthermore, weighed food diaries were used and therefore compliance is questioned due to problems with the burden of weighing food.

Eating rate also changes with location. In an observational study Bell & Pliner (2003) examined the effects of location on meal duration during a lunch time period and found that meal length was shortest in fast food restaurants compared to a moderately priced restaurant and a work cafeteria. Meal durations were longest in the moderately priced restaurant. Researchers began timing once food had been served and individuals were observed during the consumption of their meals. Service time may have impacted the overall meal duration in the moderately priced restaurant as observers began timing once individuals began eating the initial course of bread. Moreover, this study was only carried out during a lunch time period and therefore individuals being observed may have had a limited amount of time available due to work constraints, impacting eating rate.
1.7. Food properties and eating rate

1.7.1 Eating Rate of Foods

The type and texture of a food has been shown to influence eating rate (Viskaal-van Dongen, Kok & de Graaf 2011), there is however limited information available on the eating rate of commonly consumed foods. Most studies that report the ER of foods involve model foods (edible foods manipulated for the purposes of a study, e.g. strawberry flavoured model food gels, ) or manipulated foods (pre-portioned foods to control for bite size like rice balls or tortellini pasta) (Zijlstra et al., 2008 and 2010, Lasschuijt et al., 2017).

One explanation is that different textures elicit different durations of orosensory exposure in the mouth (Hogenkamp et al., 2010). Unsurprisingly liquids are eaten at a faster rate than solids foods (Viskaal-van Dongen, Kok & de Graaf 2011, Hogenkamp et al., 2010, Zijlstra et al., 2007) likely due to a thin viscosity and the lack of mastication required. However, hot liquids such as soup have been found to elicit similar eating rates to solid foods, possibly because of its high temperature and the use of a spoon during consumption (Viskaal-van Dongen, Kok & de Graaf 2011).

Eating rate has also been found to vary between foods of the same consistency. For example, the eating rate of foods comprising a semi-solid texture was found to range from 50g/min (mashed potatoes) to 229g/min (vanilla custard) despite having a similar texture, illustrating that a particular consistency does not have a specific eating rate (Viskaal-van Dongen, Kok & de Graaf 2011). Forde et al (2013) discovered a significantly slower eating rate during a meal where the contents were whole compared to a mashed consistency. Food with a solid texture also elicited a reduced overall food intake, with less food being consumed when the meal was in a solid form (Forde et al., 2013).

As an overview; Viskaal-van Dongen et al (2011) measured the eating rate of 45 commonly consumed foods in the Netherlands, Forde et al ( 2013) measured the ER of 35 solid, savoury meal components, Ferriday et al (2016) measured the ER of 20 different commercially available pre-packed meals and finally Forde et al (2017) measured the food-specific ER of 47 commonly consumed Singaporean foods. As the above datasets did not represent a whole diet, van den Boer et al (2017) reported a more robust study where the ER of 192 foods commonly consumed in the Netherlands was recorded. Every food was eaten by at least four participants while the time spent eating and amount eaten were recorded. Furthermore, three reference foods—eaten twice by all participants—were included to correct the eating rate data for the personal eating rate of the participants. The observed eating rate (g/min) was determined by dividing the amount eaten (g) by the time spent eating (min). This number was then calibrated to correct for the personal eating
rate of the participant; the observed eating rate was divided by a calibration factor based on how fast the participant ate the reference foods relative to the rest of the participants. Finally, energy intake rate (kJ/min) was obtained by multiplying eating rate (g/min) with the energy density (kJ/g) of the corresponding product. Each volunteer’s ER was established by measuring the first 2 minutes of chewing the test foods, which might have been misleading as the eating curve (linear/decelerated) might have changed if they allowed longer time per test meal. The results showed a wide variation in both eating rate (from 2 g/min for rice waffle to 641 g/min for apple juice) and energy intake rate (from 0 kJ/min (0 kcal/min) for water to 1766 kJ/min (422 kcal/min) for chocolate milk). Eating rate was lower when foods were more solid. Moreover, eating rate was positively associated with water content and inversely with energy density. Energy intake rate differed substantially between and within food groups, demonstrating that the food availability provides opportunities for selecting/promoting food options with a lower energy intake rate.

1.7.2. Taste

Direct comparison studies have suggested sweet and savoury foods are consumed at similar rates (38 +/- 14g/min for the sweet meal; 37 +/- 14g/min for the savoury meal) (Griffioen-Roose et al., 2009), but it can be questioned whether the palatability of a food influences the rate at which it is consumed. Bobroff & Kissileff (1986) found the initial eating rate of a preferred food to be significantly higher than a disliked food. Food specific eating rates have also shown to be positively correlated to relative palatability (Westerterp-Plantenga, Wouters & ten Hoor 1991).

This is supported by Spiegel (2000), who discovered palatability to significantly affect ingestion rate, increasing the amount of food eaten in the first 5 minutes, when comparing a high and low palatability condition. Furthermore, when high and low palatability conditions were compared between lean and obese individuals, the difference in initial consumption rate between the two palatability conditions was greater in obese participants than lean (Spiegel 2000).

The addition of salt and its effect on palatability and thus eating rate has also been studied, however no significant differences were found between the eating rate of a meal with an increased salt content and that of a control meal (Forde et al., 2013). This is supported by the work of Bolhuis et al., (2010) who concluded that eating rate did not differ between the consumption of a high or low salt tomato soup consumed ad libitum. Both studies excluded participants who displayed characteristics of restrained eating to avoid the influence of this characteristic on eating rate, strengthening their findings.
1.7.3. Energy density

Eating rate has been shown to be inversely related with energy density during an investigation of 45 commonly consumed foods, with eating rate reducing by 5.1% when energy density is increased by 100kJ/100g (Viskaal-van Dongen, Kok & de Graaf 2011). A recent experiment by McCrickerd, et al (2017) investigated the combined and separate effect of manipulating the eating rate and energy density of foods on energy intake using a $2 \times 2$ design. Their results show that the combined manipulation (i.e., rice porridge with a low eating rate and low energy density) is more effective at reducing energy intake than the individual manipulations alone (i.e., rice porridge with either a low eating rate, or a low energy density). Since both energy density and ER were manipulated in the latter study, the effect of manipulating energy densities of food on ER is unclear.
2. Justification for this programme of work.

As described above, ER has the potential to facilitate weight loss through its effects on energy intake and appetite as well as its possible use as a tool to promote mindfulness. Only a few studies to date have investigated the effect of specific eating rate protocols in a free-living adult populations with most eating rate studies being either self-reported (Ekuni et al., 2015), solely clinical (Kokkinos et al., 2010) or focusing on children/adolescents [Ford et al., 2010, Hamilton-Shield et al., 2014]. Furthermore, training people to eat more slowly in everyday contexts requires creative and engaging solutions (Hermsen et al., 2016) and to the author’s knowledge there has not been a study to date, investigating the effects of applying a SER protocol in overweight free-living adults.

In 2012, as part of a BSc final year pilot project, the effect of eating rate on satiety in healthy and overweight people was studied (Koidis et al., 2014). In this pilot study, the overweight group ate significantly faster than the healthy-weight group despite being given the same instructions. There was also a trend towards increased self-reported satiety when eating slow. Furthermore, some interesting correlations between fast eating and higher body composition values (BMI, Visceral fat score and %Trunk fat) were identified.

The resultant PhD project, building on this work, was divided into two parts. Firstly, a technical development project was planned to develop and refine the methods for applying, enhancing and assessing the slow eating rate protocol in healthy participants. This developmental work took place over 5 studies and involved expert input from the department of psychology and a collaboration with the department of robotics.

- Technical development study A (Chapter 3): aimed to further develop the SER protocol (Koidis et al., 2014) through volunteer feedback and to assess if the use of Qualisys Track Manager (QTM) was a reliable method for assessing eating rate in a clinical setting.
- Technical development study B: aimed to assess if the addition of earplugs to the SER protocol will have an effect (through the occlusion effect) on perceived satiety and if it will improve protocol concordance.
- Developmental study C (Chapter 4): aimed to transform the existing paper-based SER protocol into an online user-friendly 2-minute video. This study also aimed to
incorporate the SER protocol into an online weight loss tool (dedicated website and mobile application) with the potential for roll out in the free-living population.

- Developmental study D (Chapter 4): Aimed to develop a prototype for automatic chew counting as a means of reducing the time demand and error associated with manually counting eating rate (chews counted by observer).

- Developmental study E (chapter 4): Aimed to investigate if a mindful eating questionnaire was a good marker of ER in healthy adults and assess the factors that mediate the relationship between ER and mindfulness.

The second part of this PhD programme was a community intervention study, utilising the SER protocol developed and testing its potential as a weight-loss tool in overweight adults.
2.1 Aim and hypotheses
The PhD project focuses on four major areas: eating rate its monitoring/assessment techniques, hormonal and metabolic responses to the SER protocol, appetite control associated with SER and the association between eating rate and mindfulness.

The overarching aim of the programme of work was to develop and test the SER protocol in overweight-free living adults and investigate the protocol’s effects on body weight, hormones and metabolites as well as mindfulness.

The main objectives of this PhD project were to assess the effect of the SER protocol in overweight free-living adults on:

- Body weight; (weight, BMI, %fat, visceral fat) and energy intake
- habitual eating rate; assessed by chews/minutes and meal duration
- Hormones and metabolites (plasma Glucose, Insulin, Leptin, TAGs, NEFAs, Cholesterol and HDL-Cholesterol)
- Appetite parameters, such as subjective hunger, desire to eat and fullness rating, and plasma leptin.
- Mindfulness parameters (Sensory, Awareness, Body cues, Distraction) as assessed by a Mindful Eating Questionnaire.
- Monitoring mechanisms and compliance, via a dedicated website and mobile application (Mixpanel Core Analytics).

The author hypothesise that:

- The technological developments made will enhance the acceptability and utility of the slow eating rate protocol and its applicability to a free-living population
- The SER protocol will reduce the habitual eating rate of the intervention group
- The SER protocol will facilitate weight loss in the overweight intervention group.
- The SER protocol will result in improved hormonal and metabolic responses (specifically decreased glucose, insulin, NEFA, Leptin and blood lipids) and reduced subjective hunger desire to eat and plasma leptin and increased fullness rating.
• The SER protocol will result in increased mindful eating (with increased sensory, awareness and body cues and decreased distraction scores)
2. Introduction

This chapter focuses on the laboratory and non-laboratory techniques used to analyse data after the completion of the studies in chapters 3, 4 and 5, specifically radioimmunoassay and spectrophotometry to analyse metabolites and hormones and electronic pro-diaries to record and quantify perceived appetite and satiety. For the final 2 studies (See chapters 4 and 5) a proprietary software was used to analyse eating rate via video playback, which is discussed in detail in Chapter 4. This chapter will also discuss the general statistical tests applied to analyse the data.

2.1 Radioimmunoassay

Radioimmunoassay (RIA) is a very sensitive in vitro assay methodology used to measure antigen levels (such as hormones; insulin and leptin) using specific antibodies. It is based on the use of a radiolabelled antigen and specific antibody utilising the immunochemical antigen-antibody reaction. A sample containing an unknown amount of antigen is incubated with a fixed amount of antisera specific to the antigen and a fixed amount of radio-labelled antigen. This results in a competitive reaction between labelled (Ag+) and unlabelled antigen (Ag) for the limited number of antibody (Ab) binding sites. Once equilibrium is reached, the ratio of the free (F) and antibody bound (B) fractions of the antigen will be a constant (K) described by the law of mass action [AgAb = K (Ag) (Ab)]. After an incubation period the free and bound antigens are separated using either a specific second antibody which binds to the antibody–antigen complex or non-specific separation such as dextran-coated charcoal, which will remove the unbound antigens but not the larger antigen-antibody complex.

Prior to testing unknown samples, a series of standard solutions of known antigen concentration are plotted, and then the radioactivity of free or bound antigen is measured and compared against the standard curve. Consequently, the concentration of the antigen in the samples can be determined by extrapolation (Bartalena et al., 2012).

RIA can measure hormones in a variety of body fluids such as serum, plasma, urine and saliva. In the current studies, all tubes were run in duplicate to better detect intra-assay variation on the day.
standard was prepared fresh for each set of samples and were prepared in an antigen-free matrix to minimise the presence of any substances in samples that can interfere with the binding. Standards that are used in RIA should be stable, available in large quantities, and should be highly purified (Van Vunakis 1980). The zero point on the standard curve is set up in quadruplicate as this is the anchor point for the whole standard curve and must be as reliable as possible. It also allows calculation of limit of detection for each assay (see section 2.1.1).

All samples from each participant in each study were run in duplicate and on the same day using the same reagents. Therefore, all the samples from one participant (n = 15 in Chapter 5) were analysed in one assay to minimise between-assay variability.

2.1.1 Radioimmunoassay accuracy and sensitivity

Quality control (QC) tubes were treated in a similar manner as the biological samples. QC samples were run in duplicate and included in all assays. This is essential to ensure reproducibility and to reduce the so called ‘grey’ area between definite abnormal values and values which are normal. The QC samples contain known concentrations of the antigen, in the matrix being measured, and a range of QCs (low, medium and high) were used to cover the range of the standard curve. QC samples were run in duplicate at the start, middle and the end of the samples to be measured. This determined the inter-assay and intra-assay variation (Coefficient of variation (CV) which expresses the precision, and reproducibility of each assay. Comparison of the QC values placed at different positions in the assay assesses the presence of assay drift. However, comparison of QC values between assays (Interassay CV) monitors the precision of entire clinical study samples. Inter-assay CV of less than 15% are generally acceptable, and intra-assay CV should be less than 10% (Taylor and Harris, 2014).

Intra-assay Coefficient (CV) was calculated as follows:

\[
\text{Intra-assay Coefficient} = \left( \frac{\text{QCs SD}}{\text{QCs mean}} \right) \times 100
\]

Inter-assay Coefficient was calculated as an expression of consistency between different assays, as follows:

\[
\text{Inter-assay Coefficient} = \left( \frac{\text{Mean of QCs SD}}{\text{Mean of QCs mean}} \right) \times 100
\]
To monitor the accuracy of the RIA, total count tubes which contain only radiolabel were not decanted in the separation stage. Total count tubes give the amount of radioactivity added per tube allowing calculation of maximum binding (zero standard) of the assay. Non-specific binding tubes (NSB) containing buffer, tracer, and precipitating reagent, but not antibody, yield the lowest cpm (counts per minute) in the assay. The percentage of NSB must be as low as possible (< 10%) for a high quality assay, as it indicates if binding is not due to antibody. NSB is calculated as follows:

\[
\text{NSB} = \frac{\text{Total cpm - Zero binding cpm}}{\text{Total cpm}} \times 100
\]

The zero (0 ng/ml) standard contains tracer, buffer, antibody but not antigen indicate the maximum tracer binding in the presence of antibody. The maximum binding is calculated as follows:

\[
\text{Maximum binding} = \frac{\text{Total cpm - Zero binding cpm}}{\text{Total cpm}} \times 100
\]

The limit of detection (LOD) was also calculated to determine the sensitivity (i.e. the minimum concentration that could be accurately detected) in each assay.

The following steps were used to calculate the limit of detection (e.g. insulin RIA):

- Mean cpm of 0 ng/ml standards.
- Standard deviation (SD) of cpm of 0 ng/ml standards.
- Calculate 2 x SD of 0 ng/ml.
- Calculate cpm limit of detection = mean (0 ng/ml) + 2 x SD (0 ng/ml)
- Calculate m (counts per ng) = (mean of 0.5 ng/ml standard - mean 0 ng/ml cpm) / 0.5.
- LOD = (mean cpm 0 ng/ml cpm LOD) / m

### 2.2 Plasma Leptin

The plasma leptin concentrations were assayed using human leptin RIA kits supplied by Millipore Company (Billerica, MA, USA). In this assay 125I-labeled human leptin and human leptin antiserum were used to determine the level of leptin in plasma. The assay was separated using double antibody/PEG separation. The standards vials (100ng/ml) provided were ready to use, however further serial dilutions were performed to complete a range of standard concentrations consisting of seven tubes. A series of standard concentrations were obtained in the range 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 ng/ml. All standards were set up in duplicate (total n = 14) with a
total volume of 100μl added in each tube (Table 2.1). The NSB tubes contained 300μl of 0.05 phosphosaline buffer, whereas reference (B0) tubes contained 200μl, and 100μl for total tubes.

**Table 2.1:** Standard preparation for leptin RIA

<table>
<thead>
<tr>
<th>Tube#</th>
<th>Tube Standard concentration</th>
<th>Volume of assay buffer to add</th>
<th>Volume of standard to add</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50ng/ml</td>
<td>1.0ml</td>
<td>1.0ml of 100ng/ml</td>
</tr>
<tr>
<td>2</td>
<td>25 ng/ml</td>
<td>1.0ml</td>
<td>1.0ml of 50 ng/ml</td>
</tr>
<tr>
<td>3</td>
<td>12.5 ng/ml</td>
<td>1.0ml</td>
<td>1.0ml of 25 ng/ml</td>
</tr>
<tr>
<td>4</td>
<td>6.25 ng/ml</td>
<td>1.0ml</td>
<td>1.0ml of 12.5 ng/ml</td>
</tr>
<tr>
<td>5</td>
<td>3.125 ng/ml</td>
<td>1.0ml</td>
<td>1.0ml of 6.25 ng/ml</td>
</tr>
<tr>
<td>6</td>
<td>1.56 ng/ml</td>
<td>1.0ml</td>
<td>1.0ml of 3.125 ng/ml</td>
</tr>
<tr>
<td>7</td>
<td>0.78 ng/ml</td>
<td>1.0ml</td>
<td>1.56 ng/ml</td>
</tr>
</tbody>
</table>

Plasma samples and controls were run in duplicate with a total volume of 100μl in each tube. 100μl of 125I-Human leptin was added to all assay tubes, followed by pipetting 100μl of human leptin antibody to all tubes except total tubes. All tubes were incubated at 4°C overnight (20-24h).

On day two, 1.0 ml of cold (4°C) precipitating reagent was added to all tubes (except Total count tubes) and vortexed and incubated for 20 minutes at 4°C. Then, all assay tubes (except total) were centrifuged for 20 minutes at 2000-3000 rpm. Immediately after centrifugation, the supernatant of the assay tubes was aspirated and the assay tubes were counted in a gamma counter. The concentration (ng/ml) of human leptin in the unknown samples was determined using the dose response curve and automated software Multi-Calc (Perkin Elmer, Wizard Gamma Counter, UK).

A summary of the standard assay procedure is shown in Table 2.2. Each kit was sufficient to run 250 tubes and contained the following reagents:

- **Assay buffer:** 0.05 Phosphosaline pH 7.4 containing 0.025M EDTA, 0.08% Sodium Azide, 1% RIA Grade BSA and 0.05% Triton X-100. No preparation was required and the quantity provided in each vial was 40ml.
- **Human leptin antibody:** Rabbit anti-human leptin serum in assay buffer was ready to use. The quantity provided was 26mL/vial.
- **Labelling hydrating buffer:** assay buffer containing normal rabbit IgG as a carrier (ready to use). It is used to hydrate 125I-Human leptin. The quantity provided was 27 mL/vial.
- **125I-Human leptin:** 125I-Human leptin label (27 mL/vial upon hydration), HPLC purified (specific activity 135μCi/μg) lyophilized for stability. Lyophilized contents were hydrated with entire contents of label hydrating buffer. The working solution was then incubated at room temperature for 30 minutes, with intermittent gentle mixing.
- Human leptin standards: purified recombinant human leptin (ready to use, 2 ml/vial) in assay buffer at the following concentration: 100 ng/ml. further serial dilution were performed to prepare 7 standard tubes with the following concentrations: 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 ng/ml.
- Quality controls 1 & 2: purified recombinant human leptin in assay buffer (1ml/vial).
- Precipitating reagent: Goat anti-rabbit IgG serum, 3% PEG and 0.05% Triton X-100 in 0.05M phosphosaline, 0.025M EDTA, 0.08% sodium Azide. Precipitating reagent was chilled to 4°C, and quantity provided was 260 ml/vial.

Table 2.2: Assay procedure for Leptin RIA

<table>
<thead>
<tr>
<th>Tube #</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
<th>Step 6</th>
<th>Day two</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2</td>
<td>-</td>
<td>Add standards/QC/sample</td>
<td>Add antibody</td>
<td>Add 125I-tracer</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3, 4</td>
<td>500μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5-8</td>
<td>-</td>
<td>500μl of 6ng/ml</td>
<td>200μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9, 10</td>
<td>-</td>
<td>500μl of 0.5ng/ml</td>
<td>200μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>11, 12</td>
<td>-</td>
<td>500μl of 1.0ng/ml</td>
<td>200μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>13, 14</td>
<td>-</td>
<td>500μl of 2.0ng/ml</td>
<td>200μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>15, 16</td>
<td>-</td>
<td>500μl of 4.0ng/ml</td>
<td>200μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>17, 18</td>
<td>-</td>
<td>500μl of 7.0ng/ml</td>
<td>200μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>19, 20</td>
<td>-</td>
<td>500μl of 10ng/ml</td>
<td>200μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>21, 22</td>
<td>-</td>
<td>500μl of 20ng/ml</td>
<td>200μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>23, 24</td>
<td>-</td>
<td>500μl of 50ng/ml</td>
<td>200μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>25, 26</td>
<td>-</td>
<td>500μl of QC medium</td>
<td>200μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>27, 28</td>
<td>-</td>
<td>500μl of QC high</td>
<td>200μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>29, 30</td>
<td>-</td>
<td>500μl of sample</td>
<td>200μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>31-n</td>
<td>-</td>
<td>500μl of sample</td>
<td>200μl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

2.3 Plasma Insulin

The plasma insulin samples were assayed by utilising 125I-labeled human insulin and a human insulin antiserum supplied by Millipore Company (Billercia, MA, USA) following the double antibody/PEG technique. Insulin RIA procedures were similar to leptin RIA and variations are shown in table 2.3.
Table 2.3: Standard preparation for insulin RIA

<table>
<thead>
<tr>
<th>Tube#</th>
<th>Tube Standard concentration</th>
<th>Volume of assay buffer to add</th>
<th>Volume of standard to add</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100μU/ml</td>
<td>1.0ml</td>
<td>1.0ml of 200μU/ml</td>
</tr>
<tr>
<td>2</td>
<td>50μU/ml</td>
<td>1.0ml</td>
<td>1.0ml of 100μU/ml</td>
</tr>
<tr>
<td>3</td>
<td>25μU/ml</td>
<td>1.0ml</td>
<td>1.0ml of 50μU/ml</td>
</tr>
<tr>
<td>4</td>
<td>12.5μU/ml</td>
<td>1.0ml</td>
<td>1.0ml of 25μU/ml</td>
</tr>
<tr>
<td>5</td>
<td>6.25μU/ml</td>
<td>1.0ml</td>
<td>1.0ml of 12.5μU/ml</td>
</tr>
<tr>
<td>6</td>
<td>3.125μU/ml</td>
<td>1.0ml</td>
<td>1.0ml of 6.5μU/ml</td>
</tr>
</tbody>
</table>

Preparation of insulin reagents (Millipore Company (Billerica, MA, USA):

- Assay buffer: 0.05 phosphosaline pH 7.4 containing 0.025M EDTA, 0.08% Sodium Azide, and 1% RIA Grade BSA. No preparation was required and the quantity provided in each vial was 40ml.
- Human insulin antibody: Guinea Pig anti-human insulin specific serum in assay buffer was ready to use. The quantity provided was 26ml/vial.
- Label hydrating buffer: assay buffer containing normal Guinea pig serum as a carrier. It was used to hydrate $^{125}$I-insulin. This reagent was ready to use and the quantity provided was 27ml/vial.
- $^{125}$I-Insulin: $^{125}$I-insulin label, HPLC purified (specific activity $367\mu Ci/\mu g$) lyophilized for stability. Lyophilized contents were hydrated with entire contents of label hydrating buffer. The working solution was then incubated at room temperature for 30 minutes, with intermittently gentle mixing.
- Human insulin standards: purified recombinant human insulin (ready to use, 2 ml/vial) in assay buffer at the following concentration: 200μU/mL. Further serial dilutions were performed to prepare 7 standard tubes with the following concentrations: 200, 100, 50, 25, 12.5, 6.25, 3.125 μU/mL (Table 2.4).
- Quality controls 1 & 2: purified recombinant human insulin in assay buffer (1 ml/vial).
Precipitating reagent: Goat anti-Guinea pig IgG serum, 3% PEG and 0.05% Triton X-100 in 0.05M phosphosaline, 0.025M EDTA, 0.08% Sodium Azide. Precipitating reagent was chilled to 4 °C, and quantity provided was 260 mL/vial.

### Table 2.4: Assay procedure for Insulin RIA

<table>
<thead>
<tr>
<th>Tube #</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
<th>Step 6</th>
<th>Step 7</th>
<th>Step 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2</td>
<td>-</td>
<td>-</td>
<td>100μl</td>
<td>-</td>
<td>Add precipitating reagent</td>
<td>-</td>
<td>1.0ml</td>
<td></td>
</tr>
<tr>
<td>3,4</td>
<td>300μl</td>
<td>-</td>
<td>100μl</td>
<td>-</td>
<td></td>
<td>1.0ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5,6</td>
<td>200μl</td>
<td>-</td>
<td>100μl</td>
<td>-</td>
<td></td>
<td>1.0ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,8</td>
<td>100μl</td>
<td>100μl of 0.78μg/ml</td>
<td>100μl</td>
<td>100μl</td>
<td>Vortex, cover, and incubate 20-21 hrs at 4°C</td>
<td>1.0ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,10</td>
<td>100μl</td>
<td>100μl of 1.50μg/ml</td>
<td>100μl</td>
<td>100μl</td>
<td>Vortex, cover, and incubate 20 min at 4°C</td>
<td>1.0ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11,12</td>
<td>100μl</td>
<td>100μl of 3.12μg/ml</td>
<td>100μl</td>
<td>100μl</td>
<td>Vortex, cover, and incubate 20 min at 4°C</td>
<td>1.0ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13,14</td>
<td>100μl</td>
<td>100μl of 6.25μg/ml</td>
<td>100μl</td>
<td>100μl</td>
<td>Vortex, cover, and incubate 20 min at 4°C</td>
<td>1.0ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15,16</td>
<td>100μl</td>
<td>100μl of 12.5μg/ml</td>
<td>100μl</td>
<td>100μl</td>
<td>Vortex, cover, and incubate 20 min at 4°C</td>
<td>1.0ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17,18</td>
<td>100μl</td>
<td>100μl of 25μg/ml</td>
<td>100μl</td>
<td>100μl</td>
<td>Vortex, cover, and incubate 20 min at 4°C</td>
<td>1.0ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19,20</td>
<td>100μl</td>
<td>100μl of 50μg/ml</td>
<td>100μl</td>
<td>100μl</td>
<td>Vortex, cover, and incubate 20 min at 4°C</td>
<td>1.0ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21,22</td>
<td>100μl</td>
<td>100μl of 100μg/ml</td>
<td>100μl</td>
<td>100μl</td>
<td>Vortex, cover, and incubate 20 min at 4°C</td>
<td>1.0ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23,24</td>
<td>100μl</td>
<td>100μl of QC1</td>
<td>100μl</td>
<td>100μl</td>
<td>Vortex, cover, and incubate 20 min at 4°C</td>
<td>1.0ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25,26</td>
<td>100μl</td>
<td>100μl of QC2</td>
<td>100μl</td>
<td>100μl</td>
<td>Vortex, cover, and incubate 20 min at 4°C</td>
<td>1.0ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27-n</td>
<td>100μl</td>
<td>100μl of sample</td>
<td>100μl</td>
<td>100μl</td>
<td>Vortex, cover, and incubate 20 min at 4°C</td>
<td>1.0ml</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 2.4 Measurement of Plasma metabolites

Plasma metabolites were analysed using a centrifugal analyser. The assay principle is as follows: metabolite concentrations are determined after an enzymatic reaction which releases hydrogen peroxide to form a dye with the pigment reagents. The colour intensity of the final product is measured at a specific wavelength and is directly proportional to the analyte concentration in the sample. Glucose, TAGs, NEFAs Cholesterol and HDL-Cholesterol were measured by colourimetric assay using an ILab650 (Instrumentation Laboratory, Warrington, UK).

All plasma samples were initially thawed and centrifuged at 1500 g for 5 min at 4 °C. To optimise the clarity of the samples the supernatants were then re-aliquoted into new plain tubes and placed in the I-Lab 650. Calibration of the I-Lab650 was carried out every day before the measurements were taken, and the quality control with a known concentration was measured and checked before each run of samples. QCs (low and high) were run in duplicate at the beginning and at the end of each batch of samples to assess intra- and inter assay variability. All samples from one participant were randomised and run in one assay to minimise the effect of assay variation.
2.4.1 Plasma glucose
Plasma glucose was determined using the GLUOX analysis kit (IL TestTM, catalogue number 0018250840, Instrumentation Laboratory, Werfen Ltd, Warrington, UK). Glucose was oxidised by glucose oxidase forming hydrogen peroxidase, which under catalysis of peroxidase with phenol and 4-amiophenazonne forms a quinoneime dye. The absorbance of quinoneime was measured biochromatically at a wavelength of 510nm and also at blanking wavelength of 600nm (see below).

\[
\begin{align*}
(\beta-D-glucose) + (O_2 + H_2O) &\xrightarrow{\text{glucose oxidase}} \text{gluconic} + H_2O \\
2H_2O + 4\text{-amino antipyrine} + \text{phenol} &\xrightarrow{\text{peroxidase}} \text{quinoneimine} + 4H_2O
\end{align*}
\]

2.4.2 Plasma NEFAs
Non-esterified fatty acids (NEFAs) were measured using Randox NEFA analysis kit (Randox Laboratories Ltd., Crumlin, County Antrim, UK). NEFA was converted to acetyl-CoA by acyl-CoA synthase enzyme to produce Acyl CoA. Acyl CoA is oxidised then to produce hydrogen peroxidase which was then condensed by peroxidase to form a quinoneime dye which is directly proportional to the NEFA concentration. The absorbance of the quinoneime was measured at 550nm (see below).

\[
\begin{align*}
NEFA + ATP + CoA &\xrightarrow{\text{acyl CoA synthase}} \text{acyl CoA} + AMP + PPi \\
\text{Acyl CoA} + O_2 &\xrightarrow{\text{acyl CoA oxidase}} 2,3 \text{ trans enoyl CoA} + H_2O \\
H_2O + \text{TOOS} + 4\text{-amino antipyrine} &\xrightarrow{\text{peroxidase}} \text{quinoneimine dye} + H_2O \\
\text{TOOS} &= \text{N-ethyl-N-(2hydroxy-3-sulphopropyl) m-toluidine}
\end{align*}
\]

2.4.5 Plasma TAGs
Triglyceride (TAG) was measured using IL TestTM TAG analysis kit (Catalogoue number 0018255-640, Instrumentation Laboratory, Werfen Ltd, Warrington, UK). TAG in EDTA plasma was converted to quineoneime by a series of chemical reactions. TAG was lipolysed into glycerol and fatty acids by lipoprotein lipase. Glycerol was converted by glycerol kinase to form glycerol-3-phosphate, which was oxidised to dihydroxyacetone phosphate. The last step was the production of
quineoneime dye by peroxidase. The absorbance of quineoneimine dye (measured biochromatically at 505/692nm was directly proportional to the TAG concentrations (see below).

\[
\text{Triglycerides} \xrightarrow{\text{lipoprotein lipase}} \text{glycerol + fatty acids} \\
\text{glycerol} + \text{ATP} \xrightarrow{\text{glycerol kinase}} \text{glycerol-3-phosphate + ADP} \\
\text{glycerol-3-phosphate} + \text{O}_2 \xrightarrow{\text{glycerophosphate oxidase}} \text{dihydroxyacetone phosphate} + \text{H}_2\text{O} \\
\text{H}_2\text{O} + 4\text{-chlorophenol} + 4\text{-amino antipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine dye} + \text{H}_2\text{O}
\]

2.4.6 Plasma Cholesterol

Cholesterol was measured using IL TestTM Cholesterol analysis kit (kit code:981812 and 981812 instrumentation Laboratory, Werfen Ltd, Warrington, UK). Cholesterol esters are enzymatically hydrolysed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide combines with HBA and 4-aminoantipyrine to form a chromophore (quinoneimine dye) which may be quantitated at 500-550 nm (Allain et al., 1974) see below:

\[
\text{Cholesterol esters} \xrightarrow{\text{CE}} \text{Cholesterol} + \text{Fatty Acids} \\
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{CE}} \text{Cholest-4-en-3-one} + \text{H}_2\text{O}_2 \\
2 \text{H}_2\text{O}_2 + \text{HBA} + 4\text{AAP} \xrightarrow{\text{POD}} \text{Quinoneimine Dye} + 4 \text{H}_2\text{O}
\]

Footnote: CE: cholesterol fatty acid ester, POD: Peroxidase enzyme solution.

2.4.7 Plasma HDL-Cholesterol

High density lipoprotein cholesterol (HDL-Cholesterol) was measured using IL TestTM HDL-Cholesterol analysis kit (kit code:981823 and 981824, instrumentation Laboratory, Werfen Ltd, Warrington, UK). This test is homogeneous enzymatic colorimetric test, where in the presence of magnesium sulfate, dextran sulfate selectively forms water- soluble complexes with LDL, VLDL and chylomicrons, which are resistant to PEG modified enzymes. The cholesterol concentration of
HDL-cholesterol is determined enzymatically by cholesterol oxidase coupled with PEG to the amino groups (approx. 40%).

\[ \text{HDL-Cholesterol esters} + \text{H}_2\text{O} \xrightarrow{\text{PEG cholesterol esterase}} \text{HDL-cholesterol} + \text{RCOOH} \]

\[ \text{HDL-cholesterol} + \text{O}_2 \xrightarrow{\text{PEG cholesterol oxidase}} \Delta^4\text{-cholestenone} + \text{H}_2\text{O}_2 \]

\[ 2\text{H}_2\text{O}_2 + 4\text{-amino-antipyrine} + \text{HSDA} + \text{H} + \text{H}_2\text{O} \xrightarrow{\text{peroxidase}} \text{purple blue pigment} + 5 \text{H}_2\text{O} \]

### 2.5 Appetite assessment via food Visual Analogue Scale
In studies A (Chapter 3) and final (Chapter 5), participants were asked to complete the questionnaires right before the test meal (T=0), and then every 15 minutes after the test meal for the first hour, and then at 90, 120 and 180 minutes. A total of 8 readings were obtained. In the final study (Chapter 5) participants completed the questionnaire only once, right before the test meal (T=0) in order to establish baseline appetite levels. All appetite data were then exported onto a CSV file, prior to statistical analyses such as paired t-tests, one way and repeated measures ANOVA.

### 2.6 Screening Questionnaire

#### 2.6.1 Dutch Eating Behaviour Questionnaire (DEBQ)
The Dutch Eating Behaviour Questionnaire (DEBQ) was developed by Van Strien et al (1986) for the assessment of restrained, emotional and external eating behaviour. It was used in all three studies (Chapters: 3, 4 & 5) and measured eating habits by three subscales (Hamilton, 2007), including 33 items [Appendix 4]. The first subscale assesses disinhibition; the inability to stop eating even when full (Hamilton, 2007), evident in questions such as “When you have eaten too much, do you eat less than usual the following days?” The second factor evaluates eating in response to negative emotional states, implied in questions such as “Do you have a desire to eat when you are bored or restless?” Lastly, the DEBQ tests eating in response to environmental cues denoted by questions such as “If you see others eating, do you have also the desire to eat?” The DEBQ has been used previously in eating rate studies (Framson et al., 2009; Warren et al., 2017), and it has demonstrated adequate external, internal validity and test-retest reliability. The questionnaire’s answers are composed of a rating scale of frequency, from ‘never’ to ‘very often’.
In the current programme of work the total scores of volunteers were summed and those with a high level of restrained eating (score > 16) were excluded from all three studies (Chapters: 3, 4 & 5). In chapter 4, data collection from the DEBQ items was undertaken automatically via the Typeform online software tool (Typeform, 2018).

2.6.2 General Health Questionnaire:
A general health questionnaire, commonly used in the Faculty of Health and Medical Sciences, University of Surrey, was used and altered for each study depending on the inclusion and exclusion criteria. See Appendix 5 for an example of a General Health Questionnaire used as part of this thesis.

2.6.3 Mindfulness Eating Questionnaire (MEQ)
In chapters 4 and 5 participants completed the Mindful Eating Questionnaire based on that being developed at the time of the study by Winkens et al (2018). This questionnaire was derived from a combination of existing questionnaires (Hulbert-Williams et al., 2014; Van Diest, 2013; Framson et al., 2009) [Appendix 6]. It was composed of 14 items, generated from the original question battery by factor analysis and reliability analyses (Winkens et al., 2018). Items focused only on one part of the definition of mindful eating, which is “deliberately eating consciously, with attention and awareness, without distraction” (Brown and Ryan, 2003). Items addressed either awareness or behaviour associated with four constructs; sensory eating, awareness, body cues, and distraction. For example, the item «I notice how my food looks» addresses sensory eating. Possible answers were comprised of a rating scale of frequency, namely ‘never’, ‘seldom’, ‘sometimes’, ‘often’, and ‘very often’. For sensory eating and body cues items, ‘never’ and ‘very often’ answers were manually awarded 1 and 5 points respectively, and the opposite for awareness and distraction questions. As directed by the creator of the questionnaire (Winkens et al., 2018) each category was analysed separately across all participants using t-tests and ANOVA’s.

2.7 Statistical Analysis
Statistical software packages used were SPSS 22 (SPSS Inc., IBM Company, Chicago, Illinois, USA), Graphpad Prism 7 (GraphPad Software Inc., La Jolla, San Diego, California, USA) and PS software (Vanderbilt University, Tennessee, USA). Analysis of variance (ANOVA and repeated measures ANOVA) were performed using SPSS and Prism, but post hoc tests (Tukey test) were
only performed using SPSS. One-way ANOVA, Two-tailed paired t-test and area under the curve (AUC) were carried out in GraphPad Prism, and the power size calculation was performed using PS software.

2.7.1 Normality tests
In order to inform the choice of statistical tests normality tests were initially carried out in all datasets to assess the data distributions. All data were checked for normality using D’Agostino and Pearson omnibus normality test in Graphpad Prism and Kolmogorov-Smirnova as well as Shapiro-Wilk test using SPSS. Normality tests were carried out per group, time and treatment to make sure that none of these interventions or factors had influenced the normal distribution of the data. A p value ≤ 0.05 indicates that the distribution is significantly different from normal. When all data were normally distributed, parametric tests were carried out whereas non-parametric tests were carried out for non-normally distributed data. Variance, outliers, skewness and kurtosis were also checked before running any parametric tests. Variance between groups across variables were similar (variance ratio 1:1), skewness and kurtosis was between -3 and +3, and no influential outliers were defined for any of the variables in any of the datasets tested. Histograms were also drawn to visually assess the data distribution (Bland, 2015).

2.7.2 Missing values
For hormone and metabolite measures, missing values were replaced by either using linear interpolation for the middle missing values, or linear extrapolation for missing values at the beginning or end of the clinical sessions. The percentage of values which needed to be inter-/extrapolated in Chapter 5 were glucose: 2.5%, insulin: 2.1%, leptin 1.3%, NEFA: 3.1%, TAGs 2.8%, Cholesterol 1.4% and HDL-Cholesterol 2.1%. Inter-/extrapolation has many limitations especially when the missing data occurs where the postprandial peak is expected, however, in chapters 3 and 5 there were no missing values during postprandial peaks.

2.7.3 Intention to Treat
Intention to treat (ITT) is the recommended standard approach to analyse data from randomised controlled trials. This method requires that patients are analysed according to their original random allocation to preserve the prognostic balance, thereby minimising selection bias and cofounding. According to the Cochrane handbook (Cochrane handbook for systematic reviews and interventions, 2011) to fulfil the principle of ITT, (1) participants in a trial need to be retained in the groups in which they were originally allocated, (2) the outcome data need to be measured on all
participants and (3) all participants need to be included on the analysis. ITT analysis was carried out in the final intervention study (Chapter 5), as 3 participants dropped out after (24h) the intervention had started.

2.7.4 Analyses of variance (ANOVAs)
Prior to performing analysis of variance (ANOVA), the datasets were tested for violation of sphericity which refers to the condition where the difference in variance between all related groups (levels) are equal (i.e., an increase in the type 1 error rate). Mauchly’s test of sphericity is a formal way of testing the violation of assumption of sphericity using SPSS. If sphericity is not violated, then the F-statistic is valid and can be used in statistical significance. However, if sphericity is violated, then the F-statistic is invalid, the type 1 error rate is increased and corrections to the degrees of freedom (df) must be applied. In these circumstances the Greenhouse-Geisser correction was chosen to counter violation of sphericity (Bland, 2015). Two types of analysis of variance were used in this project: one-way ANOVA and repeated measures ANOVA. One-way ANOVA was used in Chapters 3 and 5 when comparing three treatment factors [Chapter 3: Normal eating rate (NER), slow eating rate protocol with earplugs in (SER-in), slow eating rate protocol with earplugs out (SER-out); Chapter 5: NER, control group (Cont group), Intervention group (Int group)]. Analyses of variance were conducted in SPSS. One-way ANOVA was used to investigate the difference in baseline samples for: appetite scores (Chapters 3, 4, 5), hormones and metabolites (chapter 5) and eating rate parameters (chapters 3, 4 and 5), total area under the curve (TAUC) and incremental area under the curve (IAUC). In the final study (Chapter 5), a more complex design of several factors (eating rate parameters, group, time, body composition, appetite measures and MEQ results) required repeated measures ANOVA with between subject factors, using SPSS.

Tukey's honest significance difference (Tukeys HSD) post hoc tests were performed after ANOVA tests (repeated measured ANOVA and one-way ANOVA) to identify significant differences between group, treatment and time. This test was only used if a significant difference was shown in ANOVA test (Bland, 2015).

2.7.5 Students t-tests
Students t-test is used to test a hypothesis of normally distributed samples when the standard deviation is unknown. T-tests determine whether there is difference in the mean values between two groups of samples (Bland, 2015). In the current project, paired t-tests were used in chapters 3,4 and 5 when the same measurements were tested in the same individuals under two different conditions (i.e Study B: SER-In, SER-Out ).
2.7.6 Correlation and multiple regression analysis
Pearson product-moment correlation coefficient, is a statistical value used to measure the linear dependence between two variables X and Y. The correlation coefficient value ranges between -1 to +1. +1 implies a positive relationship between variables, Y increases as X increases. -1 implies a negative relationship, Y increases as X decreases, whereas a value of 0 represent no linear correlation (relationship) between the variables (Bland, 2015). In (Chapter 5) correlation analyses were performed to measure associations between independent variables (age, BMI, body-fat percentage and manual ER in chews per minute) and the primary outcomes (automatic ER and the MEQ summary score). Following this, multiple regression was performed to investigate the factors predicting the dependent variables. For all data, the accepted level of significance was (p≤0.05).

In chapter 5, a mixed model analysis was performed using SPSS in order to correlate data extracted from the study’s dedicated website with all of the main outcome parameters (body composition, eating rate, energy intake, appetite, mindfulness). Once the outcomes with the highest correlation values were identified, a backward linear regression was used to create a modelling of change for the intervention group using the website's monitoring output data.

2.7.7 Area Under the Curve
Area under the curve (AUC) is primarily used to quantify the measurable effects of a phenomenon. In this project, AUC was used to measure the area above the X-axis that represents the concentration of various metabolites and hormones as well as change over time for perceived measures of appetite, using Graphpad Prism. Prism draws a straight line between adjacent points (point of (x,y)) to define the curve. Then, the curve is divided into regions which can be calculated using the trapezoid rule. The trapezoid rule used in Prism is simply defined by this formula (X * (Y1+Y2)/2). The area is computed from the user specified baseline and the curve starting from the first X values to the last X value (Bland, 2015). Total area under the curve (TAUC) was used to estimate the area above X axis (Y = 0), whereas Incremental AUC (IAUC) as used to estimate the area above baseline point (Y = first collected sample).
Chapter 3

Development and testing of a slow eating rate protocol
3.1 Introduction
Eating rate (ER) has been strongly implicated in appetite control (Robinson et al., 2014) and when manipulated it impacts on meal portion size, energy intake and satiety (Krop et al., 2018). We previously developed a slow eating rate protocol [SER] (Koidis et al., 2014) and showed that overweight adults in a fast eating condition consumed a meal at a faster eating rate than healthy weight adults given the same fast eating instructions, with a trend towards reduced perceived fullness when eating at a fast eating rate for the overweight group.

With the recent increase in supply of smartphones and MP3 players, the usage of personal sound equipment (earphones) has also been increasing (Daniel, 2007, Hong et al., 2013). Ear plugs, or inserted earphones with a foam tip, block the ear canal which lowers the intensity required to find bone-conduction thresholds when an ear is occluded, as opposed to when it is uncovered, known as the ‘occlusion effect’ (Dean and Martin 2000). This effect enhances sounds made by the body such as swallowing and chewing (Feldman et al., 1972). Enhancing oral processing, which includes chewing and swallowing, can affect appetite by accelerating satiation, reducing energy intake and enhancing meal memory (See chapter 1) though the evidence and underlying mechanisms are unclear (James, 2018).

Thompson et al (2010) in their overview of dietary assessment methods, highlighted the necessity of the use of new technological approaches for objective and accurate assessment of free-living food intake (FI) patterns for the monitoring of eating behaviours. To date no commercially available or validated way of measuring ER- in a non-disruptive manner exists (see chapter 1, section 1.4 for further details on ER monitoring techniques).
3.1.1 Technical development study (A): Development and testing of a slow eating rate protocol

3.1.2 Aim

- To further refine a previously developed slow ER protocol through volunteer feedback
- To assess if the use of Qualisys Track Manager (QTM) is a reliable method for measuring eating rate in a clinical setting

3.1.3 Objectives

- Recruit a sample of 6-8 healthy volunteers in order to receive feedback on the protocol’s acceptability and feasibility
- Identify factors affecting protocol compliance
- Refine the slow eating rate protocol on the basis of the study findings for use in future eating rate studies.
- Explore the use of movement lab technology to monitor eating rate in the lab
- Assess the reliability of QTM for measuring eating rate when compared to manual counts by observers
3.1.4 Methods

3.1.4.1 Ethical approval
A favourable ethical opinion was obtained from the University of Surrey Ethics Committee (EC/2014/102/FHMS, Appendix 7).

3.1.4.2 Participants
Inclusion and exclusion criteria were used as part of the pre-study screening process to ensure the eligibility and suitability of the study participants:

Inclusion Criteria:
- Healthy people aged 18-70 years
- Male or female
- Able to complete health and demographic questionnaires in English
- Willing to maintain a regular sleep cycle, with sleep duration 6.5-8h a night before each visit
- Body Mass Index: >18m/kg²

Exclusion Criteria:
- Restrained eaters as assessed by the Dutch Eating Behaviour Questionnaire
- BMI below 18 m/kg²
- Irregular sleep cycle
- Any medication affecting appetite/satiety

3.1.4.3 Recruitment
The studies were advertised via email to all students and staff at the university and to the general public.

3.1.4.4 Facilities
The studies were carried out in the Clinical Investigation Unit (CIU) and the Movement laboratory (07DK 00), at the University of Surrey, Guildford, UK. The CIU unit is provided with all facilities such as communication (telephone and internet), sleeping and toilet/shower as well as light and temperature controlled. Participants were supervised by research investigators throughout the study.
3.1.4.5 Eating Rate Instructions
The eating rate instructions developed for the pilot study were used as a starting point in this study (Koidis et al., 2014).

**Slow eating rate original instructions:**

- Chew each mouthful more than 40 times
- Put your utensils down after each bite
- If you lose count of chews, pause for 10 seconds, swallow and then take your next mouthful.
- You are only allowed 250ml of water, please drink only in-between mouthfuls, unless necessary.

3.1.4.6 Eating rate detection software
A real-time motion capture software (Qualysis, Gothenburg, QTM 2.0) was used in this study, following input from the Department of Mechanical Engineering Sciences, Centre for Biomedical Engineering, University of Surrey. QTM has the potential for automating the procedure of counting and detecting counts which could be used in future work instead of the labor-intensive manual counts.

3.1.4.7 Experimental Procedures

3.1.4.7.1 Pre-study procedures
Written informed consent was obtained from each participant prior to the study (Appendix 8) after they had read the participant information sheet (Appendix 9). Participants had the right to withdraw from the study at any time without stating a reason. Participants’ results and data were stored in strictest confidence in compliance with the General Data Protection Regulation (GDPR) and the UK Data Protection Act 2018, in locked cabinets.

All selected participants received an oral and written explanation of this developmental study requirements and design. Participants were also given an opportunity to ask questions of the researchers prior to giving informed written consent. As this was a protocol development study, we aimed to recruit between 6-8 participants until the eating rate methodology was refined to the point that participants could comfortably follow the ER instructions.
3.1.4.7.2 Experimental Design
Each participant attended the CIU/Movement laboratory, FHMS on one occasion and had to follow specific eating rate instructions whilst being filmed. Each participant was asked to provide feedback on their overall eating experience in the study, which contributed towards the protocol development.

3.1.4.7.3 Study Session

i) Study meal
The test meal consisted of one portion of spinach and ricotta tortellini (570 kcals, 15.9% protein, 50.5% CHO, 31.6% Fat). This meal was chosen as each tortellini is the size of a mouthful, therefore aiding in protocol compliance and monitoring of the eating rate procedure. Water intake was controlled throughout the meal with participants only consuming 250ml.

ii) Study Protocol

![Protocol Schematic](image)

Participants consumed breakfast at their own home between 07:30-08:00. Participants were then asked to attend the movement laboratory between 12:30h-15:00h and were asked to eat and drink nothing except water between breakfast and lunch. Arriving at the movement lab each participant had markers attached at various places on the face, neck and hand with hypoallergenic double-sided tape. Marker 1: Approx. 1cm from the upper lip corner Marker 2: Chin, Marker 3: Larynx, Marker 4: Hand, which will be used to hold the utensil with which the test meal will be consumed (see Figures 3.1.2 & 3.1.3). These thresholds were used as per the guidance of the movement lab expert. A fixed-portion controlled lunch (Study meal) was then consumed under normal eating rate conditions. The optical motion tracking system [Qualysis, Gothenburg] recorded the movement of the jaw, neck and hand and two video cameras were also recording (neck, mouth and jaw only). The recording was analysed using QTM 2.0, a data capture software and MATLAB software.
The participant with the 4 monitors attached was seated in a comfortable position on a table with movement lab cameras in the periphery. The monitors reflect light back to the periphery cameras and that is how the movement of the eating rate procedure is captured (Figure 3.1.3). The data was then transmitted to the QTM software, where the movement is reproduced on screen with only the monitors and diameters showing (Figure 3.1.4). In-between the motion detection cameras there were two video cameras on tripods (front and side view) (Figure 3.1.3) that feedback real-time footage of the volunteer consuming a meal. The side view camera was used as a backup chew-capture method in case of a machine malfunction of the front-view camera. Two researchers counted the chews independently from the side and front recordings. The results were then compared using Pearson’s correlation.

At the time of the study, QTM software had never been used to detect chewing movement, and therefore had no recommended pre-set thresholds. Following guidance from the movement lab expert, four different chewing threshold levels (≥0.5cm, ≥0.65cm, ≥0.75cm and ≤0.90cm) were tested in the QTM software.
3.1.5 Results

3.1.5.1 Chew Count Correlation
The chew counts for both observers were correlated to investigate inter-investigator reliability. There was strong positive correlation between the 2 front chew counts (See table 3.1.1) so 1 set of data which was the average of the two investigators were used for analysis.

Table 3.1.1. Pearson correlation between the front and side chew count from one observer and front chew counts from 2 observers.

<table>
<thead>
<tr>
<th>Side Chews observer 1 vs Side Chews observer 2</th>
<th>Front Chews observer 1 vs Front Chews observer 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Correlation Coefficient (r)</td>
<td></td>
</tr>
<tr>
<td>0.989</td>
<td>0.991</td>
</tr>
<tr>
<td>Significance Level (p)</td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>N</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 3.1.2 shows the correlation between all participants across different jaw-opening thresholds as set via the QTM software. As it can be seen from the table above, chew counts, both side and front view correlate strongly and significantly between the two observers.

Table 3.1.2. Pearson correlation between QTM counts across all participants for different threshold levels versus manual chew Counts.

<table>
<thead>
<tr>
<th>QTM Counts (threshold)</th>
<th>QTM Counts (threshold)</th>
<th>QTM Counts (threshold)</th>
<th>QTM Counts (threshold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0.5cm threshold)</td>
<td>(0.65cm threshold)</td>
<td>(0.75cm threshold)</td>
<td>(0.90cm threshold)</td>
</tr>
<tr>
<td>Pearson Correlation Coefficient (r)</td>
<td>0.459</td>
<td>0.344</td>
<td>0.499</td>
</tr>
<tr>
<td>Level of significance (p)</td>
<td>0.792</td>
<td>0.782</td>
<td>0.592</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>
In table 3.1.3 below, results are depicted in 2 minute recoding intervals as per QTM software monitoring standards. On one column the counts by an observer are depicted (mean values of 2 different observers). The other 4 columns are the chewing counts picked up by the software, with different allocated minimum thresholds of mouth opening.

**Table 3.1.3: Count of chews: manual vs. QTM software at different mouth opening thresholds.**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Counts by Observers</th>
<th>QTM Counts with 0.5cm threshold or below</th>
<th>QTM Counts with 0.65cm threshold</th>
<th>QTM Counts with 0.75cm threshold</th>
<th>QTM Counts with 0.90cm threshold or above</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td>102</td>
<td>240</td>
<td>223.5</td>
<td>165</td>
<td>103</td>
</tr>
<tr>
<td>2-4</td>
<td>102</td>
<td>202.5</td>
<td>185</td>
<td>140</td>
<td>72</td>
</tr>
<tr>
<td>4-6</td>
<td>105</td>
<td>127</td>
<td>103</td>
<td>72.5</td>
<td>38</td>
</tr>
<tr>
<td>6-8</td>
<td>105</td>
<td>99</td>
<td>84.5</td>
<td>56.5</td>
<td>31</td>
</tr>
<tr>
<td>8-10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean</td>
<td>103.5</td>
<td>167.2</td>
<td>149</td>
<td>108.5</td>
<td>61</td>
</tr>
<tr>
<td>STDDEV</td>
<td>1.7</td>
<td>65.3</td>
<td>66.1</td>
<td>52.2</td>
<td>33.2</td>
</tr>
<tr>
<td>Subject 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td>124</td>
<td>121</td>
<td>99</td>
<td>91</td>
<td>55</td>
</tr>
<tr>
<td>2-4</td>
<td>114</td>
<td>143.5</td>
<td>86</td>
<td>77</td>
<td>41</td>
</tr>
<tr>
<td>4-6</td>
<td>117</td>
<td>117.5</td>
<td>100</td>
<td>86</td>
<td>47</td>
</tr>
<tr>
<td>6-8</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>8-10</td>
<td>-</td>
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<tr>
<td>Mean</td>
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<td>127.3</td>
<td>95</td>
<td>84.7</td>
<td>47.7</td>
</tr>
<tr>
<td>STDDEV</td>
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<td>14.1</td>
<td>7.8</td>
<td>7</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>0-2</td>
<td>103</td>
<td>364</td>
<td>277</td>
<td>165</td>
<td>109.5</td>
</tr>
<tr>
<td>2-4</td>
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<td>339</td>
<td>219.5</td>
<td>155</td>
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<td>4-6</td>
<td>87</td>
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<td>228</td>
<td>142</td>
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<td>6-8</td>
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<td>362</td>
<td>238</td>
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<td>-</td>
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</tr>
<tr>
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<td>100</td>
<td>342.5</td>
<td>240.6</td>
<td>151.5</td>
<td>95.7</td>
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<tr>
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<td>25.4</td>
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<td>10.8</td>
</tr>
<tr>
<td>Time (min)</td>
<td>Counts by Observers</td>
<td>QTM Counts with 0.5cm threshold or below</td>
<td>QTM Counts with 0.65cm threshold</td>
<td>QTM Counts with 0.75cm threshold</td>
<td>QTM Counts with 0.90cm threshold or above</td>
</tr>
<tr>
<td>-----------</td>
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<td>----------------------------------------</td>
<td>----------------------------------</td>
<td>----------------------------------</td>
<td>------------------------------------------</td>
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<tr>
<td>Subject 4</td>
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</tr>
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<td>180</td>
<td>139</td>
<td>112</td>
</tr>
<tr>
<td>4-6</td>
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<td>333</td>
<td>290</td>
<td>178</td>
<td>95</td>
</tr>
<tr>
<td>6-8</td>
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<td>253.2</td>
<td>160.5</td>
<td>95.4</td>
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<tr>
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<td>18.1</td>
<td>42.8</td>
<td>17.9</td>
<td>17.5</td>
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<tr>
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<td>320</td>
<td>246</td>
<td>135</td>
<td>79</td>
</tr>
<tr>
<td>2-4</td>
<td>88</td>
<td>314</td>
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<td>139</td>
<td>112</td>
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<td>290</td>
<td>168</td>
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<tr>
<td>6-8</td>
<td>91</td>
<td>311.5</td>
<td>275</td>
<td>144</td>
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<td>8-10</td>
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</tr>
<tr>
<td>Mean</td>
<td>92.4</td>
<td>311.2</td>
<td>219.1</td>
<td>181</td>
<td>99.2</td>
</tr>
<tr>
<td>STDEV</td>
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<td>15.1</td>
<td>45.1</td>
<td>17.2</td>
<td>17.5</td>
</tr>
<tr>
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<tr>
<td>2-4</td>
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<tr>
<td>Subject 6</td>
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</tr>
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<td>4-6</td>
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<td>333</td>
<td>290</td>
<td>159</td>
<td>110</td>
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<tr>
<td>6-8</td>
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<td>331.4</td>
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<td>144</td>
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<td>8-10</td>
<td>103</td>
<td>350</td>
<td>255</td>
<td>161.2</td>
<td>120</td>
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<tr>
<td>Mean</td>
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<td>325.7</td>
<td>253.2</td>
<td>160.5</td>
<td>95.4</td>
</tr>
<tr>
<td>STDEV</td>
<td>6.2</td>
<td>15.5</td>
<td>41.1</td>
<td>21.4</td>
<td>17.1</td>
</tr>
</tbody>
</table>

Table 3.1.3 suggests that the QTM count reproducibility is poor, as none of the tested thresholds correlated well against the counts by observer, with a substantial variation in mean chews detected for the different threshold cut offs.
3.1.5.2 Protocol Improvements based on Participant’s Feedback

The feedback provided from the participants is presented in table 3.1.4. This feedback helped develop the form of the final version of the protocol that was used in study B below and Chapter 5.

**Table 3.1.4. Participants’ feedback on ER Protocol**

<table>
<thead>
<tr>
<th>Participant Feedback</th>
<th>Change in Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participant 1:</strong> Original pasta used, too large to fit into mouth at once</td>
<td>Change to 4-cheese tortellini that have a smaller size</td>
</tr>
<tr>
<td><strong>Participant 2:</strong> Feeling very thirsty with the pasta, needed a sip of water after every mouthful, so 250 ml restriction did not suffice.</td>
<td>Added 2 table spoons tomato and basil sauce to make the meal more palatable and reduce the sense of thirst.</td>
</tr>
<tr>
<td><strong>First 3 participants:</strong> 40 chews seem too many, usually felt ok at about 25-30 chews.</td>
<td>Chew each mouthful for 30 times until you swallow for the first time. Then chew 10 more times the food that has remained in your mouth</td>
</tr>
<tr>
<td><strong>Participant 4:</strong> Difficulty to place and detect monitor no 3 (throat) as participant had excess skin in the throat area. This resulted in the loss of some data through the QTM detecting system as monitor no 3 was not clearly visible.</td>
<td>Ensure monitors are clearly visible before start recording the study and use bigger size monitor for the throat.</td>
</tr>
<tr>
<td><strong>Participant 5:</strong> Reported that although they were aware of study instructions, sometimes they just swallowed food after a few bites by &quot;habit&quot;</td>
<td>Have the eating rate protocol printed on the side of the participant’s plate to help maintain focus and act as a reminder of the instructions</td>
</tr>
</tbody>
</table>
3.1.5.3 Revised SER protocol

Table 3.1.5 below shows the revised slow eating rate protocol instructions after taking into consideration participant’s feedback.

Table 3.1.5: Revised SER protocol

- Before you start eating, take a comfortable position that you usually eat in and try to maintain that position until you finish your meal.

- Take one piece of pasta each time

- After each mouthful place your fork down

- Chew each mouthful for 30 times until you swallow for the first time. Then chew 10 more times the food that has remained in your mouth

- If you lose count of chews, pause for 10 seconds, swallow and then take your next mouthful.

- You are only allowed 250ml of water, please drink only in-between mouthfuls, unless if necessary.

- Eat until you are comfortably full. The remaining of the pasta will be given to you to take away in a plastic container.

- Please make a note of how many times you missed chewing count here _______
3.1.6 Discussion
This study aimed to further develop a slow eating rate protocol, by utilising kinetics software (QTM and MATLAB) and volunteers’ verbal feedback.

3.1.6.1 Data produced via QTM and MATLAB
QTM count reproducibility was poor, as none of the tested thresholds correlated well against the counts by observer. There was substantial variation within participants which could be due to certain jaw movements not picked up by the software’s camera’s suggesting that a large amount of chewing data should be first added to the software so it could then recognise chew movements more efficiently.

3.1.6.2 Volunteer Verbal Feedback
According to the National Institute for Health Research (NIHR 2014), it is advisable to involve patients and members of the public (NIHR 2014) before setting up a research study. Patient and Public Involvement (PPI) when designing, implementing and evaluating research invariably makes studies more credible, effective and cost efficient (Brett et al., 2010, McMillan et al., 2018).

Based on the volunteer’s feedback, the protocol was amended by changing the meal provided (Tesco 4 cheese tortellini) and making mouthfuls easier. Tomato sauce was added to improve lubrication (See chapter 1 for importance of lubrication in oral processing) and the chewing rate instructions were changed from 40 chews per mouthful to 30 chews before the first swallow and then another 10 for the remainder food.

3.1.7 Conclusion
In conclusion, this technical development study resulted in an improved ER protocol through the volunteer’s feedback. Furthermore, manual counts by an observer proved to be a more reliable technique for measuring ER when compared to the QTM software, and were therefore used in the subsequent studies. Using QTM software for assessing ER needs further validation before it can be used in an eating rate study.
3.2.1 Technical development study (B): Assessment of the use of ear plugs on protocol effectiveness in an eating rate study.

3.2.1.2 Aims:

Following the results of the above developmental study, and in an attempt to reinforce and improve concordance with the SER protocol, earplugs were incorporated into a second developmental study.

1. Assess the effects of adding earplugs to the previously developed SER protocol on perceived satiety
2. Assess the effects of adding earplugs to the previously developed SER protocol on protocol adherence.

3.2.1.3 Objectives:

- Recruit 12 healthy volunteers (6 males 6 females)
- Measure the effect of earplugs on perceived satiety
- Measure protocol compliance in subjects with and without ear plugs

3.2.1.4 Hypothesis: The use of earplugs will increase perceived satiety and improve adherence to the slow eating rate protocol.

Null Hypothesis: The use of earplugs will not improve adherence to the slow eating rate protocol or increase perceived satiety.
3.2.2 Methods

3.2.2.1 Ethical application
The University of Surrey Ethics Committee gave a favourable opinion for this study (EC/2014/102/FHMS, Appendix 7)

3.2.2.2 Participants
Inclusion criteria included good general health, aged 18-70 years, females and males, BMI >18m/kg². Exclusion criteria were restrained eaters, BMI ≤18m/kg² or taking any medication that might affect their appetite. Written consent was obtained from all participants prior to the start of the study. Participants also completed a general health questionnaire and a Dutch Eating Behaviour Questionnaire (DEBQ) (Van Strien et al., 1986).

3.2.2.3 Study Meals
Standardised breakfast: 2 chocolate muffins and a 200ml carton of orange juice (see Table 3.2.1), consumed between 06:30-08:00 for all three visits.

Lunch (test meal): One portion (200g dry weight) of a supermarket own brand 4 cheese pasta with 1.5 tablespoons of tomato and basil sauce prepared as per packet instructions (560kcal, 10.3% protein, 34.3% fat and 55.4% CHO) consumed between 12:00-12:30 for all three visits.

Water intake was limited to 250ml during test-meal consumption and participants were asked to eat until comfortably full.

Table 3.2.1: Study meals

<table>
<thead>
<tr>
<th>Meal</th>
<th>Portion (g)</th>
<th>Energy (kcal)</th>
<th>Protein (g)</th>
<th>CHO (g)</th>
<th>Sugars (g)</th>
<th>Fat (g)</th>
<th>Fibre (g)</th>
<th>Total GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standardised meal (Breakfast)</td>
<td>300</td>
<td>425</td>
<td>5.4</td>
<td>70.5</td>
<td>38.6</td>
<td>12.4</td>
<td>2.4</td>
<td>55.2</td>
</tr>
<tr>
<td>Test-Meal (Lunch)</td>
<td>200</td>
<td>606.5</td>
<td>37.1</td>
<td>85</td>
<td>42.9</td>
<td>12.2</td>
<td>5.8</td>
<td>56.4</td>
</tr>
</tbody>
</table>

GI: Glycaemic Index, calculated via the glycaemic index calculator, University of Sydney: [www.glycemicindex.com](http://www.glycemicindex.com)
3.2.2.4 Study Protocol
This study was a randomised crossover design. Following screening, where the normal eating rate (NER) was established via the use of cameras, participants attended the Clinical Investigation Unit (CIU), at the University of Surrey, on three occasions a minimum of one week apart. Two cameras (front and side view) were used, which recorded the participants. Visit 1 was a screening visit and visits 2 and 3 were experimental with instruction on, and assessment of, a slow eating rate protocol with (SER-In) and without (SER-Out) earplugs (delivered in a random order). They attended between 12:30h-14:30h. Two camcorders (Polaroid iD1880) were set up to focus on the face of the participants from the front and right side views during their meal consumption. The videos produced were played back and chews/minute were independently counted by two researchers. Eating rate was measured as the amount of chews per minute from the first bite until the end of the meal. Participants completed a satiety Visual Analogue Scale (VAS) before the meal (assessing perceived hunger, desire to eat and perceived fullness on a line from 0-10mm) and then the cameras were turned on and participants were left without interruption to eat their meal. Once participants had finished their meals they completed further satiety VAS 15, 30, 45, 60 and 90 minutes after their meal.

3.2.2.5 Video analysis
The video data were analysed by 2 researchers independently. Chews and mouthfuls per minute were counted using a reduced video speed.

3.2.2.6 Statistics
All results are presented as mean ± standard error of mean (SEM) or standard deviation (SD). All data were analysed using GraphPad Prism 6 for Windows 7. Data were checked for normality using the Shapiro-Wilks test and Gaussian distribution was assumed. Pearson correlation was carried out on the total chew count data to identify if there was a linear relationship between front and side camera views. Linear regression analysis was used to establish the most appropriate line of fit from data obtained from the eating rate protocols. Meal duration data and total chew count data were analysed using repeated measures one-way ANOVA followed by multiple comparison Tukeys test to locate individual differences. Total area under the curve (TAUC) for satiety data was calculated using the trapezoid rule and repeated measures One way ANOVA and followed by multiple comparison Tukey’s test.
3.2.3 Results

This study aimed to trial the previously developed ER protocol with and without earplugs and assess if the addition of earplugs would affect perceived satiety.

Table 3.2.2: Demographic data of participants (n=12) taking part in the study (mean ± SD)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>BMI (kg/m²)</th>
<th>Fat (%)</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>24.58 ± 6.6</td>
<td>66.08 ± 12.3</td>
<td>172.0 ± 8.5</td>
<td>22.23 ± 3.1</td>
<td>18.85 ± 7.6</td>
<td>6: Female 6: Male</td>
</tr>
<tr>
<td>Range</td>
<td>18-37</td>
<td>45.6-92.8</td>
<td>157-190</td>
<td>18-30.28</td>
<td>2.7-29.5</td>
<td>n/a</td>
</tr>
</tbody>
</table>

3.2.3.1 Chew Count Correlation

The video data from the two researchers were compared using Pearson’s correlation. There was a significant strong positive correlation (table 3.2.3) between the front and side chew counts from both observers. The front chew counts for both observers were correlated. There was strong positive correlation between the 2 front chew counts (See table 3.2.3) therefore one set of data were used for further analysis.

Table 3.2.3. Pearson correlations between front and side chew count by one observer and between front chew counts by two observers.

<table>
<thead>
<tr>
<th>Front chews observer 1 vs side chews observer 1</th>
<th>Front chews observer 1 vs front Chews observer 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Correlation Coefficient (r)</td>
<td>0.995</td>
</tr>
<tr>
<td>Significance Level (p)</td>
<td>0.013</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
</tr>
</tbody>
</table>
3.3.3.2 Meal Duration

Figure 3.2.1 shows mean meal duration. Meal duration during the NER (7.34 ± 0.63 mins) was significantly shorter (p<0.00001) than both SER protocols. (SER-In, 15.11 ±1.30mins; SER-Out, 15.46±0.89 mins).

![Figure 3.2.1](image)

**Figure 3.2.1.** Mean meal duration adjusted for interruption by VAS in NER, SER-In and SER-Out (mean ± SEM, N=12). Bars with identical superscript letters are significantly different at p<0.0001

3.2.3.3 Total Chew Count

Figure 3.2.2 shows a histogram of total chew count. One-way ANOVA showed a significant difference (p<0.029) between mean total chew counts across the three groups (NER, SER-In, SER-Out). A multiple comparison Tukey’s test showed a significant difference (p=0.05) between NER and SER-In and between NER and SER-Out (p=0.031) but no significant difference between SER-IN and SER-Out (p= 0.112).

![Figure 3.2.2](image)

**Figure 3.2.2:** Mean total chew count in NER, SER-In and SER-Out (mean ± SEM, N=12). Bars with identical superscript letters are significantly different.
3.2.3.4 Eating Rate

Mean mouthfuls per minute were plotted for the first 6 minutes (Figure 3.2.3). Only the first 6 minutes were comparable of the meal as that was the minimum duration time for normal eating. A repeated measures one way ANOVA on the raw data showed a significant effect of time (p = 0.0002), group (p= 0.009) and time by group interaction (p=0.034) between the eating protocols. A multiple comparisons Tukey test showed significant differences (p = 0.05) between NER and SER-In and between NER and SER-Out.

![Figure 3.2.3](image)

Figure 3.2.3. Mean mouthfuls/minute for the first 6 minutes across NER, SER-In and SER-Out (N=12). *Lines with identical superscript letters are significantly different.

3.2.3.5 Cumulative chews per mouthful

The expected cumulative chew count per mouthful for the two SER conditions was plotted and fitted with a linear regression line shown in Figure 3.2.4. Analysis with repeated measures ANOVA showed a significant effect of time and no significant effect of group or group by time (p=0.48).

![Figure 3.2.4](image)

Figure 3.2.4. Linear regression lines for expected cumulative chews /mouthful (as per the protocol) vs the mean expected cumulative chews for SER-In and SER-Out. ANOVA showed no significant difference between the three linear regression lines (p = 0.48), N=12.
### 3.2.3.6 Eating Rate and Satiety

The satiety variables ‘How hungry do you feel?’ (perceived hunger), ‘How much do you feel you can eat?’ (desire to eat) and ‘How full do you feel?’ (perceived fullness) were analysed with TAU Cs plotted in Figure 3.2.5. One way ANOVA was calculated from visual analogue data to compare the different eating conditions. Perceived hunger (p=0.034) and desire to eat (p=0.005) were significantly lower and perceived fullness significantly greater (p = 0.03) in SER-In compared to NER, with no significant differences between SER-In and SER-Out.

![Figure 3.2.5](image-url)

**Figure 3.2.5.** Mean total area under the curve (TAUC) for perceived hunger, desire to eat and perceived fullness across three eating rate conditions, range 0-100mm; NER, SER-In and SER-Out (Mean ±SEM, N=12). * p<0.05.

Significant differences in perceived hunger (p=0.03), desire to eat (p=0.005) and perceived fullness (p=0.03) were identified. Post hoc tests identified that the SER-In condition significantly reduced hunger and desire to eat and increased satiety relative to the NER condition. No significant differences were observed between SER-In and SER-Out for perceived desire to eat (p= 0.101) perceived hunger (p= 0.103) or perceived satiety (p= 0.131).
3.2.4 Discussion

This study aimed to assess the addition of earplugs to our previously developed SER protocol, the hypothesis being that the earplugs would enhance satiety.

This is the first study of its kind to investigate the potential additive effect of ear plugs on compliance to a slow eating rate protocol and on subsequent perceived satiety. Our results show that the addition of earplugs to the SER protocol did not significantly affect meal duration nor enhance satiety although there was a positive effect on the SER-In group. This result concurs with our previous study showing that a SER had an effect on satiety (Koidis et al., 2014) however as there is no significant difference in meal duration between SER-In and SER-Out, this result is intriguing.

None of the participants reported any discomfort caused by the earplugs, for the duration of the study.

Although an impact on the primary outcome of meal duration was not seen, differences in the perceived satiety measures with and without earplugs suggest that wearing earplugs may exert a psychological rather than a physiological effect. This psychological effect of SER was previously hypothesised by Ferriday et al (2015) who suggested that slower eating might affect episodic memory (making the memory of the meal eaten slower more vivid), though the results were inconclusive and their protocol did not accommodate earplugs. Furthermore, ear occlusion enhances the auditory sense (Feldman., 1972), which is one of the main stimuli of the cephalic-phase response (Smeets et al., 2010). It therefore could be argued, that perceived satiety was enhanced in this study, due to the effects that the earplugs exert on the cephalic phase response. A recent review by James (2018) suggests that several studies have demonstrated that enhancing/prolonging oral processing time can accelerate satiation, though the evidence and underlying mechanisms are unclear, therefore making such link is challenging since somatosensory stimulation, chewing and salivation interact in complex ways (Smeets et al., 2010).

Despite the instructions to eat until comfortably full all participants consumed the entire test-meal, which provided approximately 25% of the daily estimated energy requirements for males and 30% for females (SACN 2010). Whilst portion size could be expected to effect the relationship between ER and satiety (Krop et al., 2018) in the current study the two groups had equal numbers of males and females and no significant differences in body composition parameters. Therefore the test-meal provided was not adjusted for participants’ weights in concordance with the methodology of previous ER studies (Almiron Roig et al., 2015, Ferriday et al., 2015 & 2016).
The secondary aim of this study was to assess the effects of adding earplugs to the previously developed SER protocol on protocol adherence. In both conditions (SER-In and SER-Out) adherence protocol instructions was good and very similar, which further shows that irrespectively of earplugs earplugs or not the participants were able to follow the protocol instructions well, with the earplugs having no added benefit. Consequently, due to the nature of the study design it was not possible to assess if the addition of earplugs has an added effect as participants were following the same instructions in both conditions.

3.2.5 Conclusion
In conclusion the addition of earplugs to our original SER protocol did not significantly increase measures of self-reported satiety though a positive effect was observed for the SER-in group only. Further work should investigate if the slow eating rate protocol exerts its satiating effects through objective markers of satiety (i.e. appetite hormones) and whether the SER protocol with earplugs could be used as an effective weight loss tool in the community. We acknowledge that these results cannot be directly generalised to the free-living setting but are the first step in establishing proof of concept and a robust methodology for testing in other scenarios.
Chapter 4

Development of monitoring tools and protocol enhancement, in preparation for a free-living eating rate study.
4.1 Introduction
Healthcare represents one of the most important economic and social challenges that every country faces: today researchers, healthcare administrators and clinicians as well as other field practitioners are dealing with an increased pressure generated by the growing expectations from both the private and public sector. The continuously growing and ageing population affects health care demands and highlights the need for new and more advanced scientific solutions (Chiuchisan et al., 2014, Omanović-Miklićanin et al., 2015). Alongside this the growing popularity and capability of the e-health (healthcare practise using the internet, Watson 2004) sector has also attracted the public interest in recent years (Germanakos et al., 2005). Advances in new technology have had some negative effects on health, such as weight gain and reduced exercise due to a more sedentary home and market production and lower and easier accessible food prices (agricultural revolution) (Lakdawalla and Philipson, 2009). However new technology has also the potential to improve health (Thomas and Bond, 2014). This includes the unprecedented spread of mobile and wireless technologies among the poorest populations, indicating the growth of this technology and its potential applications (Aceto et al., 2018). Technological advancements, alongside the rising quality and availability of medical software applications (often coming in the shape of mobile applications) have driven the rapid integration of mobile devices into clinical practise (Ventola 2014). Jacobs et al (2017) in the first stand-alone study examining the effects of a popular weight loss application (Noom) on weight loss, concluded that smartphone apps offer effective low-cost, user-friendly ways for people to learn about and track their behaviour change and progress. Self-monitoring has been shown to be the cornerstone of behavioural weight loss, so it follows that adherence to self-monitoring should lead to a more successful treatment intervention.
4.2 Development study C: Transforming the SER protocol into an online tool for weight management.

Puente and Martinez-Marcos (2018) in their recent review of the effectiveness of interventions in overweight and obese adults, concluded that online interventions may promote integration of advice, with better access to information and lower cost compared to traditional approaches (see chapter 1 for further details on online weight loss interventions) and that intensive (defined as regular, brief individualised advice) online programs could achieve clinically significant weight losses (i.e. 5%). Harvey-Berino (2010), randomised healthy overweight adults to either attend weekly meetings in person, online or in hybrid format (combined online and face-to-face). After 26 weeks, reduction in weight was; -8.0kg for those receiving face to face support, -5.5kg for online and -6.0 for the hybrid group. The above findings were confirmed in two systematic reviews of web-based interventions for the management of overweight and obese adults by Neve et al (2010) and Armen and Irwin et al (2011) which conclude that online weight loss interventions are effective in achieving weight loss, are of low cost and are also user friendly. To date, there has only been one weight loss intervention in a free-living overweight adolescent population specifically targeting eating rate, the Mandometer method (see Chapter 1, section 1.4), and none for adults. As Hermsen et al (2016), noted in their recent study utilising the automatic vibration feedback fork to reduce eating rate, training people to eat more slowly in everyday contexts requires creative and engaging solutions. Given the effectiveness for weight loss reduction shown by online tools and the promising outcomes for weight loss and perceived satiety (see chapter 1, section 1.2) when reducing eating rate, we aimed to combine these two approaches and to develop our existing SER protocol into a user-friendly online weight loss tool.
4.2.1 Aims

- Transform the existing SER protocol into an online-friendly video.
- Incorporate the SER video into an online weight loss tool with the potential for roll out in a free-living population

4.2.2 Objectives:

- Convert the paper version of the previously developed SER protocol into a user-friendly 2-minute video
- Develop a website and mobile application in order to improve accessibility of the SER protocol to the free-living population
- Investigate non-invasive ways by which SER protocol concordance can be monitored in the community via the website and mobile application

4.2.3 Methods

Following the development and testing of the slow eating rate protocol (SER) (Appendix 10) in a clinical setting (See chapter 3), we proceeded with researching mechanisms by which this protocol could be easier adopted, supported and monitored in the community.

4.2.3.1 SER Protocol Enhancement and Psychology Input

Following discussion with behavioural psychology experts, the SER protocol was converted in an audio-visual tool. Both our expert consultants and recent studies have concluded that receiving explanation via video is more powerful than receiving written instructions (Hoogerheide et al., 2014 and 2016, Shoufan 2018). The instructions of the SER protocol were therefore converted into a 2-minute video, with an actor re-enacting the procedure and infographics highlighting the guidelines (i.e. number of chews). The full-length video is available here: [https://www.youtube.com/watch?v=9_bQGZ85pJY&t=3s](https://www.youtube.com/watch?v=9_bQGZ85pJY&t=3s) and extracts of the video can be seen in Figure 4.2.1. As eating behaviour and eating rate specifically are strongly linked with mindfulness and mindful eating (see Chapter 1, section 1.5 Eating rate and mindful eating) input was sought from the Department of Psychology (University of Surrey) in order to add a mindfulness measure to our SER protocol. The psychology expert group suggested adding a measure of mindful eating, specifically a questionnaire (under development at the time) by Laura Winkens from the Department of Health Sciences in Vrije University, Amsterdam. For further information on the
Mindful Eating Questionnaire please see section 4.4.1.1. Furthermore, given the success rate of online weight loss tools (Chapter 1, section 1.2) and the popularity of mobile applications for weight management, a dedicated website and iOS/Android application were created.

4.2.3.2 Website and Application Creation for SER Protocol Monitoring

A dedicated website (www.meetstudy.co.uk, see Figure 4.2.1) as well as a mobile application for iOS and Android (http://play.google.com/apps/details?id=meetstudy.meetstudy) were created in WordPress (www.wordpress.com). The video-version of the SER protocol was integrated (both website and application) and a Hotspots plug-in (Hotspots Analytics for WordPress) as well as Mixpanel (®Mixpanel) were added in order to allow user activity monitoring and event tracking.

Figure 4.2.1. Dedicated Website landing page for the community intervention study.
4.2.3.2.1 Participant user pathway

The addition of the HotSpots plug in allowed for monitoring of user concordance with the SER protocol in the following way:

a) The participant was given a unique password and username to access the “participant log in” part of the app/website
b) The application was downloaded and installed on the participant’s mobile phone or tablet
c) The participant was asked to open the app/access the website immediately before consuming a meal
d) The participant was asked to “click” on the SER protocol video and to watch this again which logs the start of their meal (length of video subtracted from their total time) allowing for calculation of meal duration.
e) Once the video is finished the participant was asked to put their device (laptop/mobile) aside and eat according to the SER protocol
f) Once the meal was finished the participant was asked to re-open the app/website and press the “submit” button which logs the end of their meal allowing for calculation of meal duration.
Figure 4.2.2: Participant user pathway including the animated SER protocol, from 2.2.1 step a to f.

The above participant user pathway allowed data on the following variables to be captured:

- number of times the app/website was accessed by the particular user.
- number of times the SER protocol video was played.
- duration (minutes and seconds) of website/app ‘visits’.
- meal duration defined as: time period between the end of the video (step d) and the end of the meal (step f).
4.2.3.3 Participant user pathway – pilot test

The participant user pathway, including the SER protocol video, was piloted with volunteers from the Biosciences department, during their lunch time for a week. In total 5 people volunteered and at the end of the week were asked to give feedback on:

a) Ease of access to the website/application

b) Usefulness of the SER protocol video in explaining and encouraging adherence to the guidelines

c) Intrusiveness of following the SER protocol for a week and impact on daily routine

4.2.4 Results

All five volunteers reported that they could access the website and application with ease, that the video SER protocol was useful in reminding them of the SER guidelines and caused no significant interruption to their daily routine or natural meal flow. Three volunteers also feedback that a reminder to log in to the website/application would be beneficial if the trial was to run for longer than a week.

In the website/application backend, all data outlined in the participant user pathway section 4.2.3.2.1 above, were successfully gathered with no data loss or software malfunctions.
4.2.5 Discussion

The aim of this development study was to transform the existing SER protocol into an online-friendly video. The use of an instructional video stems back to Schön (1987) where “novices learn from observing how experts or non-experts behave in complex real situations in order to elicit reflection on practise”. A video lacking specific instructions and tasks to perform may elicit an illusion of understanding and shallow processing (Lowe 2004) which is why the video we created had specific instructions and processes which the participants in this study could comfortably mimic.

In a recent commentary on “why and when does instructional video facilitate learning” Bétrancourt and Benetos (2018) concluded that despite the omni-presence of instructional videos (especially in the last decade) the diversity of purposes, formats and contexts makes it difficult for research to accumulate findings. Consequently, although all volunteers in this study reported following the SER protocol instructions with ease, the researchers cannot identify particular video-design factors that have contributed to this. A second aim of this study was to incorporate the SER video into an online weight loss tool with the potential for rollout in a free-living population. The website and mobile application built for this study showed no functionality issues and successfully gathered all data outlined in the participant user pathway section 4.2.3.2.1. Furthermore, the website and application were structurally [simple website landing page with subsections including information about the study, researchers and volunteer resources (questionnaires, information sheets) and a unique log in access panel for the participants] based on a previous randomised controlled social and mobile weight loss trial by Patrick et al (2014) which successfully engaged users in the community.

4.2.6 Conclusion

In conclusion, the SER protocol was successfully transformed into an online-friendly video which was then incorporated into an online (website and application) weight loss tool capable of being rolled out in the community.
4.3 Development study D: Automating ER data extraction and analysis

As reviewed in chapter 1, monitoring and analysis of ER data has occurred to date either: a) manually (manual counts made by an observer), which requires a significant amount of time spent on analysis by researchers; b) machine-automatically via the use of machinery like the UEM and the Mandometer, which have been characterised as cumbersome and disruptive to the natural feeding rhythm, or c) completely automatically like AIM (Automatic Ingestion Monitor) or the Augmented fork with vibrotactile feedback, which are yet to be validated for broader population use. Therefore, in order to automate ER data analysis and extraction from video recordings (the methodology used in the previous study, Chapter 3, and proven to work efficiently to date) in the least invasive way, a cross-departmental collaboration was established with an MSc student in the Department of Electronic Engineering, robotics division (Faculty of Engineering and Physical Sciences, University of Surrey) to contribute the necessary technical expertise for the automated analysis of the video-recordings for eating rate parameters.

4.3.1 Healthcare monitoring applications

As stated by Goldstone (2010) life expectancy rates will increase dramatically by 2050, and approximately 30% of Europeans, Canadians, Chinese, and Americans will be over the age of 60 years. This will lead to higher demands for medical personnel which may be impossible to supply in the near-future. Hence, researchers are trying to enhance the existing healthcare monitoring approaches that would handle urgent medical situations and shorten the hospital stay and regular medical visits of a patient.

In simple terms, healthcare monitoring systems are designed based on the combination of one or more AR components such as fall detection, human tracking, security alarm and cognitive assistance components. Most of the healthcare systems use body-worn and contextual sensors that are placed on users’ bodies and in their environment. Once help is needed, the system notifies the relevant parties (i.e. medical personnel) about the situation to assist the patient quickly. For example the E-safe fall detection and notification system (Gannapathy et al., 2013) has used the zigbee-based wearable sensor system to automatically detect fall situations and notify the in-house correspondents via zigbee technology. Then the external correspondents are also notified via Short Message Service (SMS) and email.

The smart assisted living (SAIL) system was introduced by Zhu et al (2011) using human–robot interaction (HRI) to monitor the health condition of an elderly or disabled individual. SAIL consists
of a body sensor network, a companion robot, a smartphone, and a remote health provider. Based on the sensor data, the robot assists the human or the help is provided by a remote health provider contacted through a smartphone gateway.

CAALYX (http://www.ij-healthgeographics.com/content/6/1/9), a European Union (EU)-funded healthcare project, aims at assisting elderly people using a wearable device that is capable of measuring vital signs and fall detection events and of notifying care providers automatically in an emergency situation. Most importantly, the CAALYX is able to report the current medical status of the patient together with his or her current location that helps the emergency team to provide immediate assistance (Ranasighe et al., 2016). Computer vision and specifically, facial action recognition is the construct of HAR deals with automated analysis of ER videos which will be the focus of the study in the section below.

4.3.1.1 Automated Eating Rate Identification via Visual Analysis of Facial Behaviour

Although there is a large body of work on facial action recognition in the field of computer vision, very few techniques focus on chewing recognition, with most published papers focusing on wearable sensors i.e Dong et al (2012), in a study focusing on recognition of gestures via the use of wearable sensors, developed a watch-like device that contained a miniature gyroscope, measuring intake via an automatic tracking of wrist motions during hand-to-mouth movements (bites) (for more examples see section 1.4, Chapter 1) (Berdi et al., 2015, Hermans et al., 2016). In a collaborative effort with the robotics department (University of Surrey), an MSc project (undertaken by Mr Cheng Zhao, CZ) was undertaken utilising video data from our previous study (Chapter 3) to develop an automated ER video detection system. The author (FK) provided videos and raw data from Chapter 3 (both studies A and B) in the form of number of chews observed. These were then measured via a semi-automated purpose-designed software within the robotics department (led by CZ), which allowed video manipulation (slow motion for easier chew/swallow detection) and counted a chewing action every time the “space button” was pressed. Initially data on total chews/video and total swallows per video for all videos captured during study 1 (Chapter 3) were extracted by FK and inputted to the semi-automatic system testing. However, swallowing detection proved particularly challenging and therefore the remainder of the study focused only on automating the chewing detection process.
4.3.1.2 Human Activity Recognition (HAR)

Human activity recognition (HAR) is a highly dynamic and challenging research topic. It aims at determining the activities of a person or a group of persons based on sensor and/or video observation data, as well as on knowledge about the context within which the observed activities take place. In the ideal case, an activity is recognized regardless of the environment it is performed in or the performing person (Ranasighe et al., 2016).

In general, the HAR process involves several steps—from collecting information on human behavior out of raw sensor data to the final conclusion about the currently performed activity. These steps are as follows: (1) pre-processing of the raw data from sensor streams for handling incompleteness, eliminating noise and redundancy, and performing data aggregation and normalization; (2) segmentation—identifying the most significant data segments; (3) feature extraction—extracting the main characteristics of features (e.g. temporal and spatial information) from the segmented data using, for example, statistical moments; (4) dimensionality reduction—decreasing the number of features to increase their quality and reduce the computational effort needed for the classification; and (5) classification, the core machine learning and reasoning—determining the given activity (Krishnan et al., 2014).

The main goals of HAR systems are to observe and analyse human activities and to interpret ongoing events successfully. Using visual and non-visual sensory data, HAR systems retrieve and process contextual (environmental, spatial, temporal, etc.) data to understand the human behavior. There are several application domains where HAR concepts are investigated and the systems are developed. These can be divided roughly into four categories: active and assisted living (AAL) systems for smart homes, healthcare monitoring applications, monitoring and surveillance systems for indoor and outdoor activities, and tele-immersion (TI) applications (integration of audio and video conferencing and virtual reality to ultimately reproduce a “real face-to-face meeting in every detail).

Traditionally, the task of observing and analysing human activities was carried out by human operators, for example, in security and surveillance processes or the processes of monitoring a patients’ health condition. With the increasing number of camera views and technical monitoring devices, however, this task becomes not only more challenging for the operators but also increasingly cost-intensive, in particular, since it requests around-the-clock operation. In practice, for the case of home care, personnel deployment for such tasks often cannot be financially feasible. Moreover, HAR systems within these fields are able to support or even replace human operators in order to enhance the efficiency and effectiveness of the observation and analysis process. As an
example, with the help of sensory devices, an HAR system can keep track of the health condition of a patient and notify the health personnel in case of an urgent need.

On the other hand, scientific and technical progress continuously improves the living conditions of humans. This causes a dramatic societal change as it comes with decreasing birth rates and increasing life expectancy, which together turn the age pyramid upside down (Michael et al., 2012). Intensive research and development in the field of Active and Assisted Living (AAL) (Menschner et al., 2012) focuses on mastering one of the consequences of this change: the increasing need of care and support for older people. The goal of AAL systems, therefore, is to provide appropriate unobtrusive technical support enabling people to live as independently as possible for as long as possible in their homes. To be able to provide such support, an AAL system needs to know about a person’s behavior; that is, it depends on powerful HAR systems for obtaining, collecting, compiling, and analysing such knowledge. Similarly, TI systems also make use of HAR systems to track and simulate human behaviors in a virtual environment in order to build attractive game interfaces or to enhance the existing communication methods.

Despite the technological advancements mentioned above, detection of ER is still done through cumbersome UEM-style monitors (section 1.4 Chapter 1), through highly specialised and still under development wearable sensing devices (see section 1.4, Chapter 1) or through counts by observer which is time consuming and requires the use of research staff. Consequently, there is a clear need for an automatic detection of ER through easy to use and affordable technology that is not cumbersome or invasive.
4.3.2 Aim
To automatically compute the chewing times and chewing rates in eating video clips.

4.3.3 Objectives
The mastication processes are segmented into two categories of closing and opening the mouth, annotated as ‘chewing’ and ‘non-chewing’ respectively in this project. For this reason, the key challenge addressed by this project was to automatically distinguish between chewing and non-chewing frames in eating videos. Specifically, the problem was transformed into an image classification task with the following seven objectives:

- To automatically compute the chewing times and chewing rates in eating video clips.
- To acquire and analyse sample data from existing videos.
- To apply facial landmark technique and extract point tracks to new videos.
- Based on annotations from Objective 1, to train a machine learning system [support vector system (SVM) decision forest or similar].
- To train a convolutional neural network CNN to recognise the chewing actions from video frames (instead of from the point tracks) in either DIGITS or MATLAB.
- To design a chewing rate estimation system.
- To apply the automated method developed to measure the eating rate in chews/minute via the data gathered from study E, section 4.4 (n = 60).
4.3.4 Methods

To address objective 1 existing eating rate videos were annotated manually via a semi-automated purpose-designed software within the robotics department. Eating episodes from different people were recorded in 5-minute videos by the author. Some examples of chewing annotations were recorded to indicate which frames were considered as chewing in eating videos. Mastication is a continuous process while annotations can only highlight some instantaneous frames, chewing frames were determined by randomly selecting a video frame in each mouth closure process. Moreover, some confused annotations were repaired through checking all eating videos. Based on video analyses, the average time of chewing cycles ranged from 11 frames to 20 frames. The frame rate of these videos was 25 frames per second.

To address objective 2, a facial landmark technique (CLandmark program) was applied by CZ to detect and track facial key points on video frames which were then used to perform image descriptors and then applied to the image classification. CLandMark, is a program where the position information of human faces are extracted frame by frame from training videos. Every key point is located in the coordinate with origin of the top left pixel. The total facial parts are denoted by 68 key points, involving cheeks, chin, eyebrows, eyes nose and lips (Uřičář et al., 2015). Position information of human faces were extracted frame by frame from the ER videos. Every key point was located in the coordinate with origin of the top left pixel. Key point trackers were applied on both SVM and CNN classification. Following this, two classification algorithms, including SVM and CNN, were trained independently by CZ to address objectives 3 and 4.

Specifically, for objective 3 the SVM classification was conducted using Classification Learner in MATLAB. Based on repaired annotations, the facial landmarks on both chewing and non-chewing frames were extracted to build a set of vectors for SVM training. The aim was to check the separability of position information extracted from each video frame in objective 2. As a result, the best accuracy of the SVM classifier was at around 62.4% of mastication recognition, which was deemed unsatisfactory given that other similar automatic detection systems achieve 80% accuracy or higher (Chapter 1). A significant prediction based on this result was that the key point features were separable and deep learning with CNN would improve the accuracy. Therefore, as a next step, CNN classifiers were trained based on three types of input data. DIGITS is a powerful application for deep learning research which was employed to train CNN classifiers. Three types of training data involved: original images, key point maps and optical flow maps. Each frame of the testing video was classified by the pretrained CNN model. The closing and non-closing processes of mouths were packed as numbers of chewing and non-chewing blocks containing several video
frames. The chewing time was computed by counting the chewing blocks and the chewing rate was obtained by dividing by the mastication durations.

To address objective 4, deep learning systems based on three types of input data were trained combining with the annotations obtained from objective 1. The facial landmarks from objective 2 were also applied to CNN classification for one type of input data. The final objective was to design and establish a chewing rate estimation system which could return corresponding chewing rates for the inputs of eating videos.

Finally, to address objective 6, the automated chewing count system developed in the previous 5 objectives was pilot-tested on video-data collected from participants in study E (section 4.4). Manual ER was also calculated by counting the chews/minute manually (video playback) in the same methodology as in Chapter 3 and then used to compare to automatically estimated eating rates via paired t-tests. Also, a Bland-Altman plot, the gold standard of comparing two measurement techniques (Bland and Altman, 1985), was derived to compare the two ER techniques (automatic and manual) via Microsoft Excel.

### 4.3.5 Statistical analysis

Statistical analysis was performed on data gathered in Study E, section 4.4.4 below.

Normality tests were performed for the two ER data sets, as well as for age, BMI, body-fat percentage and visceral fat level. The mean, and standard deviation (SD), or median and 25% and 75% percentiles were calculated for normally and non-normally distributed datasets respectively. The accepted level of significance was $p \leq 0.05$. All data were then entered into Microsoft Excel to be split into subgroups (see Table 4.3.1 below) and then collated in a Statistical Package for the Social Sciences (SPSS) 24 spreadsheet (IBM Corp, 2016) for further analysis.

Mean values of automatic ER and manual ER were compared between these demographic and anthropometric subgroups to deduce population norms. Statistical tests used were Independent t-tests and one-way ANOVA for normally distributed data, or Mann Whitney U tests and Kruskal Wallis tests for non-normally distributed data.

Correlation analysis was performed to measure associations between independent variables (age, BMI, body-fat percentage, manual ER in chews per minute, and ER in grams per minute) and the primary outcome, automatic ER. Lastly, multiple regression was performed to investigate the factors predicting the dependent variables. For all data, the accepted level of significance was ($p \leq 0.05$).
4.3.6 Results

4.3.6.1 Automated eating rate detection

By end of the project the collaboration between FK & CZ had achieved all proposed objectives. The final output of this project was the establishment of a chewing rate estimation system. The highest accuracy of the chewing detection system was 80±1.5%, based on the inputs of optical flow maps which were finally applied on the chewing rate estimation system. The final step to calculate the chewing rate is depicted in the equation below, where the number of chewing blocks is divided by mastication duration with a 25 frame rate:

\[
Chewing\ Rate = \frac{Number\ of\ Chewing\ Blocks}{(\text{The end frame} - \text{The first frame} + 1)/25}
\]

As an example, a twelve-minute video containing 537 chewing annotations is tested in the chewing calculation system. The calculation returns 599 estimated chewing annotations, parts of which are shown in Figure 4.3.1:

![Figure 4.3.1: Example of the association between real (first row) chewing annotations and their estimated (second row) chewing annotations © Zhu et al., 2017.](image)

The first row depicts real chewing annotations recorded by human effort. The second row indicates the estimated chewing annotations resulting from automatic chewing rate calculations. Some of the real chews are missing in estimated annotations such as the first and the fifth real annotations. Some redundancies occur at the fourth, the eleventh and the fourteenth estimated annotations. In general, the offset of estimated annotations is around 3 frames which is considered accurate (Zhu et al., 2018).
4.3.6.2 Manual and automatic ER correlations

Table 4.3.1: Automatic ER (chews/minute) and manual ER (chews/minute) from study E (n=60).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (±SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean automatic ER (chews/minute) n =60</td>
<td>57.4 (±13.8)</td>
<td></td>
</tr>
<tr>
<td>Mean manual ER (chews/minute) n =60</td>
<td>53.4 (±15.6)</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Gender n = 60</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female n = 37</td>
<td>58.7 (±14.4)</td>
<td></td>
</tr>
<tr>
<td>Male n=23</td>
<td>55.2 (±12.8)</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-34 n=56</td>
<td>57.6 (±14.0)</td>
<td></td>
</tr>
<tr>
<td>35-64 n=4</td>
<td>53.9 (±11.5)</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24.9 n=42</td>
<td>55.7 (±14.0)</td>
<td></td>
</tr>
<tr>
<td>25-29.9 n=16</td>
<td>61.2 (±13.4)</td>
<td></td>
</tr>
<tr>
<td>&gt;30 n=2</td>
<td>61.8 (±8.49)</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Fat% in females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-31% n=35</td>
<td>57.2 (±13.3)</td>
<td></td>
</tr>
<tr>
<td>&gt;32% n=2</td>
<td>55.8 (±0.849)</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>Fat% in males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-22% n=10</td>
<td>66.3 (±14.4)</td>
<td></td>
</tr>
<tr>
<td>&gt;23% n=13</td>
<td>51.3 (±13.2)</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Mean ER values for Gender, Age and Fat% were compared using t-test and BMI using one-way ANOVA.
Table 4.3.2: Correlation coefficients and their significance values for automatic eating rate (chews/minute), and manual ER (chews/minute) against age (years), BMI (kg/m\(^2\)), body-fat content (%), (n=60).

<table>
<thead>
<tr>
<th></th>
<th>Primary Outcome:</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>(r)</td>
<td>(p)</td>
</tr>
<tr>
<td>Automatic eating rate (chews/ min)</td>
<td>Manual ER (chews/ min)</td>
<td>0.549</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Secondary Outcomes:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>(r)</td>
<td>0.28</td>
<td>0.75</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>(r)</td>
<td>0.131</td>
<td>0.29</td>
</tr>
<tr>
<td>Body-fat %</td>
<td>(r)</td>
<td>0.311</td>
<td>0.09</td>
</tr>
</tbody>
</table>

BMI, Body Mass Index; \(r\), Pearson correlation value; \(p\), significance value; Auto, automatic; min, minute; ER, Eating Rate.
4.3.6.3 Assessment of automatic eating rate

Mean automatic ER was higher than mean manual ER, see Table 4.3.1. Automatic ER was positively, strongly ($r \geq 0.500$), and significantly ($p<0.01$) correlated to manual ER, see Table 4.3.2. However, the paired t-test performed revealed a significant difference between the two ($t=2.217$, $p=0.03$). A Bland-Altman plot, see Figure 4.3.2 below, deduced a lower limit of agreement (LOA) of -23.0 chews/minute (% error -0.23%), an upper LOA of 30.9 chews/minute (%error 0.58%), and an average bias of 3.93 chews/minute ($\pm 13.7$). Nonetheless, there was no apparent consistency in the variability across the graph.

Figure 4.3.2: Bland-Altman plot between automatic and manual eating rate (chews/minute) (Bland and Altman, 1985) SD, standard deviation.
4.3.7 Discussion
This developmental study aimed to develop a tool for automatically computing the chewing times and chewing rates in eating video clips. This was successful with automatic computation of chewing through ER videos, with an average of 80% accuracy.
A total of 9 hours’ worth of unannotated chewing videos were processed in this study. The robotics department deemed the automatic system acceptable although noted that further refinements were possible and necessary. System errors that could be targeted are mainly derived from the wrong classification which results from imperfections of the classification model. Mouth closures are sometimes confused with other human actions such as drinking and rising heads which can result in erroneously counted chews. On the other hand, pre-set thresholds of chewing might filter out some chewing blocks and result in a reduced number of chews detected.
Chewing sequences were obtained by applying the CNN classifier on the video frames. A number of classification errors occurred in the chewing sequence due to the following three reasons: Firstly, the training data of CNN classifier was only extracted from mastication processes. The errors may occur in the range of before or after the eating actions. In other words, we only focused on classifying the processes of closing and the opening during mastication rather than distinguishing mastication from numerous other human actions. Such errors cannot be totally avoided unless an improved classification model is trained. In this project, the range of mastication was artificially partitioned by denoting the first and the final frames of chewing. Secondly, some errors also present within the mastication processes because of uncertainties of the classifier. Such errors are always accompanied by low confidence scores so that they can be improved by setting a higher threshold.
So far, the chewing rate estimation system has the ability to calculate chewing rates from new eating videos with various errors. Based on previous evaluations, the system could be further optimised through the following steps: Firstly, the size of training data should be expanded as more ER annotated videos will result in better training of the automated system. This step can be achieved through the collaboration of the two departments in future ER studies. Secondly, the outside range of mastication should be considered as well, which can be achieved by adding an extra category or designing a new CNN classifier. Thirdly, the facial detection and tracking algorithm could be optimised for more accurate facial patches. Finally, more new eating videos with annotations should be tested and evaluated to prove that the estimation systems of chewing and mouthful rate can be applied in generic videos and not pre-annotated ones.
4.3.6.1 Assessment of automatic ER
Despite the system being deemed adequately accurate, and the strong positive correlations achieved between automatic and manual chews counts a statistical, significant difference between the means of the techniques was still identified. Despite this the Bland-Altman plot suggested that there was a small average bias, and small percentage errors, indicating that differences between the techniques are likely small. The clinical significance of these differences is unknown, as there are no set cut-off points for ER characterisation. It has been shown that there is a linear relationship between the size of experimental manipulation to eating rate (i.e. how much eating rate has been reduced by) and energy intake in an ER literature review by Robinson et al (2014) though, exact ER cut off points for slow/fast eating rate are not established to date. According to the literature review (see chapter 1) it seems that the significance of the difference in ER is dependent on a) the parameters by which ER is measured (chews/minute, grams/minute, mouthfuls, oral processing time etc.) and the outcomes of each study (energy intake, satiety, mindfulness, body composition change etc.). Furthermore, body-fat content was the strongest predictor of ER in the multiple regression analysis, and it was positively, but weakly associated to ER, thus being consistent with other studies (Robinson et al., 2014, Llewellyn et al., 2008).

4.3.7 Conclusion
The system developed was based on two classification approaches; support vector machine (SVM) and convolutional neural network (CNN). A SVM classifier was trained based on facial landmark vectors which achieved an accuracy of 62.4% for automated chewing detection. As an improvement, CNN classifiers were trained using three types of feature maps, containing original images, key point maps and optical flow maps. The end software used was AlexNet, which by the end of the project was successfully identifying chewing rate with an 80% accuracy. Furthermore, AlexNet chew counts correlated well with manual chew counts by an observer and given its high accuracy of chewing rate identification, it will be built-into the chewing rate estimation system for the free-living intervention study (see Chapter 5). The factors affecting automatic ER in this study warrant further investigation in a more diverse population as they seem to contradict current literature.
4.4 Development study E: Mindful Eating Questionnaire

4.4.1 Introduction-Mindful eating and ER
As discussed in chapter 1, behavioural therapists have been suggesting slower eating as a method of combating obesity since the 1970’s. The practise of mindful eating is considered an emerging weight management approach (Kristeller and Wolever 2010, Lofgren 2015, Clementi et al., 2017) that is based on awareness of emotional and physical sensations associated with eating, and individuals adopting it might better recognise internal cues of satiety and hunger (Framson et al., 2009). By adopting these mindfulness skills someone can potentially reduce their ER (Kristeller and Wolever, 2011), lower their energy intake (Kristeller et al., 2006), and thus maintain or ideally lose weight (Dalen et al., 2010; Smith 2006).

However mindful eating lacks a precise definition (Warren et al., 2017), although it is conceptualised as being aware of the present moment when one is eating (Hendrickson and Rasmussen 2013) and paying close attention to the effect of the food on the senses and noting the physical and emotional sensations in response to eating (Kristeller et al., 2014). In a recent literature review by Warren et al (2017) on the effects of mindful eating on behaviour change, the role of mindfulness in weight management was less apparent than had previously been postulated (Katterman et al., 2014) although this may have been due to methodological issues, specifically the high level of heterogeneity between studies (Godsey 2013). Indeed, Olson et al (2015), in their systematic review of mindfulness and weight loss, highlighted several methodological weaknesses amongst the studies conducted to date which hinder a strong correlation between mindful eating and weight loss and make it difficult to draw any definitive conclusions. Non-dieting interventions that encourage eating as a response to internal hunger and satiety cues have shown improved eating patterns (Clifford et al., 2015). Effective and easy-to adopt tools and methods manipulating ER, as a means to cultivate mindful eating, and conversely inhibit impulsive eating (Teper et al., 2013), therefore could be developed, tested and brought to everyday life as another option in the weight management ‘toolbox’.
4.4.1.1 Assessment of mindful eating

Despite the various tools developed to allow self-report of mindfulness (Park et al., 2013, Sauer et al., 2013) it is often argued in the literature (Clementi et al., 2017, Warren et al., 2017) that a measure developed in a specific domain may be more appropriate as mindfulness is a learned skill (Bishop et al., 2011). At the time of the study there were two specific mindful eating scales available, the Mindful Eating Questionnaire (MEQ) developed by Framson et al (2009) and the Mindful Eating Scales (MES) developed by Hulbert-Williams et al., (2014). On the advice of our psychology advisors (see section 4.2.1) it was suggested that MEQ and MES were not appropriate tools to measure eating rate (ER) as they don’t distinguish mindful eating from emotional, external and restrained eating. The expert group facilitated a collaboration with the Department of Health Sciences in Vrije University (Department of Health Sciences, Faculty of Science, Vrije Universiteit Amsterdam, Amsterdam Public Health Research Institute, Amsterdam, The Netherlands) where a mindful eating questionnaire more appropriate for measuring eating rate was currently under development by L Winkens.

The Mindful Eating Questionnaire Under Development (MEQ-UD) (Winkens et al., 2018), was a questionnaire derived from a combination of existing tools (Hulbert-Williams et al., 2014; Van Diest, 2013; Framson et al., 2009) (see Chapter 2, section 2.6 for more details).

Another questionnaire important for ER studies, is the Dutch Eating Behaviour Questionnaire (DEBQ) (Van Strien et al., 1986) (see Chapter 2, section 2.6 for more details. The questionnaire’s answers were again composed of a rating scale of frequency, from ‘never’ to ‘very often’. Automatic summary scoring of those, from 0% to 100%; with higher percentages meaning more mindful, was performed in this study via Typeform data collection pool (Typeform, 2018).

In this study, we therefore aimed to investigate whether the Mindful Eating Questionnaire Under Development (MEQ-UD) by Winkens was a good marker of ER in a cross-sectional sample of adults.
4.4.2 Aims
- Investigate if the Mindful Eating Questionnaire Under Development (MEQ-UD) is a good marker of eating rate in healthy adults.
- Assess statistically the potential mediators of eating rate and mindfulness and the factors that mediate the relationship between the two.

4.4.3 Objectives
- Recruit a sample of free-living adults
- Investigate the role of age, gender and Body Mass Index (BMI) as mediators of mindfulness

4.4.4 Methods
Prior to the study the Faculty of Health and Medical Sciences Ethics Committee at the University of Surrey (UEC/2016/020/FHMS) (Appendix 11) gave a favourable opinion for the study. All participants signed a consent form prior to participating in the study. The study was carried out in the Clinical Investigation Unit (CIU) at the University of Surrey, Guildford, United Kingdom.

4.4.4.1 Study population
A power calculation was not performed for this study, as there were no normative data available for the Mindful Eating Questionnaire being tested. However, a target of 60 was deemed feasible based on financial and time constraints. Participants were healthy, female and male volunteers who lived in the Surrey area. They were invited to participate through posters distributed around the University of Surrey campus, and Guildford town, via a study-specific Facebook account and via local community Facebook pages. The recruitment criteria were intentionally broad to enable recruitment of a representative sample of adults, for further details please see Table 4.4.11. Volunteers were screened against the eligibility criteria using an online questionnaire prior to the study session. Participants were sent a participant information sheet (Appendix 12), in which they were asked to refrain from alcohol, caffeine and heavy exercise 24 hours before their study session, as well as to fast 4 hours prior to it.
Table 4.4.1: Study inclusion/exclusion criteria:

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Mass Index &gt; 15.9kg/m²</td>
<td>BMI ≤ 15.9kg/m²</td>
</tr>
<tr>
<td>Age: 18 and 70 years old</td>
<td>Allergy or intolerance to any of the test meal ingredients vegetarian stuffed pasta</td>
</tr>
<tr>
<td>Willing to fill out health and demographic questionnaires</td>
<td>Currently following any form of weight reduction diet</td>
</tr>
<tr>
<td>Full set of dentures and able to eat normally</td>
<td>Present psychiatric disorders, Epilepsy, Sleep disorders,</td>
</tr>
<tr>
<td>Willingness to eat a free meal provided while being video recorded</td>
<td>Drug, Alcohol, dependence, Taking experimental or prescribed drugs</td>
</tr>
<tr>
<td>Able to give verbal and written consent</td>
<td>Endocrine disorders, Diabetes, Cardiovascular disease, Hypertension, Kidney disease, Liver disease, Gastrointestinal disease</td>
</tr>
</tbody>
</table>

4.4.4.2 Data collection

4.4.4.2.1 Anthropometrics:

Anthropometric measurements were taken following standard protocols. Standing height was measured using a wall mounted stadiometer (to the nearest 0.1cm) with participants’ shoes removed. BMI, total body-fat percentage and visceral fat level were measured using a stand-on electrical bioimpedance machine (TANITA Corporation, Japan) with participants wearing light clothing.

4.4.4.2.2 Satiety measurements:

Prior to eating participants were asked to drink 250ml of water to minimise confounding of feeling full prior to eating (Andrade et al., 2012). Then, a short questionnaire was completed, in the form of a compact wrist-worn electronic diary; called Patient Reported Outcome (PRO-diary, CamNtech Ltd, 2013). This tool assessed self-reported hunger and appetite by asking three questions; «How hungry do you feel?», «How full do you feel?», and «How much do you think you can eat?». The electronic diary included a semantic differential scale with end-points labelled ‘not at all’ to ‘extremely’. Automatic scoring of the answers from 0.0 to 10.0 was performed via CamNtech Ltd (2013) with higher scores indicating a higher level of hunger, a higher level of fullness and a higher perception of how much can be eaten.
4.4.4.2.3 Eating Rate Measurements:

Participants were instructed to eat a test meal at their own pace until comfortably full. The standardised portion was 20 pieces, approximately 248 grams, of Italian Spinach and Ricotta Tortellini, whose bite size allowed for easier monitoring of the eating topography. The meal was served at lunch time, with two tablespoons of tomato and basil sauce, to aid lubrication. The nutritional content of the meal (495 kcal, 17 grams of fat, 65 grams of carbohydrates, and 17 grams of protein) provided volunteers approximately a fifth of their daily energy requirements, estimated by the Henry equation (Todorovic and Micklewright, 2011). Pre and post-consumption food weight was measured by a kitchen scale (to the nearest 1 gram). Meals were video-recorded from the front view, and participants ate in isolation, with closed curtains, and with no access to their mobile phone or books, to avoid any distractions. Eating rate, in chews per minute, was then calculated automatically via the facial recognition software AlexNet (see section 4.3). Participants were categorised as slow or fast eaters, according to their distance from the median of eating rate.

4.4.4.2.4 Questionnaires:

Subjects completed two online questionnaires on mindfulness in eating (MEQUD and DEBQ), after the completion of their meal, to ensure that the questions would not bias their eating behaviour, and thus their ER outcome (Zandian et al., 2008).

4.4.4.3 Statistical analysis

The mean, and standard deviation (SD), or median and 25% and 75% percentiles were calculated for normally and non-normally distributed data sets respectively. Normality and frequency tests were performed for age, BMI, body-fat percentage and visceral fat level, as well as for their subgroups. Also, normality tests were performed for the three appetite scores, for eating rate, as well as for the summary scores of DEBQ, MEQ-UD and their sub-scales. The accepted level of significance was $p \leq 0.05$. All data were firstly entered into Microsoft Excel for them to be split into subgroups (see Table 4.2 below) and then collated in a Statistical Package for the Social Sciences (SPSS) 24 spreadsheet (IBM Corp, 2016) for further analysis.

Mean values of baseline appetite levels were compared between sub-groups of gender, age, BMI and body-fat percentage (separated by gender due to gender-specific reference ranges), to check for any potential confounding. Mean values of eating rate, MEQ-UD and DEBQ summary scores were compared between these demographic and anthropometric subgroups to deduce population norms.
Statistical tests used were Independent t-tests and one-way ANOVA for normally distributed data, or Mann Whitney U tests and Kruskal Wallis tests for non-normally distributed data.

Correlation analysis was performed to measure associations between independent variables (age, BMI, body-fat percentage and the DEBQ score) and the primary outcomes (ER and the MEQ-UD summary score). Lastly, multiple regression was performed to investigate the factors predicting the dependent variables.

4.4.5 Results

4.4.5.1 Participant characteristics
Sixty volunteers participated in the study, none of whom were excluded from the analysis. Detailed participant characteristics are given in Table 4.2. Study participants were predominately women (62%), white (82%), current university students (90%), and ranged from 19 to 64 years of age, with a median of 22 years (21, 24). Mean BMI was 23.0 kg/m² (±4.17), and the majority of the participants (70%) were within the healthy BMI range (WHO, 2000). Mean body-fat content in women was 20.8% (±5.89%), with the majority of them (95%) lying in the healthy range (TANITA, 2018), while mean body-fat content in men was 22.9% (±8.69), with the minority of them (43%) in the healthy range (TANITA, 2018). Visceral fat level was within the healthy range for almost all the participants (98%), thus visceral fat content was not included in any statistical analysis. The mean DEBQ summary score was 64% (±6.5%), with a high score representative of greater mindfulness.
Table 4.4.2 Participant characteristics: Frequency, mean, standard deviation or median, and 25%,75% for gender, age (years), BMI (kg/m²), body-fat content (%), visceral fat level and for their subgroups (n=60).

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
<th>Mean ±SD</th>
<th>Median (25%, 75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>37</td>
<td>62</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Male</td>
<td>23</td>
<td>48</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>*Age (years)</td>
<td>60</td>
<td>100</td>
<td>-</td>
<td>22 (21, 24)</td>
</tr>
<tr>
<td>*19-34</td>
<td>56</td>
<td>93</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>*35-64</td>
<td>4</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BMI (kg/m²)¹</td>
<td>60</td>
<td>100</td>
<td>23.0 (±4.17)</td>
<td>-</td>
</tr>
<tr>
<td>&lt;24.9</td>
<td>42</td>
<td>70</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25-29.9</td>
<td>16</td>
<td>27</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>&gt;30</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fat%</td>
<td>60</td>
<td>100</td>
<td>21.6 (±7.10)</td>
<td>-</td>
</tr>
<tr>
<td>Fat% in females²</td>
<td>37</td>
<td>62</td>
<td>20.8 (±5.89)</td>
<td>-</td>
</tr>
<tr>
<td>10-31%</td>
<td>35</td>
<td>95</td>
<td>n-</td>
<td>-</td>
</tr>
<tr>
<td>*&gt;32%</td>
<td>2</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fat% in males²</td>
<td>23</td>
<td>38</td>
<td>22.9 (±8.69)</td>
<td>-</td>
</tr>
<tr>
<td>2-22%</td>
<td>10</td>
<td>43</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>&gt;23%</td>
<td>13</td>
<td>57</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>*Visceral fat level²</td>
<td>60</td>
<td>100</td>
<td>-</td>
<td>1 (1, 3)</td>
</tr>
<tr>
<td>*Normal</td>
<td>59</td>
<td>98</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>*Excessive</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Body Mass Index; kg, kilogram; m, metres; n, sample size; SD, Standard Deviation; *, not normally distributed, (p≤0.05).¹ WHO, 2000. ²TANITA, 2018.
4.4.5.2 Appetite ratings

Within a potential appetite score range of 0.0 to 10.0mm, mean or median scores for appetite questions «How hungry do you feel?», «How full do you feel?», and «How much do you think you can eat?» were 6.5mm (4.9,7.4), 2.7mm (1.7,4.6) and 7.0mm (±1.8), respectively, with higher scores indicating more hunger, more fullness, and greater perception of how much could be eaten. For more details in appetite scores, see Table 4.3.

**Table 4.4.3:** Appetite scores for the sample as a whole (n=60) and by gender, age, BMI, body-fat content. Mean, (standard deviation) or median (25%,75%).

<table>
<thead>
<tr>
<th></th>
<th>Hunger</th>
<th>Fullness</th>
<th>Satiety</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole sample (n=60)</strong></td>
<td>6.5 (4.9, 7.4)*</td>
<td>2.7 (1.7, 4.6)*</td>
<td>7.0 (±1.8)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (n=37)</td>
<td>p= 0.74</td>
<td>p= 0.46</td>
<td>p= 0.19</td>
</tr>
<tr>
<td>Male (n=23)</td>
<td>7.8 (±0.91)</td>
<td>2.9 (±2.0)</td>
<td>2.9 (±2.0)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-34 (n=56)</td>
<td>p= 0.97</td>
<td>p= 0.94</td>
<td>p= 0.15</td>
</tr>
<tr>
<td>35-64 (n=4)</td>
<td>6.4 (± 1.1)</td>
<td>2.9 (±1.9)</td>
<td>2.9 (±1.9)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24.9 (n=42)</td>
<td>p= 0.17</td>
<td>p= 0.28</td>
<td>p= 0.20</td>
</tr>
<tr>
<td>25-29.9 (n=16)</td>
<td>7.8 (±1.4)</td>
<td>2.7 (±1.9)</td>
<td>2.5 (±1.8)</td>
</tr>
<tr>
<td>&gt;30 (n=2)</td>
<td>4.9 (±2.1)</td>
<td>3.6 (±2.0)</td>
<td>3.3 (±1.3)</td>
</tr>
<tr>
<td><strong>Fat% in females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-31% (n=35)</td>
<td>p= 0.38</td>
<td>p= 0.69</td>
<td>p= 0.81</td>
</tr>
<tr>
<td>10-31% (n=35)</td>
<td>6.0 (± 1.8)</td>
<td>3.3 (±2.0)</td>
<td>5.8 (±2.5)</td>
</tr>
<tr>
<td>&gt;32% (n=2)</td>
<td>5.8 (±0.64)</td>
<td>2.8 (±2.1)</td>
<td>3.4 (±2.4)</td>
</tr>
<tr>
<td><strong>Fat% in males</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-22% (n=10)</td>
<td>p= 0.51</td>
<td>p= 0.66</td>
<td>p= 0.75</td>
</tr>
<tr>
<td>2-22% (n=10)</td>
<td>6.3 (±2.7)</td>
<td>2.5 (±2.0)</td>
<td>7.5 (±1.8)</td>
</tr>
<tr>
<td>&gt;23% (n=13)</td>
<td>6.1 (±2.1)</td>
<td>2.8 (±1.7)</td>
<td>7.5 (±2.1)</td>
</tr>
</tbody>
</table>

*Median (25%, 75%)
4.4.5.3 Eating rate
Median ER was 58 chews/minute (42.5, 69.1), see Table 4. Participants who were below the median were categorised as slow eaters, whereas those who were above it were categorised as fast eaters. Median values of ER were compared between subgroups of gender, age, BMI, and body-fat (according to gender specific cut offs). There were no significant differences between the groups, see Table 4.4. Those who had a higher ER were women [59 chews/minute (44, 72.1)], younger [56.1 chews/minute (43.2, 73.8)], obese [65.4 chews/minute (68.7)], and in the higher body-fat content range [58.9 chews/minute, (62.2) for women, and 59.7 chews/minute, (44.1, 73) for men].

**Table 4.4:** Eating rate for the sample as a whole (n=60) and by gender, age, BMI, body-fat content, median (25%,75%).

<table>
<thead>
<tr>
<th>Value</th>
<th>Median (25%, 75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER (chews/minute) n =60</td>
<td>58 (42.5, 69.1)</td>
</tr>
<tr>
<td>Gender n = 60</td>
<td>p= 0.46</td>
</tr>
<tr>
<td>Female n = 37</td>
<td>59 (44, 72.1)</td>
</tr>
<tr>
<td>Male n=23</td>
<td>55 (41, 69)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>p= 0.23</td>
</tr>
<tr>
<td>19-34 n=56</td>
<td>56.1 (43.2, 73.8)</td>
</tr>
<tr>
<td>35-64 n=4</td>
<td>52.5 (40.3, 69.4)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>p= 0.25</td>
</tr>
<tr>
<td>&lt;24.9 n=42</td>
<td>53.8 (40, 68.1)</td>
</tr>
<tr>
<td>25-29.9 n=16</td>
<td>65 (46.1, 73.6)</td>
</tr>
<tr>
<td>&gt;30 n=2</td>
<td>65.4 (68.7)</td>
</tr>
<tr>
<td>Fat% in females</td>
<td>p= 0.12</td>
</tr>
<tr>
<td>10-31% n=35</td>
<td>57 (44.3, 70.1)</td>
</tr>
<tr>
<td>&gt;32% n=2</td>
<td>58.9 (62.2)</td>
</tr>
<tr>
<td>Fat% in males</td>
<td>p= 0.76</td>
</tr>
<tr>
<td>2-22% n=10</td>
<td>57.2 (40.6, 71.8)</td>
</tr>
<tr>
<td>&gt;23% n=13</td>
<td>59.7 (44.1, 73)</td>
</tr>
</tbody>
</table>
4.4.5.4 Mindfulness Eating Questionnaire (Under Development) scores

The mean or median scores for the four MEQ-UD constructs are shown in Table 5 below. The highest median score for the MEQ-UD subscales was that for sensory construct (85%, 75,95) and the lowest was that for distraction (50%, 40,60).

**Table 4.4.5:** MEQUD score% for the whole population (n=60) and subgroups for: gender, age (years), BMI (kg/m²), body-fat content (%).

<table>
<thead>
<tr>
<th></th>
<th>MEQUD Sensory (%)</th>
<th>MEQUD Awareness (%)</th>
<th>MEQUD Body Cues (%)</th>
<th>MEQUD Distraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Score (n=60)</strong></td>
<td>85 (75, 94)</td>
<td>80 (67, 92)</td>
<td>67 (±16)</td>
<td>50 (40, 60)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (n=37)</td>
<td>15 (±3.4)</td>
<td>12 (±1.9)</td>
<td>14 (±3.2)</td>
<td>12 (±2.2)</td>
</tr>
<tr>
<td>Male (n=23)</td>
<td>16 (±2.9)</td>
<td>11 (±2.1)</td>
<td>15 (±2.7)</td>
<td>12 (±1.4)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-34 (n=56)</td>
<td>13 (±2.8)</td>
<td>12 (±2.1)</td>
<td>16 (±2.0)</td>
<td>12 (±2.2)</td>
</tr>
<tr>
<td>35-64 (n=4)</td>
<td>14 (±2.2)</td>
<td>12 (±1.1)</td>
<td>17 (±1.8)</td>
<td>12 (±1.7)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24.9 (n=42)</td>
<td>17 (±2.3)</td>
<td>13 (±1.2)</td>
<td>16 (±1.8)</td>
<td>12 (±1.7)</td>
</tr>
<tr>
<td>25-29.9 (n=16)</td>
<td>18 (±1.9)</td>
<td>13 (±1.9)</td>
<td>15 (±1.7)</td>
<td>13 (±1.8)</td>
</tr>
<tr>
<td>&gt;30 (n=2)</td>
<td>17 (±2.1)</td>
<td>12 (±2.3)</td>
<td>16 (±2.1)</td>
<td>12 (±1.8)</td>
</tr>
<tr>
<td><strong>Fat% in females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=37)</td>
<td>p= 0.27</td>
<td>p= 0.25</td>
<td>p= 0.34</td>
<td>p= 0.33</td>
</tr>
<tr>
<td>10-31% (n=35)</td>
<td>17 (±2.8)</td>
<td>12 (±1.8)</td>
<td>15 (±1.2)</td>
<td>12 (±2.1)</td>
</tr>
<tr>
<td>&gt;32% (n=2)</td>
<td>16 (±2.2)</td>
<td>13 (±1.9)</td>
<td>15 (±1.5)</td>
<td>13 (±1.8)</td>
</tr>
<tr>
<td><strong>Fat% in males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=23)</td>
<td>p= 0.13</td>
<td>p= 0.19</td>
<td>p= 0.23</td>
<td>p= 0.17</td>
</tr>
<tr>
<td>2-22% (n=10)</td>
<td>17 (±2.3)</td>
<td>12 (±2.2)</td>
<td>16 (±2.1)</td>
<td>12 (±1.2)</td>
</tr>
<tr>
<td>&gt;23% (n=13)</td>
<td>16 (±1.7)</td>
<td>11 (±1.9)</td>
<td>17 (±1.9)</td>
<td>12 (±1.8)</td>
</tr>
</tbody>
</table>

BMI, Body Mass Index; kg, kilogram; m, metres; MEQUD, Mindfulness Eating Questionnaire Under Development; n, sample size; SD, standard deviation; ER, eating rate; p, significance value.
4.4.5.5 Correlation analysis

4.4.5.5.1 MEQ-UD Sensory

The scores of the MEQUD sensory and the DEBQ, were positively correlated with each other; thus implying consistency between the two, despite the absence of the external cues construct in the MEQUD.

Table 4.4.6: Correlation coefficients and their significance values for automatic eating rate (chews/minute), and MEQUD-Sensory score (%), against age (years), BMI (kg/m$^2$), body-fat content (%), DEBQ summary scores (%), manual ER (chews/minute), (n=60).

<table>
<thead>
<tr>
<th>Eating rate (chews/ min)</th>
<th>Primary Outcome:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEQUD (Sensory) score (%)</td>
<td>0.046</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Secondary Outcomes:

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.28</td>
<td>0.75</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>0.131</td>
<td>0.29</td>
</tr>
<tr>
<td>Body-fat %</td>
<td>0.311</td>
<td>0.09</td>
</tr>
<tr>
<td>DEBQ scores %</td>
<td>-0.037</td>
<td>0.84</td>
</tr>
</tbody>
</table>

MEQUD Mindfulness Eating Questionnaire Under Development; BMI, Body Mass Index; kg, kilogram; m, metres; DEBQ, Dutch Eating Behaviour Questionnaire; r, Pearson correlation value; p, significance value; min, minute; ER, Eating Rate.
### 4.4.5.5.1 MEQ-UD Awareness

**Table 4.4.7:** MEQUD-Awareness score correlation (%), against age (years), BMI (kg/m²), body-fat content (%), DEBQ summary scores (%), manual ER (chews/minute), (n=60).

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEQUD Awareness score (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER (chews/min)</td>
<td>0.039</td>
<td>0.29</td>
</tr>
</tbody>
</table>

**Secondary Outcomes:**

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.079</td>
<td>0.87</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.041</td>
<td>0.61</td>
</tr>
<tr>
<td>Body-fat %</td>
<td>-0.022</td>
<td>0.63</td>
</tr>
<tr>
<td>DEBQ scores</td>
<td>0.046</td>
<td>0.61</td>
</tr>
</tbody>
</table>

MEQUD Mindfulness Eating Questionnaire Under Development; BMI, Body Mass Index; kg, kilogram; m, metres; DEBQ, Dutch Eating Behaviour Questionnaire; r, Pearson correlation value; p, significance value; Auto, automatic; min, minute; ER, Eating Rate.

### 4.4.5.5.3 MEQUD Body Cues

**Table 4.4.8:** MEQUD-Body cues score (%), against age (years), BMI (kg/m²), body-fat content (%), DEBQ summary scores (%), and manual ER (chews/minute), (n=60).

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Automatic eating rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEQUD (Sensory) score (%)</td>
<td>0.055</td>
<td>0.21</td>
</tr>
</tbody>
</table>

**Secondary Outcomes:**

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.021</td>
<td>0.61</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.134</td>
<td>0.23</td>
</tr>
<tr>
<td>Body-fat %</td>
<td>0.181</td>
<td>0.09</td>
</tr>
<tr>
<td>DEBQ scores %</td>
<td>-0.029</td>
<td>0.78</td>
</tr>
</tbody>
</table>

MEQUD Mindfulness Eating Questionnaire Under Development; BMI, Body Mass Index; kg, kilogram; m, metres; DEBQ, Dutch Eating Behaviour Questionnaire; r, Pearson correlation value; p, significance value; Auto, automatic; min, minute; ER, Eating Rate.
4.4.5.3 MEQUD Distraction

Table 4.4.9: MEQUD-Distraction score (%), against age (years), BMI (kg/m²), body-fat content (%), DEBQ summary scores (%) and manual ER (chews/minute), (n=60).

<table>
<thead>
<tr>
<th>Eating rate (chews/ min)</th>
<th>MEQUD (Sensory) score (%)</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEQUD (Sensory) score (%)</td>
<td>0.045</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Secondary Outcomes:

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.020</td>
<td>0.64</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.112</td>
<td>0.22</td>
</tr>
<tr>
<td>Body-fat %</td>
<td>0.223</td>
<td>0.08</td>
</tr>
<tr>
<td>DEBQ scores %</td>
<td>-0.037</td>
<td>0.87</td>
</tr>
</tbody>
</table>

MEQUD Mindfulness Eating Questionnaire Under Development; BMI, Body Mass Index; kg, kilogram; m, metres; DEBQ, Dutch Eating Behaviour Questionnaire; r, Pearson correlation value; p, significance value; Auto, automatic; min, minute; ER, Eating Rate.

4.4.5.6 Mixed Models analysis

Several mixed models analyses were constructed to assess the prediction of ER by the independent variables [MEQ-UD (Sensory, awareness, Body cues, Distraction), gender, age, BMI, body-fat content, and DEBQ scores]. The adjusted $R^2$ was 0.021, meaning that only 2.1% of the variance was accounted for by these variables. The analysis found that the most important factor; positively, and significantly, correlated to ER was body-fat content (unstandardised $\beta= 0.538$, $t=2.03$, $p=0.05$). Multiple regression analyses were also performed to assess the prediction of the MEQ-UD variables by the independent variables (ER, the demographic and anthropometric measurements mentioned, and to the DEBQ summary score). It was shown that the adjusted $R^2$ was 0.074, thus only 7.40% of the variance was accounted for by these variables.
4.4.6 Discussion
This developmental study aimed to investigate if the MEQ-UD was a good marker of eating rate in healthy adults. The statistical tests between ER and the MEQ-UD showed that their relationship was non-significantly positive. Whilst this may suggest that the more mindful someone is the faster they eat, the lack of significance and fact that this opposes the previous literature (Warren et al., 2017, Teper et al., 2013) weakens any such conclusions. Furthermore, the MEQ-UD provided by Winkens and their team, was not the final version used in their recent publication (Winkens et al., 2018), as three extra questions have been added to it since this study was conducted which, if included in our study, may have resulted in a positive relationship with ER. On the other hand, the relationship between automatic ER and DEBQ; a well-established mindfulness questionnaire, was negative and non-significant. This agrees with literature findings (Warren et al., 2017), which support that slow ER is a characteristic of mindfulness, although the relationship may not be as strong as implicated. The MEQU correlations well for all 4 sub-scales with the DEBQ, which is not surprising since their mindful eating domains are similar. It should be noted, that Winkens et al (2018), in an internal factor analysis on abbreviated versions of the DEBQ (22 questions instead of 33) and MEQ-UD [called Mindful Behavior Eating Scale (MBES) in the paper], showed that mindful eating items are pure indicators of mindful eating and can be measured independently from the eating styles emotional, external and restrained eating. Consequently, although the MEQU subscales correlate strongly with the DEBQ, they should be measured separately (and not combined into one questionnaire) as the MEQU constructs could be related to different health outcomes (Winkens et al., 2018).

A secondary aim of this study was to assess potential mediators of ER and mindfulness and the factors that mediate the relationship between the two. Concerning the factors affecting mindfulness in eating, the most important predictor of all four MEQ-UD subscales was found to be age. As the population in our study was of a limited age range (predominantly 93% young, 19-33 years), age cannot be expected to successfully discriminate between the ERs and therefore to be a good predictor of the MEQ-UD subscales. Alispahic and Hasanbegovic-Anic (2017) showed that the most mindful participants in their study were older (two age groups of 33-49 years and 50+, both included in the “older group” population), agreeing with the notion that mindfulness as measured by MEQ-UD, is a characteristic of older populations when compared to younger ones. Furthermore, the most important, negative, but weak, predictor of MEQ-UD scores was body-fat content, which was evident in males, but not in females. If body fat content were proven to be a strong predictor of mindfulness and this relationship was also examined in a cause-effect study, then strategies to
increase mindful eating might be of most benefit to the male population though such link was not established in this study.

It was observed that some subgroups of the sample with higher MEQ-UD scores, had a lower ER, in line with expected associations between the two variables. This pattern appeared to hold true for men and older participants more so than women and young participants although the effects failed to achieve statistical significance. As mentioned above, Alispahic and Hasanbegovic-Anic (2017) showed that older participants were more mindful in their study though contrary to our findings women also scored higher in the mindfulness subscale. As reviewed in chapter 1, there remains insufficient evidence to support differences in ER due to gender or age.

Obese participants were found to be both more mindful and faster eaters than those in lower BMI groups. In line with these findings, is well documented in the literature (see chapter 1) that overweight and obese people have a faster ER (Hill and McCurchon 1984, Laesle et al., 2007, Bolhuis and Keast 2015). However only 3% of the study’s population were obese compared to 26% men and 27% for women in the UK (Health Survey for England 2016), again suggesting the lack of variation within our sample may have hindered our ability to differentiate between different eating rates.

4.7.7 Study Limitations
Some important limitations of this project relate to the sample used. First of all, it was not possible to calculate statistical power a priori for the size of the sample. A study population of 60 participants may or may not be responsible for the non-significant results of the study, but this remains to be determined. Also, despite attempts to recruit a wide demographic the sample was predominately young, white, and educated adults of normal BMI and body-fat content. Whilst this was a convenient starting point for this research work, it was not representative of the broad age, race, and educational diversity that portrays the UK population (Public Health England, 2017). With such populations, the results obtained are difficult to be applied outside the study population (Clementi et al., 2017). Weight-wise, findings cannot necessarily be generalised to those who would most benefit from weight management interventions. Given that mechanisms concerning overeating and excess weight may vary with BMI (Davis and Fox, 2008), we should be cautious about assuming that the concluded weak associations of ER and the MEQ-UD amongst normal weight individuals would apply to all individuals equally, and that they would not necessarily rule out different effects amongst overweight or obese individuals (Tapper, 2017). Concerning recruitment,
if the budget had been available, a monetary reward could have been offered to volunteers so that the attention of a wider and more diverse group would be attracted. Similarly, weight loss trials have reported difficulty in recruitment (Blanton et al., 2006) with local community recruitment strategies yielding the highest recruitment rates, and specifically posters in the community (Griffin et al., 2013, Bergmann et al., 2017). Although local posters were used as a recruitment strategy in the current study, other reported methods such as advertisements in the local health service intranet and local metropolitan newspapers could have contributed to a more successful recruitment rate (Griffin et al., 2013).

There is also a possibility of sampling error as the convenience sample used was local volunteers, who were willing to participate in a mindfulness eating study. This means that participants were likely to have been relatively more motivated to check their self-regulatory skills (Tapper, 2017), which may again make them non-representative of the general population. In particular, if participants were aware how they were meant to be eating, their ER could have been subject to desirability bias (Robinson et al. 2014; Grossman 2011). Besides, although participants were allowed to take leftovers home, having lunch for free may have also affected their eating behaviour (Bolhuis and Keast, 2015).

Another important point to note (discussed in Chapter 1) is that assessing mindfulness with the use of a questionnaire is challenging. The questions of similar tools have been shown to have low convergent validity and they may be interpreted in different ways according to the subjects’ experience on mindfulness practices (Tapper, 2017). In order to avoid these issues, participants could have been asked whether they have had mindfulness practice experience in the past so that they would be allocated in different groups prior to the statistical analysis, although this would again have necessitated a larger sample size. Also, the answers given could have been biased, as these were completed at the location of the research, away from the comfort of the participants’ own home, with research assistants present (Dalen, 2010). An improvement to this would be for participants to complete the online questionnaires from the comfort of their own home after their session, however this could decrease the response rate.

What is more, the use of an unpublished questionnaire, which was still under development at the start of the study, may constitute a major weakness by itself. Indeed, the final version of the MEQ, recently published as the «Mindful Eating Behaviour Scale» (Winkens et al., 2018), was slightly different to the version used here. Specifically, the new version included 3 more questions on mindfulness. Another limitation in using unpublished information is that the lack of agreed ranges and definitions, makes it difficult to standardize and compare the results. This does not only apply
to the use of the unpublished MEQ, but to the automated ER software for which slow and fast ER ranges had not been defined.

Observational studies may be fraught with confounding factors (Robinson et al., 2014); in this study some of them were controlled for. For example, water intake prior to eating was fixed for all participants so that gastric distention would not affect their appetite sensation (Zhu and Hollis, 2013). However, participants were not asked to refrain from water prior to their session, and thus some participants may have come less thirsty than others, which may have affected results. As a future improvement, participants could be asked to completely refrain from fluids prior to the session. Apart from that, the amount of the starting food offered was the same for all participants, so that the number of their chews would not be affected by the mass of food consumed. Lastly, the research questionnaires were answered after eating the test meal offered, so that the knowledge of the mindfulness questions would not interfere, neither consciously nor unconsciously, with the participants’ eating behaviour.

4.7.8 Conclusion
The current study failed to show statistically significant results which could warrant using the MEQ-UD a valid proxy for measuring ER, though valuable information concerning mindfulness subscales could be gathered in an ER study. Furthermore, an ER study with more diverse age population and the finalised version of the MEQ-UD (currently published, Winkens et al., 2018) would be interesting in order to further investigate the link between ER and mindfulness.
Chapter 5

M.E.E.T - Mindful Eating and Eating Topography

Investigation of the short and longer-term effects of a slow eating rate protocol in overweight people, in a community intervention study.
5.1 Introduction

The worldwide prevalence of overweight and obesity are cause for concern (Chapter 1, section 1.2). Reducing eating rate may be a promising tool in combating obesity (Chapter 1, Section 1.3) as people who eat quickly tend to consume more energy, be overweight and have lower satiation after a meal (Chapter 1, Section 1.3).

Eating rate may influence satiation levels and energy intake through a number of mechanisms. When people eat slowly, this influences the secretion of satiety hormones such as insulin and leptin (Chapter 1, Section 1.6). Circulating leptin has been found to play a role in regulating energy expenditure, food intake and adiposity by acting at sites primarily located within the central nervous system (Campfield et al., 1995, Pellemounter et al., 1995). During weight maintenance, plasma leptin reflects total body mass whereas during weight loss decreasing plasma leptin increases appetite and decreases energy expenditure (Anubhuti 2008). Slower eating also increases food oral exposure (Chapter 1, section 1.3) and the number of chews per unit of food, which have both been shown to lower energy intake (Chapter 1, section 1.3). Finally, slower eating may decrease feelings of deprivation by enhancing and prolonging pleasurable aspects of eating (Section 1.11.1).

One barrier to changing eating rate is that it may be a highly automatic behaviour, making eating rate difficult to change (Chapter 1, Section 1.3). However, recent research suggests that real-time feedback can interrupt the execution of deeply engrained habitual behaviours and make them available for conscious scrutiny and behaviour change (Chapter 1, section 1.4). Techniques which cultivate mindfulness; a focused, non-judgemental attention to the present moment (Brown et al., 2007), may offer a potential approach to facilitate changes in eating behaviour. These may help people recognise and respond to satiety, or even enable them to not respond to inappropriate cues for eating; for example stress, boredom, advertisements, which make people consume energy in a dissociative and automatic manner (Brown et al., 2007). By adopting these mindfulness skills someone can potentially reduce their ER (Kristeller and Wolever, 2011), lower their energy intake (Kristeller et al., 2006), and thus maintain or even lose weight (Dalen et al., 2010; Smith 2006). In contrast to energy restrictive diets, mindfulness-based interventions try to reconstruct cognition (Bishop, 2004) by cultivating awareness of physical sensations, environmental cues and sensory properties of food (Tapper, 2017). Emotionally, mindfulness-based practices promote self control and strength by disrupting emotionally related habits, and also by reducing psychological distress (Alberts, 2012). However, the mechanisms by which these interventions bring about their effects are not totally understood yet, and it is not always clear whether all components are responsible for
the claimed benefits (Tapper, 2017). Effective and easy-to adopt tools and methods manipulating ER, as a means to cultivate mindful eating, and conversely inhibit impulsive eating (Teper et al., 2013), therefore need to be developed, tested and brought to everyday life.

Few studies have investigated the effect of specific eating topography protocols in a community population since most eating topography studies are either self-reported (Ekuni et al., 2012) solely clinical or focused on children (Hamilton-Shield et al., 2014). Hence, this study aims to investigate if a SER protocol can facilitate increased mindful eating and decreased weight in free living overweight/obese adults.
5.2 Aims
To investigate the feasibility and efficacy of a SER protocol in a free-living community setting to facilitate weight loss in overweight adults and to investigate the potential mechanism of action.

5.3 Objectives
- To recruit a sample of free-living overweight adults (N= 20) and randomise them to control or intervention groups
- To assess if the SER protocol is associated with changes in body composition (BMI, weight, fat%, visceral fat), energy intake, perceived satiety (subjective via visual analogue scales and hormones) if followed for 6 weeks by overweight free-living adults when compared to a control group not following the SER protocol.
- To investigate if the SER protocol can reduce the habitual eating rate of overweight adults in a free-living community setting after a 6-week intervention.
- To assess if the SER protocol will result in a more mindful eating as assessed by MEQ [Mindful Eating Questionnaire] and an increased meal duration.
- To assess the intervention group’s compliance with the SER protocol via data gathered from the study-dedicated website and changes in habitual eating rate after the 6-week intervention.
- To assess the feasibility of administering the SER protocol in a community setting.
- To assess if the SER protocol exerts any metabolic and hormonal changes in the test subgroup, after the 6-week intervention.
5.4 Hypothesis

It was hypothesised that:

- The SER protocol will facilitate greater weight loss in the Intervention-group as compared to the control group after the 6-week intervention.
- The SER protocol will reduce the habitual eating rate of the intervention group after 6 weeks.
- The SER protocol will result in improved hormonal and metabolic changes in the intervention group as compared to the control groups.
- The SER protocol will result in increased mindful eating in the intervention group as compared to the control group, with increased sensory, awareness and body cues and decreased distraction scores.
- The study’s dedicated website will be a useful monitoring tool and the data produced will be informative of future studies on eating rate interventions.

5.5 Methods

5.5.1 Participants

A power calculation (power of 0.80, α = 0.05) was performed using data from the previous developmental study-B (Chapter 3) in order to calculate the sample size necessary for this 10-week parallel, open label randomised controlled trial. The developmental study-B showed that with an 80% power considering the change in satiety (perceived hunger) [Chapter 3, Section 3.6.1] a sample of eight participants was adequate to detect differences (p<0.05). Therefore, we aimed to recruit a total of 20 overweight/obese participants (BMI>25 kg/m²), i.e. ten in each control and intervention group to allow for 20% drop out. The only existing community intervention manipulating ER is that of Ford et al (2010) where their main outcome was change in BMI, similarly change in BMI was selected as our primary outcome. Secondary outcomes included changes in: body composition (Weight, %fat loss, visceral fat level), energy intake, habitual ER, appetite, hormones and metabolites and effect of website monitoring tool.

The participants were recruited via email and posters distributed to University staff and students and the local community. Participants who met the BMI criteria received a copy of the Participant Information Sheet containing details about the study procedure. Those met the inclusion and exclusion criteria (table 1) provided informed written consent prior to study initiation. The study protocol received ethical approval from the University of Surrey Ethical Committee (UEC/2016/020/FHMS).
Table 5.1. Inclusion and Exclusion criteria.

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>• BMI &gt; 25 kg/m².</td>
<td>• Restrained eaters as assessed by DEBQ.</td>
</tr>
<tr>
<td>• Age between 18 and 70 years old.</td>
<td>• Currently following any form of weight reducing diet.</td>
</tr>
<tr>
<td>• Free of medications except minor analgesics and contraceptive pill (for at least a year).</td>
<td>• Family or personal history of psychiatric disorders.</td>
</tr>
<tr>
<td>• Willing to be filmed (mouth and jaw) for the purposes of the study.</td>
<td>• History of infectious conditions such as hepatitis, jaundice, tuberculosis.</td>
</tr>
<tr>
<td>• Complete set of dentures and able to eat.</td>
<td>• HIV positive.</td>
</tr>
<tr>
<td></td>
<td>• History of drug or alcohol abuse.</td>
</tr>
<tr>
<td></td>
<td>• Food allergies or intolerance to test meal ingredients.</td>
</tr>
<tr>
<td></td>
<td>• Allergic to any types of medications except Penicillin.</td>
</tr>
<tr>
<td></td>
<td>• Use of any prescribed medication within two weeks prior to the study.</td>
</tr>
<tr>
<td></td>
<td>• Use of any recreational drugs or been involved in any experimental drug trial within the past 3 months.</td>
</tr>
</tbody>
</table>

BMI, body mass index; DEBQ, Dutch Eating Behaviour Questionnaire; HIV, human immunodeficiency virus.

5.5.2 Meals

5.5.2.1 Test Meals

The test meal was a fixed portion (20 pieces) of spinach and ricotta tortelloni with two tablespoons of tomato and basil pasta sauce (651.8kcal, 79.14g CHO, 20.96g protein, 26.08g fat). This meal provided approximately 30% and 24% of energy requirement for female and male participants respectively (based on energy requirements of the 19 to 24 years age group). Meal preparation involved boiling one portion of pasta for two minutes, thoroughly draining the water and mixing the pasta and sauce evenly. This was served at an appropriate temperature with a fork and 250ml water measured with a measuring cylinder (Fisherbrand 250ml FB55211 ±2.0ml).

5.5.2.2 Ad libitum meal

A digital scale (James Martin collection by WAHL, ±1.0g) was used to weigh out 400g dry weight of spaghetti to the nearest gram. Spaghetti was boiled for five minutes and served with the same pasta sauce used in test meal. The ad libitum meal (5052kcal, 948g CHO, 168g protein, 57.6g fat) was quadruple the amount of the test meal.
5.5.3 Study Protocol

The study design (Figure 5.1) was divided into three parts: pre-study (visit 1), during the study (visits 2 and 3) and post-study (visit 4). Visits 1 and 4 involved the same procedure and visits 2 and 3 were procedurally identical but different from 1 and 4. Participants were invited to the Clinical Investigation Unit (CIU), University of Surrey for a screening session (visit 1) to complete several questionnaires: i) General Health Questionnaire, ii) Dutch Eating Behaviour Questionnaire (Van Strien et al., 1986) (DEBQ) to assess restrained eating behaviour by calculating emotional, external and restraint scores (participants with a score of >16 was excluded) and iii) Mindful Eating Questionnaire (Winkens et al., 2018) (MEQ) to assess mindful eating (higher mean score, more mindful). Eligible participants then completed a 24 hour recall with a trained researcher according to a standardised Multiple Pass recall (Raper et al., 2004) methodology. Height was measured using a stadiometer to the nearest 0.1cm with shoes removed; weight, BMI, % fat and visceral fat were measured using an electrical bio-impedance machine (TANITA Multi-frequency body composition analyser MC-180MA III) (Kyle et al., 2014). Participants then consumed the test meal whilst a high definition web camera (Canon LEGRIA HF R76) recorded the front view of jaw area to measure baseline chewing rate (as an objective measure of ER). Participants were informed that they did not need to finish the meal but to eat until they felt comfortably full. They were instructed to minimise movements and to only start eating when the researcher exited the room. Participants were then randomly allocated into control (n=10) or intervention groups (n=10). A simple randomisation was carried out by a blinded researcher using computer generated numbers. Each participant was coded alphanumerically (e.g. MS005) to maintain confidentiality.

Before attending visits 2 and 3, participants were asked to complete a 3-day food diary to verify adherence to the pre-study day conditions. These included the consumption of a standard breakfast between 07:30h and 08:00h and the avoidance of alcohol, caffeine and heavy exercise 24 hours before each visit. Participants were not allowed any food or drink (except water) between breakfast and lunch (test meal) on study days. At 12:00 h, participants arrived at the CIU where anthropometric measurements were taken. Participants were trained to complete a set of appetite ratings on a line from zero (“Not at all”) to 100 (“Extremely”) at 0 (at first bite), 15, 30, 45, 60, 90, 120, 180 minutes, using a validated electronic PRO-Diary VAS device (CamNtech Ltd, Cambridge). Participants in the intervention group were then provided with the SER protocol (Appendix 10) and were asked to note the frequency of missed chew counts as a means of reporting adherence and to complete a tick-sheet to ensure completion of VAS at specified intervals. All participants were video recorded during the test meal consumption, each placed in an area that is visually isolated from others. All participants remained in the unit until ad libitum meals were
served and consumed 3.5 hours after the test meal. They were free to eat any desired amount at their own pace within 30 minutes. This meal was weighed before and after consumption to assess food intake.

Participants in the intervention group were briefed about the six-week community intervention and trained on how to access the study website; participants in control group were given no instructions.

Figure 5.1: Protocol schematic. GHQ, General Health Questionnaire; DEBQ, Dutch Eating Behaviour Questionnaire; Mindful Eating Questionnaire; VAS, Visual Analogue Scale; SER, Slow Eating Rate.
5.5.4 Website monitoring

Whilst free-living in the community between visits 2 and 3 for six weeks, participants in the intervention group were monitored through a website created for this study: www.meetstudy.co.uk and its phone application https://play.google.com/store/apps/details?id=meetstudy.meetstudy. Automatic email reminders were sent twice every week including instructions on how to securely log in to the study website to watch the SER video. Participants were then expected to do as instructed, start eating lunch according to the protocol and click the ‘submit’ button once the meal was finished allowing measurement of meal duration [For further information see Chapter 4, Study C]. Participants returned to the CIU for visit 3 after the six-week intervention and again for visit 4 one week later for a re-assessment of anthropometrics and habitual ER.

5.5.5 Statistical analysis

All statistical analyses were run using SPSS 23.0 (IBM Statistical Package for the Social Sciences, US). VAS hunger, desire to eat and fullness scores were analysed as incremental area under the curve (iAUC), calculated using the trapezoid rule. Absolute change in anthropometric measurements (baseline vs. visit 4) and absolute change in ER (as represented by chews per minute (CPM)) (baseline vs. visit 2; baseline vs. visit 4) were calculated. Data from the 3-day food diaries at visit 2 and 3 were entered into Nutritics [Nutritics Software by Nutritics Limited, using 2015 CoFIDS including McCance and Widdowson 7th edition, 2015] to generate energy intake and macronutrient intakes for each participant. Data were checked for normality using Shapiro-Wilk test. Results were analysed using independent t-tests or the non-parametric alternative, Mann-Whitney U test to compare differences between groups. Chi-square test was performed to check for associations between groups and gender. Paired t-tests and non-parametric alternative, Wilcoxon matched pairs, were performed to compare differences between visits within the intervention group. This includes anthropometric measurements (baseline vs. visit 4), energy intake (baseline vs. 4), AUC and IAUC hunger, desire to eat and fullness (visit 2 vs. 3). Repeated measures ANOVA was used to compare differences over time between the groups for: Body composition, eating rate parameters, energy intake, metabolites and hormones, measures of appetite and measures of mindfulness.

Postprandial data for hormones and metabolites were assessed at visit 2 as a baseline and then again at visit 3 at the following time points 15, 30, 45, 60, 90, 120, 180 mins. (For further details on sample collection methodology and analysis see Chapter 2 section 2.1). Repeated measures ANOVA was applied to compare the two visits followed by Tukeys honest significance difference (Tukeys HSD) post-hoc tests in order to locate differences between time points, only when
statistically significant differences were detected. TAUC and IAUC were applied to compare the results obtained from the ANOVA analyses. TAUC was used to calculate the area above the X axis (Y=0), whereas IAUC was used to calculate the area above the baseline point (Y = first collected sample). Refer to section 2.2.3 for further details on this test.

A mixed model analysis was performed in order to correlate data extracted from the study’s dedicated website with all of the main outcome parameters (body composition, eating rate, energy intake, appetite, mindfulness). Once the outcomes with the highest correlation values were identified, a backward linear regression was used to create a model of change for the intervention group using the website’s monitoring output data. A P-value of <0.05 was considered statistically significant. Intention to treat analysis (ITT) was carried out to account for participants lost to follow up (see chapter 2 for ITT analysis).
5.5 Results

5.6.1 Consort Flow Diagram

The number of participants analysed at each visit are illustrated in Figure 5.2. Baseline characteristics of participants in control and intervention groups are presented in Table 5.2. At baseline, no significant differences were found between the two groups. The scores from DEBQ indicated no participants were restrained eaters.

**Figure 5.2.** CONSORT flow diagram.
5.6.2 Baseline Measurements

5.6.2.1 Baseline (Visit 2) Body Composition & Anthropometrics

Table 5.2: Baseline body composition and anthropometrics in control and intervention group, significance of treatment in completers and intention-to-treat group.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n=9)</th>
<th>Intervention (n=9)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.1 ±4.9</td>
<td>31.8 ±5.6</td>
<td>0.40</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 3</td>
<td>4</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Female 6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>93.8±9.2</td>
<td>88.1±12.1</td>
<td>0.28</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.72 ± 0.9</td>
<td>1.75 ± 0.11</td>
<td>0.98</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.4±3.3</td>
<td>31.28±4.1</td>
<td>0.61</td>
</tr>
<tr>
<td>% fat</td>
<td>32.15±11.1</td>
<td>33.8±6.9</td>
<td>0.48</td>
</tr>
<tr>
<td>Visceral fat</td>
<td>7.5±3.4</td>
<td>8.6±3.9</td>
<td>0.44</td>
</tr>
</tbody>
</table>

SD, standard deviation; BMI, body mass index; MEQ, Mindful Eating Questionnaire. *(P≤0.05 is significant)*

At baseline there were no significant differences between the two groups for any anthropometric measures. The participants were predominantly between the ages of 25-35 years [Median age =29 for the intervention group and 31 for the control group], 84% white Caucasian, current university students and staff 79%. The intervention group had more participants than the control group in the overweight category (60% and 55% respectively) and less participants in the obese category (40% versus 55%) as defined by the WHO, p=0.38). The total body fat % was above the healthy range for all participants (TANITA, 2018) and 79% of the intervention group participants had unhealthy levels (TANITA, 2018) of visceral fat vs 75% in the control group (p=0.44).
5.6.2.2 Baseline Eating Rate

Baseline eating rate (chews/minute) was assessed during visit 1 for both groups, when participants were asked to eat a meal without instructions whilst video recorded. Average ER, in chews per minute, was then calculated automatically via AlexNet [Chapter 4]. Participants were categorised as linear or decelerated eaters, according to the slope created when plotting chews per minute over time as demonstrated in previous eating rate studies (Kokkinos et al., 2010).

**Table 5.3:** Baseline eating rate characteristics of participants in control and intervention groups, as measured by AlexNet.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n=9)</th>
<th>Intervention (n=9)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean meal duration (mins)</td>
<td>8.2±3.1</td>
<td>8.5±3.8</td>
<td>0.38</td>
</tr>
<tr>
<td>Mean Chews/min</td>
<td>62.4 ± 19.1</td>
<td>66.2±16.5</td>
<td>0.47</td>
</tr>
<tr>
<td>Linear Eaters (n= 11) (73.3%)</td>
<td>7 (77.8%)</td>
<td>7 (77.8%)</td>
<td>-</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Male</td>
<td>2</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Decelerated Eaters (n = 4) (26.7%)</td>
<td>2 (22.2%)</td>
<td>2 (22.2%)</td>
<td>-</td>
</tr>
<tr>
<td>Female</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

SD, standard deviation.  
Linear and Decelerated eaters measured in average chews per minute/time. Meal duration and mean Chew/minute were normally distributed.

The two groups had the same numbers of linear [Control n= 7, Intervention n=6] and decelerated eaters [n= 2 Control, n= 2 Intervention] at baseline, with the control group having more female (n= 5) linear eaters than the intervention group (n =4) see Figure 5.3 below.
Figure 5.3: Baseline ER (chews per minute/time) for both groups represented with lines of best fit. A: Control group baseline eating rate n=9, B: Intervention group baseline eating rate n=9. Female (–) Male (—). (—) ( — ) linear eating rate, (*) decelerated eating rate.

5.6.2.3 Baseline Dietary Intake
At baseline (pre-visit 2), eight out of 9 participants in the intervention group and all participants from the control group completed their 3-day food diaries. The food diaries were analysed for energy (kcal), protein(g), Fats(g), total Carbohydrates (CHO) and total Fibre, using Nutritics (Nutritics Software, by Nutritics Limited).

Table 5.4. Baseline Dietary intake, 3-Day Diary: Control VS Intervention Group.

<table>
<thead>
<tr>
<th>Dietary Intake and % energy contribution</th>
<th>Control (n=9) Median (25%, 75%)</th>
<th>Intervention (n=9) Median (25%, 75%)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>1940 (1390,2450)</td>
<td>2055 (1430, 2600)</td>
<td>0.12</td>
</tr>
<tr>
<td>Protein(g)</td>
<td>70.1 (52, 96.1)</td>
<td>85.5 (59.5, 106.1)</td>
<td>0.17</td>
</tr>
<tr>
<td>Protein (% total energy)</td>
<td>14.5%</td>
<td>16.0%</td>
<td></td>
</tr>
<tr>
<td>Total Fat(g)</td>
<td>87 (68.7, 94.1)</td>
<td>95 (72.1, 105.1)</td>
<td>0.13</td>
</tr>
<tr>
<td>Fat (%total energy)</td>
<td>41.0%</td>
<td>43.0%</td>
<td></td>
</tr>
<tr>
<td>CHO(g)</td>
<td>220.5(189.1, 251.5)</td>
<td>225.1 (186.1, 255.1)</td>
<td>0.23</td>
</tr>
<tr>
<td>CHO (% total energy)</td>
<td>44.5%</td>
<td>41%</td>
<td></td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>22 (12.1, 30.1)</td>
<td>27.1 (16.6, 33.27)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

CHO, total Carbohydrates.
Energy, protein, total fat, total carbohydrates and fibre were not normally distributed, therefore median and 25% and 75% quartiles are presented in for all nutrients. *Mann-Whitney U test) was performed on all nutrients.

At baseline, there were no significant differences between the two groups for total energy, protein, fat, carbohydrates and fibre. Expressing intakes as a proportion of each participant’s dietary reference values (DRV’s, Department of Health 1991) did not change the pattern of
results seen. Overall this analysis suggests that when expressed as percentage of energy intake and compared to UK recommendations both groups reported slightly higher protein intake, higher fat intake (maximum is recommended as not more than 35% of daily energy intake) and slightly lower carbohydrate intake (DoH 2016). Reported energy intake [Estimated energy expenditure (EER) – Estimated Energy Intake (EEI), (Rennie et al., 2007)] was found to be on average 14% below estimated energy needs for the control group and 12% for the intervention group.
5.6.2.4 Baseline Metabolites and Hormones (Intervention group)
Basal (commencement of each study day) levels of plasma Glucose, Insulin, Leptin, TAGs, NEFAs, Cholesterol and HDL-Cholesterol from samples collected immediately before the test meal (T=0) for V2 vs V3, for the completers intervention group are shown in Figure 5.4.

![Graphs showing baseline metabolites and hormones](image)

**Figure 5.4:** Hormones and metabolites at basal levels at visit 2 and 3. Plasma Glucose (A), Insulin (B), Leptin (C), NEFAs (D), TAGs (D), Cholesterol (F), HDL-Cholesterol (G) (mean ±SEM) prior to the test meal, T=0, for all participants in the intervention group (n=8), V2(●), V3 (○). Independent t-test showed no significant differences between the baselines parameters.
5.6.2.5 Baseline Appetite Measures
Three aspects of self-reported satiety were analysed via the electronic visual analogue scales (see Chapter 2); ‘How hungry do you feel?’ (Perceived Hunger), ‘How much do you feel you can eat?’ (Desire to eat) and ‘How full do you feel?’ (Perceived Fullness). The basal, pre-prandial appetite scores are presented in Table 5.5 below showing no significant differences between groups at the start of the study day for visit 2. No significant basal differences between the two groups were detected for visit 3 (not depicted here).

Table 5.5: Baseline appetite scores Intervention vs Control Group.

<table>
<thead>
<tr>
<th>Appetite measures†</th>
<th>Control (n=9)</th>
<th>Intervention (n=9)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Hunger (mm)</td>
<td>0.94±0.08</td>
<td>0.92±0.1</td>
<td>0.24</td>
</tr>
<tr>
<td>Desire to Eat (mm)</td>
<td>0.88±0.08</td>
<td>0.86±0.04</td>
<td>0.22</td>
</tr>
<tr>
<td>Perceived Fullness (mm)</td>
<td>0.12±0.09</td>
<td>0.09±0.08</td>
<td>0.19</td>
</tr>
</tbody>
</table>

† Measured on a 0 (Not at all) to 10(Extremely) scale, SD standard deviation, All 3 appetite scores measured were normally distributed in both groups. *Paired t test within group.
5.6.2.6 Baseline Mindful Eating Questionnaire Responses
The four subscales of the questionnaire depicted in Table 5.6 below represent the following categories: Focused eating (Sensory), eating with awareness (Awareness), Hunger and Satiety cues (Body Cues), and eating without distraction (Distraction).

<table>
<thead>
<tr>
<th>Categories</th>
<th>Control (n=9)</th>
<th>Intervention (n=9)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensory</td>
<td>11.5 (10,13)</td>
<td>12 (11, 13)</td>
<td>0.51</td>
</tr>
<tr>
<td>Awareness</td>
<td>9.5 (8, 12)</td>
<td>8.5 (7.5, 12)</td>
<td>0.13</td>
</tr>
<tr>
<td>Body Cues</td>
<td>13 (9.5, 14.5)</td>
<td>14.0 (11.0, 16.5)</td>
<td>0.15</td>
</tr>
<tr>
<td>Distraction</td>
<td>7.5 (6.5, 8.5)</td>
<td>8.0 (6.0, 9.5)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Mindfulness categories were not normally distributed, therefore median and 25% and 75% quartiles are presented. Maximum scale ranges: sensory 4 to 20, awareness 3 to 15, Body cues 4 to 20, Distraction 3 to 15. A’Mann-Whitney U test was performed on all 4 categories.

Table 5.6 shows that at the beginning of the study both groups had similar levels of self-reported mindful eating with no significant differences between control and intervention. As the higher the scores are the more mindful the eating behaviour is, both groups seem to start the study with average levels of mindful behaviour, with the control group having higher awareness score, suggesting that they more likely to be more aware of when they are eating/snacking on food compared to the intervention group. Furthermore, the intervention group showed a slightly higher score for Body Cues, meaning that they might be able to better trust their body about appetite stimuli (i.e. what to eat, when to eat, when to stop eating).
5.6.3 Effect of Intervention (6 weeks)

5.6.3.1 Effect of Intervention: Body Composition

All fifteen completers had their body composition measured via the multifrequency bioelectrical impedance digital scales (see Chapter 2 for further details on the body composition measurement technique).

Table 5.7: Body composition measurements at baseline and after 6 weeks for adults completing a slow eating rate community intervention study, control versus Intervention.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n=7)</th>
<th>Intervention (n=8)</th>
<th>2-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Visit 3</td>
<td>Baseline</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>92.5±9.6</td>
<td>93.4±9.1</td>
<td>0.58</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.7±4.8</td>
<td>32.9±4.8</td>
<td>0.57</td>
</tr>
<tr>
<td>% Fat</td>
<td>31.25±11.5</td>
<td>30.1±10.9</td>
<td>0.60</td>
</tr>
<tr>
<td>Visceral Fat</td>
<td>7.9±3.9</td>
<td>8.1±4.0</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Data presented as Mean ± standard deviation; BMI, body mass index. \(^1\)Paired t test within group, baseline compared to visit 3. \(^a\)2-Way repeated measures ANOVA, significant effect of intervention, level of significance \(p\leq 0.05\). \(^b\)Level of significance for all initially enrolled subjects, missing data imputed using Last Observation Carried Forward imputation approach.

Repeated measures analysis of variances showed a significant effect of treatment completers group and visit number for BMI, Weight, Visceral Fat and %Fat. The intervention-completers group significantly reduced their weight (\(p=0.009\), -3%), BMI (\(p=0.009\), -2.9%), visceral fat (\(p=0.002\), -10%) and showed a trend for body fat% reduction (\(p=0.086\) -1.3%) over the intervention period. The intervention-completers group had a statistically significant reduction in all body composition measurements, compared to the control-completers group: Weight (\(p=0.025\)), BMI (\(p=0.029\)), % Fat (\(p=0.039\)), Visceral fat (\(p=0.016\)). The significant differences for all body composition parameters were maintained when both groups were treated with intention-to-treat (ITT) analysis.
5.6.3.2 Effect of Intervention: Habitual Eating Rate
Completers of both groups had their habitual eating rate measured at baseline (visit 1) and at their last visit (visit 4), where they ate a meal without any instructions whilst being video reordered. They primary components of eating rate presented below are meal duration and average chews/minute over time, both of which were automatically calculated via the SMV classifier. The participants’ average chews per minute, for both visits (V1 vs V4) (Figure 5.5 below) where then plotted in order to identify differences in eating styles (linear or decelerated).

Table 5.8. Effect of intervention on Eating Rate parameters: Control vs Intervention Group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n=7)</th>
<th>Intervention (n =8)</th>
<th>2-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Meal duration (mins)</td>
<td>8.5±3.8</td>
<td>8.7±3.6</td>
<td>0.24</td>
</tr>
<tr>
<td>Chews/min</td>
<td>58.1±17.6</td>
<td>60.2±16.81</td>
<td>0.61</td>
</tr>
<tr>
<td>Linear Eaters% (n=11)</td>
<td>71.4</td>
<td>71.4</td>
<td>-</td>
</tr>
<tr>
<td>Female (n=6)</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Male (n=5)</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Decelerated Eaters% (n=4)</td>
<td>28.6</td>
<td>28.6</td>
<td>-</td>
</tr>
<tr>
<td>Female (n=2)</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Male (n=2)</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

SD, standard deviation.
Linear and Decelerated eaters measured in average chews per minute/time. Meal duration and avg chews/minute were both normally distributed so data are presented as Mean±SD. a 2-Way repeated measures ANOVA, significant effect of intervention. b level of significance for all initially enrolled subjects, missing data imputed using Last Observation Carried Forward imputation approach.
A repeated measures ANOVA with intervention as one factor and visits (1 and 4) as the second factor was performed in a full factorial model where all interaction effects were included. The habitual eating rate did not significantly change for either group of completers, as linear and decelerated eaters maintained their eating style after the 6-week intervention. The habitual eating rate did not change for both groups following intention-to-treat analysis.

**Figure 5.5:** Eating Rate V1 vs V4 for both participant groups. Avg chews/minute over time as calculated automatically via the AlexNet. V1 =Visit 1 (Baseline), V4= Visit 4. Figure A Baseline ER vs visit 4, for the control group, n=7. B) Baseline ER vs visit 4, for the intervention group, n=8. Each participant is depicted in different colour, baseline (visit 1) measurements are represented in a continuous line and visit 4 measurements with dotted lines. (* ) decelerated eating rate.

Figures 5A and 5B above show the average chews per minute overtime for each completer at each visit and, as the statistics in Table 5.8 above suggest, there were no significant differences between baseline and visit 4, for either group. Also, as seen in figure 5.5 in both groups the number of linear [Control group: 5 (4 Female, 1 Male), Intervention group 6 (3 Female, 3 Male)] and decelerated eaters [control: 2 (1 Female, 1 Male), Intervention group: 2 (1 Female, 1 Male)] did not change after the 6-week intervention as none of the participants changed their eating style.
5.6.4.2 Effect of Intervention: Energy Intake

At visit 4, only 6 participants from the intervention-completers group and 6 from the control-completers group completed their 3-day food diaries. Participants’ 3-day food diary entries were analysed using Nutritics software and the results are displayed in table 5.9.

Table 5.9: 3-Day Food Diary Analysis Visit 2 vs Visit 3.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Control</th>
<th>Intervention</th>
<th>2-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (25%, 75%)</td>
<td>Median (25%, 75%)</td>
<td>p^1</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>Visit 2 (n=7)</td>
<td>Visit 3 (n=6)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>1890 (1320,2490)</td>
<td>1950(1350,2580)</td>
<td>0.15</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>Visit 2 (n=7)</td>
<td>Visit 3 (n=6)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>77.9 (55.2, 98.4)</td>
<td>82.5 (56.2, 99.4)</td>
<td>0.21</td>
</tr>
<tr>
<td>Protein (%) total energy</td>
<td>16.5%</td>
<td>16.9%</td>
<td>16.7%</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>Visit 2 (n=7)</td>
<td>Visit 3 (n=6)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>82 (68.7, 91.2)</td>
<td>86.5 (70.1, 94.9)</td>
<td>0.19</td>
</tr>
<tr>
<td>Fat (%)total energy</td>
<td>39.0%</td>
<td>40.0%</td>
<td>42.8%</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>Visit 2 (n=7)</td>
<td>Visit 3 (n=6)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>209.3 (188.1, 222.5)</td>
<td>210.1(190, 228.6)</td>
<td>0.23</td>
</tr>
<tr>
<td>CHO (%)total energy</td>
<td>44.5%</td>
<td>43.1%</td>
<td>40.5%</td>
</tr>
<tr>
<td>Fibre</td>
<td>Visit 2 (n=7)</td>
<td>Visit 3 (n=6)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>25 (15.12, 30.1)</td>
<td>24 (14.8, 29.51)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

CHO, Total Carbohydrates.

Energy, protein, total fat, total carbohydrates and fibre were not normally distributed, therefore median and 25% and 75% quartiles are presented for all nutrients. All data were log transformed in order to allow parametric statistics. 1 Paired t test within group, baseline compared to visit 4. 2-way repeated measures ANOVA, significant effect of intervention. 2 level of significance for all initially enrolled subjects, missing data imputed using Last Observation Carried Forward imputation approach. Significant differences presented in italics.
Paired t-tests showed no significant differences between V2 and V3 for all nutrients in the control-completers group, whereas significant differences where shown for the intervention-completers group between the two visits representing a reduced energy (p = 0.049) and reduced total CHO (p = 0.038) intake and a trend towards reduced total fat (p = 0.086). A 2-way repeated measures ANOVA showed a significant effect of time, treatment and group and a significant interaction between time and treatment. Tukey’s post hoc test showed that only the intervention-completers group had significantly less Total fat(g) p = 0.026 and Total carbohydrates (p = 0.041) and a trend towards reduced energy intake (p=0.086). Expressing intakes as a proportion of each participant’s dietary reference values (DRV’s, Department of Health 1991) did not change the pattern of results seen neither did intention-to-treat analysis.

This analysis suggests that when expressed as a percentage of energy and compared to the UK recommendations the control-completers group remained stable with a slightly high protein intake, high fat intake and slightly low carbohydrate intake (DoH 2016). In contrast, the intervention-completers group, increased their % energy from protein (+1.7%) and CHO (+3.3%) and decreased their % energy from total fat (-3.3%).

In the control-completers group, one participant (female, 22) did not submit their 3-day food diary, and at baseline this participant had a 20% under reporting EI which explains why the total average under reporting for his group was improved to 12% at visit 4. In the intervention-completers group, one participant (female, 39yrs) did not submit their 3-day food diary and was the only participant in the intervention group to gain weight (+570g). Furthermore, a second participant (male, 29yrs) who had a low under reporting score at baseline (-5% below estimated energy needs) lost 2.3kg during the 6-week intervention. The total average under reporting score for the intervention-completers group was -9% (2% improved from baseline), where the estimated energy intake was expected to be below the estimated energy expenditure since the intervention group had a statistically significant weight loss at visit 3 (p = 0.006).
5.6.4.3 Effect of Intervention: Hormones and Metabolites

Hormones and metabolite responses to a test meal before and after the intervention were assessed for intervention-completers group only (n=8) via a postprandial time course (0,15,30,60,90,120 and 180 min) after the test meal.

Figure 5.6: Plasma Glucose (A), Insulin (B), Leptin (C), NEFAs (D), TAGs (E), Cholesterol (F), HDL-Cholesterol (G) (mean ±SEM) prior to the test meal, T=0, for all participants in the intervention group (n=8), V2(●●), V3(○○).
Data from the hormone and metabolite time course figures (figure 5.6) were converted into TAUC and IAUC values (see chapter 2 for methods) and the results are presented in table 5.10 below.

**Table 5.10:** Postprandial blood plasma analysis (TAUC, IAUC): Intervention-completers group Visit 2 vs Visit 3.

<table>
<thead>
<tr>
<th>Blood Plasma</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>P ¹a</th>
<th>P ¹b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose TAUC (mmol/L.min)</td>
<td>3900±242</td>
<td>3851±278.</td>
<td>0.10</td>
<td>0.14</td>
</tr>
<tr>
<td>Glucose IAUC (mmol/L.min)</td>
<td>689±51.2</td>
<td>694±49.8</td>
<td>0.14</td>
<td>0.19</td>
</tr>
<tr>
<td>Insulin TAUC (mmol/L.min)</td>
<td>12551±3430</td>
<td>12728±3822</td>
<td>0.24</td>
<td>0.31</td>
</tr>
<tr>
<td>Insulin IAUC (mmol/L.min)</td>
<td>91121.2±9851.2</td>
<td>94340±10141.2</td>
<td>0.34</td>
<td>0.39</td>
</tr>
<tr>
<td>Leptin TAUC (ng/min)</td>
<td>7300±2951</td>
<td>8988±2420</td>
<td>0.06</td>
<td>0.1</td>
</tr>
<tr>
<td>Leptin IAUC (ng/min)</td>
<td>1129±202.3</td>
<td>1312±197.2</td>
<td>0.1</td>
<td>0.18</td>
</tr>
<tr>
<td>NEFA TAUC (mmol/L.min)</td>
<td>310±38.1</td>
<td>295±35.5</td>
<td>0.65</td>
<td>0.69</td>
</tr>
<tr>
<td>NEFA IAUC (mmol/L.min)</td>
<td>195.2±19.8</td>
<td>210±22.41</td>
<td>0.43</td>
<td>0.55</td>
</tr>
<tr>
<td>TAGs TAUC (mmol/L.min)</td>
<td>685±88.2</td>
<td>679±70.1</td>
<td>0.31</td>
<td>0.39</td>
</tr>
<tr>
<td>TAGs IAUC (mmol/L.min)</td>
<td>168.43±41.2</td>
<td>160.2±45.12</td>
<td>0.19</td>
<td>0.22</td>
</tr>
<tr>
<td>Cholesterol TAUC (mmol/L.min)</td>
<td>4951.1±328.2</td>
<td>4902.1±333.18</td>
<td>0.10</td>
<td>0.12</td>
</tr>
<tr>
<td>Cholesterol IAUC (mmol/L.min)</td>
<td>4518±208.9</td>
<td>4498.8±220.1</td>
<td>0.24</td>
<td>0.27</td>
</tr>
<tr>
<td>HDL-Cholesterol TAUC (mmol/L.min)</td>
<td>2593±220.1</td>
<td>2505±199.1</td>
<td>0.36</td>
<td>0.39</td>
</tr>
<tr>
<td>HDL-Cholesterol IAUC (mmol/L.min)</td>
<td>2218.2±180.1</td>
<td>2205.1±185.8</td>
<td>0.26</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Data presented as Mean ± standard error of the mean (SEM), TAGs = Triglycerides. ¹a Paired t test within group, visit 2 compared to visit 3. ¹b level of significance for all initially enrolled subjects, missing data imputed using Last Observation Carried Forward imputation approach. *level of significance p≤0.05.

Overall the hormone and metabolite data followed healthy patterns and showed minimal impact after the 6-week intervention. Within group paired t-tests for the intervention-completers group, showed no statistically significant differences in TAUC or IAUC for any of the hormones or metabolites between visits 2 and 3. However, this result was not
replicated by the IAUC data for leptin (p = 0.1). No significant changes within the group were seen following intention to treat analysis.

5.6.4.5 Effect of Intervention: Appetite

Three aspects of self-reported satiety were analysed; ‘How hungry do you feel?’ (Perceived Hunger), ‘How much do you feel you can eat?’ (Desire to eat) and ‘How full do you feel?’ (Perceived Fullness). The raw data and total area under the curve (TAUC) from these three variables for visits 2 and 3 were calculated for both groups (completers) and the results are depicted in table 5.11 below.

Table 5.11: Appetite (VAS) scores, effect of 6-week intervention Visit 2 vs Visit 3 for both groups-completers.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD Visit 2</th>
<th>Mean ± SD Visit 3</th>
<th>P</th>
<th>Mean ± SD Visit 2</th>
<th>Mean ± SD Visit 3</th>
<th>P</th>
<th>2-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunger (mm)</td>
<td>32±3.9</td>
<td>31.1±4.6</td>
<td>0.61</td>
<td>30.2±1.9</td>
<td>29.3±2.7</td>
<td>0.23</td>
<td>0.12</td>
</tr>
<tr>
<td>Hunger TAUC (mm*min)</td>
<td>235±21.6</td>
<td>236.1±22.1</td>
<td>0.44</td>
<td>245.1±19.8</td>
<td>239.3±20.2</td>
<td>0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>Desire to Eat (mm)</td>
<td>35.1±3.8</td>
<td>35.7±4.0</td>
<td>0.25</td>
<td>33.4±3.1</td>
<td>31.9±3.2</td>
<td>0.20</td>
<td>0.16</td>
</tr>
<tr>
<td>Desire to Eat TAUC (mm*min)</td>
<td>365.2±40.1</td>
<td>360.7±42.3</td>
<td>0.22</td>
<td>340.19±38.3</td>
<td>325.1±35.8</td>
<td>0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>Perceived Fullness (mm)</td>
<td>52.1±2.9</td>
<td>51.1±3.1</td>
<td>0.49</td>
<td>50.5±3.2</td>
<td>53.1±2.5</td>
<td>0.19</td>
<td>0.16</td>
</tr>
<tr>
<td>Perceived Fullness TAUC (mm*min)</td>
<td>528.2±29.2</td>
<td>518±29.1</td>
<td>0.33</td>
<td>510.2±33.1</td>
<td>525.41±28.1</td>
<td>0.20</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Data presented as Mean ± standard deviation; TAUC = Mean Total Area Under the Curve. \( p^1 \) Paired t test within group, visit 2 vs. visit 3. \( p^{2a} \)-2-Way repeated measures ANOVA, significant effect of intervention. \( p^{2b} \) level of significance for all initially enrolled subjects, missing data imputed using Last Observation Carried Forward imputation approach.

Overall, following the 6-week intervention, subjective appetite ratings seem to improve (reduced perceived hunger and desire to eat and increased levels of satiety) for the intervention-completers group whereas they remained fairly stable for the control-completers group, although none of these differences were statistically significant. Paired t-test showed no significant differences between V2 vs. V3 for all appetite parameters within the control-completers group or within the intervention-completers group. A 2-Way ANOVA showed no significant difference in; perceived hunger, perceived satiety and perceived fullness within or
between each group (completers) for the mean or raw data, the total area under the curve, and following intention to treat analysis. No significant differences within and in-between groups were observed for ad libitum meal intake.

5.6.4.6 Effect of Intervention: Mindfulness

The mindful eating questionnaire provided by (Winkens et al., 2018) assessed mindfulness in 4 categories (see Table 5.12).

<table>
<thead>
<tr>
<th>Categories</th>
<th>Control (n=7)</th>
<th>Intervention (n =8)</th>
<th>2-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Visit 3</td>
<td>P</td>
</tr>
<tr>
<td>Sensory</td>
<td>11±2.45</td>
<td>11.3±2.4</td>
<td>0.17</td>
</tr>
<tr>
<td>Awareness</td>
<td>7.7±1.0</td>
<td>7.4±1.3</td>
<td>1.00</td>
</tr>
<tr>
<td>Body Cues</td>
<td>13.7±1.6</td>
<td>13.9±0.99</td>
<td>0.37</td>
</tr>
<tr>
<td>Distraction</td>
<td>6.7±1.16</td>
<td>6.9±1.1</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Data presented as Mean ± standard deviation. 1Independent t test within group, baseline compared to visit 4. 2Way repeated measures ANOVA, significant effect of intervention. 3Level of significance for all initially enrolled subjects, missing data imputed using Last Observation Carried Forward imputation approach. Significant differences presented in italics.

Table 5.12 suggests that the two completers groups responded differently to the 6-week intervention in terms of their mindfulness outcomes. Specifically, the intervention-completers group shows improved values for all 4 subscales of mindfulness after the 6-week intervention, whereas the control-completers group seems to have remained stable. This observation was statistically confirmed by paired t-tests, which showed significant differences within the intervention-completers group for all four parameters of mindfulness (Sensory p = 0.018, Awareness p = 0.020, Body cues p = 0.024 and Distraction p = 0.0014) and no significant difference within the control-completers group. A 2-way repeated measures ANOVA showed that the intervention-completers group was significantly more mindful than the control-completers group after the 6-week intervention, as there was a significant effect of time and intervention and a significant time by treatment interaction. Tukey’s post hoc test, showed a significant difference between the two completers groups for measures of Sensory (p = 0.037), Awareness (p = 0.044), Body cues (p = 0.033) and a trend for distraction (p = 0.099). Following intention to treat analysis, the significant differences for
sensory, awareness and body cues were maintained though there was no longer a trend for measures of distraction.

The MEQ results from table 5.12, were converted into a MEQ subscale change (V3-V2) graph (figure 5.7) where the changes in each mindfulness subscale by group-completers are depicted next to each other for ease of comparison.

**Figure 5.7**: MEQ Questionnaire, Visit 2 vs Visit 3 for both groups. Data presented as mean±SD. Dotted lines: Intervention-completers group. Full colour: Control-completers group. Bars with identical superscript letters are significantly different at.
5.6.4.7 Eating Rate Protocol Community Monitoring

5.6.4.7.1 Baseline data of community monitoring tools
The study’s dedicated website and phone application were used to monitor the intervention group between visits 2 and 3 allowing extraction of the following data through Mixpanel core Analytics: Duration of intervention (days), % response rate to automated log in reminders (%), number of times website was visited and whether the dedicated phone application was downloaded (yes/no). Monitoring data was successfully produced for all the participants (n=8) in the intervention group.

Table 5.13: Website monitoring data extracted for the intervention group (n=8).

<table>
<thead>
<tr>
<th>Intervention group</th>
<th>Duration of Intervention (Days)</th>
<th>Response to auto-log in (%)</th>
<th>Total number of website visits</th>
<th>Total duration of all sessions (mins)</th>
<th>Mean session duration/visit (mins)</th>
<th>App downloaded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>49</td>
<td>24</td>
<td>28</td>
<td>259</td>
<td>9.4±1.9</td>
<td>7</td>
</tr>
<tr>
<td>Females (n=4)</td>
<td>45.3±3.1</td>
<td>64±24.4</td>
<td>9.5±5.5</td>
<td>87.6±22.5</td>
<td>9.2±2.0</td>
<td>50%</td>
</tr>
<tr>
<td>Males (n=4)</td>
<td>45.8±3.4</td>
<td>70±11</td>
<td>9.5±3.4</td>
<td>94±54.4</td>
<td>9.4±1.9</td>
<td>75%</td>
</tr>
<tr>
<td>P&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.412</td>
<td>0.101</td>
<td>1.0</td>
<td>0.191</td>
<td>0.541</td>
<td>-</td>
</tr>
</tbody>
</table>

Data presented as Mean ± standard deviation. <sup>1</sup> Independent t test between genders. Total number of website visits = number of times participants from the intervention group only visited the website, during the length of the intervention.

Overall, the monitoring data extracted show that the intervention group participants did engage with the website (directly or via the mobile application) during the 6-week intervention, with the male population appearing to respond better (+6%) to the weekly automated log in email reminders and showing a greater willingness to download the mobile application than the females (75% vs. 50%). Furthermore, the participants’ regular interaction with the website/app is highlighted by the fact that they spent approximately 1.5 hours in the website over the 6 weeks, where each visit averaged just under 9 minutes for both males and females.

There were no statistically significant differences between the males and females within the intervention group for any of the website monitoring parameters.
5.6.4.7.2 Impact of website engagement on primary outcomes
Several mixed model analyses were performed in order to identify the effect of the website engagement on the study’s primary and secondary outcomes [Body composition parameters: weight, BMI, %fat, visceral fat. Eating rate: chews/minute, meal duration, energy intake: kcal, total protein, CHO, fat, fibre. Perceived appetite: perceived hunger, fullness, satiety. Mindfulness subscales: Sensory, Awareness, Body cues, Distraction.] (see all website parameters in Table 5.11).

Change in BMI score \( [V3 – V2] \) was the strongest \((|r|<0.975)\) and most significant \((p =0.003)\) correlation with 3 of the website monitoring parameters [Duration of intervention (days), Total session duration (minutes), average session duration/visit (mins)] suggesting that longer sessions (total and mean) and longer intervention duration were associated with greater change in BMI score.

5.6.4.7.3 Predictive Model of Change
The change in BMI (V2-V3) was then entered into a stepwise linear regression and produced the following predictive model of change in body composition:

<table>
<thead>
<tr>
<th>Predictive model for change in body composition (visit3-visit2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ = 0.142 - 0.084 \times \text{Duration of Intervention Days} - 0.021 \times \text{Total duration of all Sessions (min)} + 0.515 \times \text{Mean Session Duration/Visit}. ]</td>
</tr>
</tbody>
</table>

Therefore, the 3 main predictors, that would best predict a change in BMI in the study’s population were:

- Duration of intervention days \( p=0.001 \),
- Total duration of all sessions, \( p=0.001 \)
- Mean session duration/visit, \( p=0.001 \).

- e.g. for 42 days with 10 sessions of 7 minutes duration each we predict change in BMI = -1.251kg/m\(^2\).

- e.g. for 42 days with 7 sessions of 10 minutes duration each we predict change in BMI = +0.294kg/m\(^2\).
According to the predictive model of change above, the best-case scenario for the biggest change in BMI would be a long duration of intervention days, a high total session minutes but low mean number of minutes spent on the website per session. Consequently, lots of short bursts on the website is better than a few long stays on the website if change in BMI is the primary outcome.

Table 5.14: Actual change in BMI vs Predicted change in BMI

<table>
<thead>
<tr>
<th>ID (SER)</th>
<th>Visit 2 BMI (Kg/m²)</th>
<th>Visit 3 BMI (Kg/m²)</th>
<th>Change in BMI (Kg/m²)</th>
<th>Predicted Change in BMI* (Kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS001</td>
<td>27.6</td>
<td>28.1</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>MS003</td>
<td>36.4</td>
<td>35.5</td>
<td>-0.9</td>
<td>-0.9</td>
</tr>
<tr>
<td>MS007</td>
<td>28.9</td>
<td>27.4</td>
<td>-1.5</td>
<td>-1.6</td>
</tr>
<tr>
<td>MS008</td>
<td>28</td>
<td>27</td>
<td>-1</td>
<td>-0.8</td>
</tr>
<tr>
<td>MS011</td>
<td>32.9</td>
<td>32.5</td>
<td>-0.4</td>
<td>-0.3</td>
</tr>
<tr>
<td>MS012</td>
<td>29.8</td>
<td>29.1</td>
<td>-0.7</td>
<td>-0.8</td>
</tr>
<tr>
<td>MS013</td>
<td>28.2</td>
<td>26.8</td>
<td>-1.4</td>
<td>-1.5</td>
</tr>
<tr>
<td>MS017</td>
<td>26.2</td>
<td>25</td>
<td>-1.2</td>
<td>-1.1</td>
</tr>
</tbody>
</table>

ChangeBMI = Actual change in BMI (Visit 1-visit4) as measured in the study. PredChangeBMI= Change in BMI using the predictive model of change.

Table 5.14 presents the data of change in BMI and the predicted change in BMI when our predictive model of change is used. These data were transformed in a graph (figure 5.8) where the actual change in BMI and predicted change in BMI for the intervention group (n=8) is clearly depicted and their close correlation is visible.

![Figure 5.8: Actual Change in BMI vs Predictive Model of Change in BMI.](image)
5.6.5 Power calculation
To assess the possibility of type two error, retrospective power calculations were conducted for a secondary outcome measure, perceived hunger. For the IAUC for perceived hunger, retrospective power calculations determined a two-sided 0.05 significance level, the study had 80% power to detect a mean difference of 4.1 mm*min between the treatment groups (Intervention vs. Control), based on a pooled standard deviation of 2.5mm*min.

5.7 Discussion
This study aimed to investigate if the SER protocol can facilitate weight loss in overweight adults in a free-living community setting. In addition, we aimed to investigate if the SER protocol can reduce the habitual eating rate of overweight adults after a 6-week intervention, and investigate the possible mechanisms of action by assessing the hormone and metabolic changes associated with the SER. This study also aimed to investigate if the SER protocol could facilitate more mindful eating in a group of overweight adults and if a dedicated website could be an effective monitoring tool for measuring eating rate in a free-living community setting. To the author’s best knowledge, this is the first study to investigate the short and long term effects of a slow eating rate protocol in the adult community, as most studies to date have been either self-reported (Ohkuma et al., 2015) only in a clinical setting (Kokkinos et al., 2010) or have focused on children/adolescents (Ford et al., 2010, Hamilton-Shield et al., 2014).

5.7.1 Demographics
Fifteen participants, 8 females and 7 males, completed this study. There were no significant differences in anthropometric data between the two genders. This is important as anthropometric differences could be expected to influence the hormone, metabolic and eating rate outcomes (Chapter 1). All participants reported no significant differences in alcohol consumption per week a month prior to the study session; however no caffeine, alcohol or carbonated drinks consumption were allowed 24-hours prior the study session in order to control for their effects. All participants had no significant medical history and food allergies and they were all non-smokers, and within the study’s criteria for age and BMI.
5.7.2 Eating rate, Body Composition and Energy Intake

The intervention completers group showed a significant change in body composition parameters by reducing (seven out of eight participants showed positive change) their weight (p= 0.006), BMI (p=0.006), body fat % (p= 0.026) and visceral fat (p= 0.007) after the 6-week intervention whereas the control completers group maintained a stable body composition, a difference which remained significant after ITT analysis. Therefore, the expected primary outcome of our hypothesis has been achieved. The observed differences in body composition parameters, could be explained by the differences seen in energy intake. Specifically, the intervention-completers group had significantly less Total fat(g) p = 0.026 and Total carbohydrates (p = 0.041) and a trend towards reduced energy intake (p=0.086) after the 6-week intervention. These differences were maintained post ITT analysis, and are of interest as the group following the SER protocol significantly reduced their total fat and carbohydrate intake without being given any particular-dietary or physical activity instructions. Slower ER is associated with lower energy intake, regardless of the type of manipulation used to change eating rate (Robinson et al., 2014). The strong influence of ER on energy intake has been suggested by several epidemiological reports, acute feeding studies and meta-analyses (Viskaal-van Dongen et al., 2011, Robinson et al., 2014, Ohkuma et al., 2015, Zhu et al., 2015, Fogel et al., 2017, van den Boer et al., 2017). However, despite apparent concordance with the previous literature and the apparent confirmation of our primary outcome, this study did not find a significant change in ER, when assessed as either chews/minute (p=0.55) or meal duration (p = 0.12) after the 6-week intervention.

Studies as early as the 1970’s (Bellack, 1975) have been advocating that reducing ER is a simple and effective method for reducing food intake and body weight (see chapter 1.3.2 for further details). Furthermore, several (acute feeding, epidemiological reports and meta-analyses) studies have highlighted that a promising means to combat overweight may lie in reducing ER (Martin et al., 2007), including a systematic review by Robinson et al., (2014) and a systematic review by Ohkuma et al (2015) highlighted that eating quickly is associated with excess body weight (see chapter 1 for further details). In addition, previous work from the author of this study (Koidis et al., 2014), showed significant correlations between a fast eating overweight group and body composition parameters (BMI, Visceral fat score and %Trunk fat) when compared to a fast eating healthy weight group, though these correlations were not likely causative rather than highlighting that overweight people have a higher ER than healthy weight people. To the author’s best knowledge, the only community weight loss
intervention manipulating ER was that of Forde et al (2010), where the Mandometer (see chapter 4.1) was used in adolescents over 12 months. Those in the Mandometer group reduced their speed of eating (measured in grams/minute) by -11% compared with a gain of 4% in the standard arm, alongside which the intervention group also significantly reduced their fat mass and BMI compared to the control group. However, although the above studies are informative to our hypothesis, the lack of change in habitual ER after the -week intervention, suggests that the effects in body composition and trend on energy intake was mediated by a different mechanism and not change in eating rate.

An acute ER study by Hermans et al (2017) which was carried in a naturalistic setting (laboratory furnished as a small restaurant), studied the effect of real-time vibrotactile feedback delivered through an augmented fork on eating rate, satiation and food intake (See chapter 1, Section 1.4). The study found that the use of the augmented fork successfully decelerated participants’ eating rates by reducing significantly (p=0.011) the number of bites/minute for the intervention group (FC: 4.55 bites/min NFC 5.28 bites/min). Furthermore, the intervention extended by +1.32mins their meal duration compared to the control group. In the current study, the intervention completers group were also able to increase their meal time by +1.7minutes vs 0.2minutes for the control-completers group (Table 5.7) though this difference was not statistically significant which, combined with a lack of accepted clinically relevant cut offs for outcomes such as meal duration, makes it difficult to draw robust conclusions as to the effect of the intervention of eating rate or the relationship between eating rate and the favourable body composition changes seen.

Andrade et al (2008) performed a study that also used a set of instructions to slow ER. The study involved 30 women (mostly healthy weight, only 4 had a BMI> 25) where an ad libitum pasta meal (870kcal) was consumed with slow eating rate instructions (small bites, small tea spoon and put it down between bites, chew each bite 20-30 times) and fast eating rate instructions (large soup spoon, eat as quick as possible, no pauses between bites). During the slow ER condition the meal duration was extended by approximately 21 minutes and the total energy intake, expressed in kcal/minute, was significantly reduced by 66kcal compared to the fast ER condition. The reduction in ER in Andrande’s study appears to have been achieved by the specific instructions to decrease bite size and utensil size which have been shown to reduce ER (Almiron-Roig et al., 2015, James et al., 2018). Interestingly, in our study, after the 6-week intervention the SER group extended their meal duration by 1.7
minutes and consequently reduced their energy intake/minute by 14kcal, a difference which was not statistically significant.

Llwellyn et al (2008), in a twin study of 10-12y old children and the first of its kind showed that faster eating was found to be a heritable behavioural phenotype related to higher weight. Sugimori et al (2004) studied 8,170 children in the Toyama Birth Cohort study at ages 3 and 6 years. Children were classified in terms of their eating speed, based on parent reports, at both assessment times. Compared to children who were normal-weight at both ages 3 and 6 years (prevalence = 5.7%), slightly rapid or rapid eating was significantly greater among children who were normal-weight at age 3 years but became obese at age 6 years (prevalence = 22.6%) as well as in children who were obese at both ages 3 and 6 years (prevalence = 26.8%). Berkowitz et al (2010) found that a faster eating rate at 4 years of age, expressed as mouthfuls of food/min at a single laboratory test meal, predicted a greater BMI and fat gain in children between ages 4 and 6 years. In our study, 73.6% (14 out of 18 participants that attended visit 2, baseline) had a high risk -linear eating style [a population percentage in agreement with Kissileff (2001) suggesting that most obese people fit a linear eating style], and all completers maintained their original eating style, despite the positive effects on body composition and energy intake seen in the intervention group. Consequently, perhaps the SER protocol could not cause a significant change in ER due to the strong genetic behavioural phenotype of ER. To the author’s best knowledge there are no studies that have followed people with specifically defined ER phenotypes over-time in order to assess their ability to change, therefore different parameters of the SER protocol like the effects of mindfulness and technological intervention are responsible for the confirmation of the study’s primary outcomes.

5.7.3 Appetite and Blood Plasma and Metabolites
Our results failed to support the hypothesis that the SER protocol will result in greater hormonal and metabolic changes in the intervention group as compared to the control groups, specifically decreased glucose, insulin, NEFA, leptin and blood lipids. A trend (p=0.0593) was observed for decreased TAUC leptin levels in the intervention group, which is in agreement with current literature as slower eating rate is associated with enhanced satiety (Andrade et al., 2008, Privitera 2012, Martin 2007, Krop et al., 2018). However, the TAUC leptin trend disappeared when analysing for ITT and IAUC analysis showed no significant
difference which weakens the observed trend. Interestingly, Murer et al., (2011) suggested that serum leptin can be a weight loss predictive factor in weight loss interventions. In his study, 203 children and adolescents participated in a 2-month inpatient weight-loss intervention (diet, exercise and behavior intervention). The authors were able to confirm that higher baseline serum leptin was associated with weight loss. At the end of the intervention, total weight loss was approximately 13.9kg and fat loss 9.2 kg with a 76% decrease in serum leptin levels. Baseline leptin also represented a negative predictor for % fat loss, even at 12 months follow up though this could be due to medical intervention following participation in the study.

Brownell et al (2000) have also suggested that eating slowly prolongs the pleasure sensations and thereby decreases the feelings of deprivation (cited in Martin et al., 2007). Krop et al., (2018) in their recent comprehensive systematic review and meta-analysis, confirmed a significant effect of oral processing oral processing aspects related to chewing on both self-reported hunger (-0.20 effect size, 95% confidence interval (CI): -0.30, -0.11) and food intake (-0.28 effect size, 95% CI -0.36, -0.19). Our subjective appetite results (perceived hunger p = 0.12, Satiety p = 0.13 and Fullness p = 0.16) are in agreement with the general literature consensus as the lack of change in ER was accompanied by no change in subjective and objective measures of satiety. A recent study by Hermans et al (2017) failed to show an increase in satiation although ER was successfully reduced and in Krop’s (Krop et al., 2018) systematic review only 10/42 studies showed an effect of chewing on appetite whereas the rest showed no effect. Consequently, there does not seem to be a clear link between ER and appetite, though the results of our study are not helpful in clarifying this relationship further.

Ford’s adolescent community ER weight loss intervention with the use of the Mandometer (Ford et al., 2010) also did not find differences in perceived levels of satiety at 6, 12 and 18 months, despite the differences detected in body composition parameters. This further highlights that changes in ER might not translate in direct changes in body composition, but rather that ER has a small contributing part of the multifactorial nature (Roberts et al., 2015) of weight loss.
5.7.4 Mindfulness and the MEQ

As it can be seen from figure 5.7, the intervention-completers group had a significantly improved sensory score compared to the control-completers group, suggesting an improved ability to be concentrated when eating as well as notice food aromas and smells. Furthermore, the intervention-completers group also showed an improved awareness score, whereas the control group’s score was reduced, suggesting less awareness when eating/snacking on food. The intervention-completers group also achieved higher Body cues score than the control-completers group, meaning an improvement to better trust their body about appetite stimuli (i.e. what to eat, when to eat, when to stop eating). Finally, the trend (p=0.099) towards a difference between the two completers groups for measures of Distraction is visible, suggesting an improved ability to concentrate on the food only when eating and not get distracted by other thoughts or multitasking in the intervention completers group.

Our results are in agreement with our hypothesis that the SER protocol will result in increased mindful eating in the intervention (completers and baseline participants) group as compared to the control group, with increased Sensory (p=0.037), Awareness (p=0.044) and Body cues (p=0.33) and a trend for improved distraction (p=0.099, no trend post ITT analysis) scores although it would appear that these changes are not mediated by a reduction in eating rate. Warren et al., (2017) in their recent literature review highlighted the lack of compelling evidence for the effectiveness of mindfulness and mindful eating in weight management, though did suggest that encouraging a mindful eating approach is a positive message to be included in general weight management advice to the public. Despite the various tools developed to allow self-report of mindfulness (Park et al., 2013, Sauer et al., 2013) it is often argued in the literature (Clementi et al., 2017, Warren et al., 2017) that a measure developed in a specific domain of mindfulness (i.e. eating rate) may be more appropriate as mindfulness is a learned skill (Bishop et al., 2011). As Tapper (2017) noted, most studies that have examined the use of mindfulness for weight loss have employed a combination of mindfulness and non-mindfulness techniques, making it difficult to establish the independent effects of the mindfulness components (see chapter 1, section 1.5 for full study analysis). In agreement with Tapper (2017) and Warren et al (2017), in the current study the ER of both groups remained (statistically significant) unchanged and so did perceived appetite though the mindfulness score of only the intervention group improved significantly. Therefore, the improvement in mindfulness seems to be a significant contributing factor to the positive body composition changes of the intervention group,
though the degree of contribution is unclear given the multifactorial design (ER, appetite, technology) of the intervention. In agreement with our findings, Jordan et al (2014) found that induced (state and trait) mindfulness predicted lower energy intake; however the results were not differentiated by how healthy the available foods were (almonds, pretzels and candy). Self-reported trait mindfulness was related to healthier food choices (fruit were selected over treats as a “thank you for participating” gift at the end of the study) a relation mediated by self-reported preferences for healthy or unhealthy foods.

Interestingly, Kristeller and Wolever (2010) have proposed a theory, whereby mindfulness enhances awareness of and responsiveness to satiety cues, and therefore functions adaptively to reduce energy intake. In our study, the intervention group that increased their mindfulness did also decrease their energy intake and achieved weight loss though no statistically significant differences were found in subjective measures of satiety or appetite hormones, suggesting that mindfulness was not mediated through satiety cues but perhaps in a more direct manner (see Figure 5.9). An example of the aforementioned direct manner can be seen in the study by Arch et al (2016) suggesting that brief mindfulness led to lower consumption of “junk” food, where brief mindfulness was achieved by making the participants (undergraduates, intervention group: focus on food, control group: distraction from food) focus on their food with a set of verbal instructions. This could explain how the intervention group in our study lost weight and consumed less fat and carbohydrates by potentially consuming less “junk” food during the 6-week intervention.

Our data also showed that the intervention group seemed to have higher baseline mindfulness (sensory, awareness and distraction) though this was not statistically confirmed. Two studies on young overweight adults by the same research group, Mantzios and Wilson (2014) Mantzios et al., (2015), have examined the ability of baseline mindfulness measures to predict weight change. In these studies, higher baseline mindfulness scores were a significant predictor of weight loss, however this hypothesis was recently disproven by Fuller et al., (2017) in a 12-month diet and exercise program of 137 overweight and pre-diabetic or type II diabetes participants, where the Five Facet Mindfulness Questionnaire was used.
Figure 5.9: Gut-brain axis and food intake regulation schematic with proposed SER effects.
5.7.5 Website Monitoring Data

Technology-based interventions, supported by electronic/technological devices (i.e., e-health interventions) are expected to increase accessibility and control for both the user and the health care professional (Eysenbach, 2001) and increase cost-effectiveness relative to the traditional in-person interventions (Smit et al., 2011). Furthermore, only in the last 3 years, (up to 2017) at least five e-health meta-analyses on weight management in adults have been published indicating that behavioural e-health interventions are adequate for weight management (e.g., Hutchesson et al., 2015; Raaijmakers, Pouwels, Berghuis, & Nienhuijs, 2015; Seo & Niu, 2015; Sherrington et al., 2016; Tang, Abraham, Greaves, & Nikolaou, 2016).

The data extracted from the study’s dedicated website and mobile application, suggested that Duration of intervention (days), Total session duration (minutes) and average session duration/visit (mins)] were the three most significant data monitoring parameters and that the change in BMI score correlated the strongest (|r|<0.975) and most significantly (p =0.003) with these parameters.

The study results confirm that data produced from our dedicated website were very informative for future studies on ER interventions in the community where the focus should be on driving the study participants often and for a short amount of time to the study website/mobile application. Furthermore, our predictive model of change could be adopted by future ER studies targeting changes in body composition as this could help them better design the frequency and duration which they will want the users to visit their website by.

As discussed in in Chapter 1, the use of specialised equipment in most ER studies has been described as too artificial’ (Allirot et al., 2012) or too cumbersome to be adopted in the community (Hermans et al., 2017). The protocol used in our study was easily accessible via the website or mobile application and arguably significantly less invasive than the Mandometer as no training or specialised equipment was necessary in order to access it. Furthermore, in the United Kingdom, overall 39% of overweight adults reported at least one weight management aid, with use of websites or mobile phone apps coming second to attending the gym or other exercise (Health Survey for England 2016) highlighting that a significant part of the general overweight population is already accustomed to using websites and apps for weight management purposes.
5.8 Study limitations

A clear limitation of the current study is its small sample size (n =15) which might be responsible for the lack of significant differences in ER and perceived appetite. However, several ER studies (de Wijk et al., 2008 and 2008, Cassaday et al., 2009, Smit et al., 2011, Forde et al., 2013 and Kommai et al., 2016) which were also included in the recent systematic review by Krop et al (2018), had the same or smaller numbers of participants. Consequently, the number of participants in this intervention is unlikely the reason why ER did not change.

Furthermore, the intervention group was contacted twice-weekly via the automatic email reminders whereas the control group was not contacted at all during the 6-week period of the intervention. There is some evidence (Digenio et al., 2009) that treatment intensity might affect the outcome of weight loss interventions and therefore contact time merits further evaluation.

Another limitation of this study was that 3 participants (2 intervention, 1 control) did not provide their 3-day food diaries after the 6-week intervention. Furthermore, energy underreporting was detected at baseline, which although was reduced following the intervention, still questions the reliability of the reported energy intake for both groups. Finally, phase of menstrual cycle was not controlled for which could have impacted on appetite measures and metabolism in general.

5.9 Conclusion

The present study indicates that a SER protocol can be effective in improving body composition parameters and improving mindfulness in a free-living overweight population however this may not be mediated by a direct impact on eating rate and instead it may be that regular monitoring and increasing attention to diet are the primary drivers for beneficial effects. However, there is a clear need for categorising the level of reduction of the habitual ER of overweight populations and its impact on weight management, appetite regulation, energy intake and mindfulness.

Furthermore, the current study showcased the usefulness of technology integration in monitoring ER in the community which can inform future ER studies.
Chapter 6

General Discussion
6.1 Introduction

The worldwide prevalence of overweight/obesity is a cause for concern (Finucane et al., 2011) as excessive weight is associated with a wide range of chronic health conditions such as diabetes (Malnick et al., 2006) and poorer mental health (Crandall et al., 2009) impacting the wider population (Dobbs et al., 2014). A promising means of combating overweight may lie in reducing eating rate (ER) (Martin et al., 2007; Robinson et al., 2014). People who eat quickly tend to consume more than slower eaters (De Graaf & Kok, 2010; Robinson et al., 2014; Viskaal-Van Dongen, Kok, & De Graaf, 2011) and feel less satiated after a meal (Rolls, 2007; Zijlstra, DeWijk, Mars, Stafleu, & De Graaf, 2009). Eating rate may influence satiation levels and energy intake through a number of mechanisms. When people eat slowly, this influences the secretion of satiety hormones and increases food oral exposure (Weijzen, Smeets, & De Graaf, 2009; Bolhuis, Lakemond, De Wijk, Luning, & De Graaf, 2011) and the number of chews per unit of food (Bolhuis, Lakemond, De Wijk, Luning, & De Graaf, 2013; 2014), which later two have been shown to lower energy intake (Bolhuis et al., 2013; 2014; Weijzen et al., 2009). Finally, slower eating may decrease feelings of deprivation by enhancing and prolonging pleasurable aspects of eating (Brownell, 2000).

Traditional diets that focus on calorie-restriction have been mostly unsuccessful in achieving long term weight loss (Schaefer et al. 2014) while non-dieting interventions that encourage eating as a response to internal hunger and satiety cues have shown improved eating patterns (Clifford et al. 2015). As such the practise of mindful eating is considered an emerging weight management approach (Kristeller and Wolever 2010, Lofgren 2015, Clementi et al 2017) that is based on awareness of emotional and physical sensations associated with eating which may allow individuals adopting it to better recognise internal cues of satiety and hunger (Framson et al 2009). However, the mechanisms by which these interventions bring about their effects are not yet fully understood, and it is not always clear whether all components are responsible for the claimed benefits (Tapper, 2017). Despite the various tools developed to allow self-report of mindfulness (Park et al 2013, Sauer et al 2013) it is often argued in the literature (Clementi et al 2017, Warren et al 2017) that a measure developed in a specific domain of mindfulness (i.e. eating rate) may be more appropriate as mindfulness is a learned skill (Bishop et al., 2011).

Combined with this a move towards web-based weight loss interventions shows promise being effective in achieving weight loss, of low cost and user friendly (Harvey-Berino 2010,
Neve et al., 2010, Armen and Irwin et al., 2011). To date, only a few studies have investigated the effect of specific eating rate protocols in a community population since most eating rate studies are either self-reported (Ekuni et al., 2015) solely clinical (Kokkinos et al., 2010) or focusing on children/adolescents [Ford et al 2010, Hamilton-Shield et al 2014]. Furthermore, training people to eat more slowly in everyday contexts requires creative and engaging solutions (Hermsen et al., 2016).

Effective and easy-to adopt tools and methods manipulating ER, as a means to cultivate mindful eating, and conversely inhibit impulsive eating (Teper et al., 2013), therefore need to be developed, tested and brought to everyday life to see if their potential to contribute to weight management can be realised.

6.2 Brief summary

This PhD project focuses on four major areas: eating rate monitoring/assessment techniques, hormonal and metabolic responses to a slow eating rate (SER) protocol, appetite control associated with SER and the association between eating rate and mindfulness. The overarching aim of the programme of work was to develop and test the SER protocol in overweight-free living adults and investigate the protocol’s effects on weight loss, and the mechanism(s) by which any such effects may be exerted, for example hormonal regulation or mindfulness.

This thesis focused on the development and testing of a specific slow eating rate protocol (SER) based on previous work by the author (Koidis et al 2014) and it can be divided into two main parts, the developmental part of the SER protocol and then its testing to the overweight population in the community. The developmental phase spanned 5 studies, focusing on; refining and finalising the protocol (Studies A and B, chapter 3), transforming it into an online weight loss tool (Study C, chapter 4), developing a prototype for automatic ER detection (Study D, chapter 4) and investigating if a mindful eating questionnaire is a good marker of ER (Study E, chapter 4). The second part of this PhD programme was a community intervention study, utilising the SER protocol developed and testing its potential as a weight-loss tool in overweight adults (chapter 5).

The overview of the findings from the studies which comprise this thesis will be discussed in the sections below, alongside their potential implications and limitations.
6.3 Developing a successful slow eating rate protocol

6.3.1 SER protocol, a set of instructions based on volunteer feedback

In the first study (technical developmental study A, Chapter 3) volunteer feedback (6 healthy volunteers) was utilised to further develop the previously developed slow eating rate protocol (Koidis et al 2014). As discussed in chapter 3.1.6.2 basing our SER protocol development on PPI did make it more effective all participants in the studies that followed (developmental studies B, C and the final community intervention) showed very good concordance in following the protocol’s guidelines. Furthermore, another strength of the SER protocol is that as it was based on a set of simple instructions, and therefore easy to convert into a 2-minute user friendly video and incorporate it into our dedicated website/app, thus creating an online weight management tool (Study C and final community study). The website/app user pathway and 2-minute SER video were piloted on five volunteers (for a week) who reported following the SER with ease, its usefulness in reminding them to follow the SER instructions daily that it caused no interruption to their daily routine or natural meal flow. We are unable to distinguish if it is the SER protocol’s video and graphics format that elicits learning (Bétrancourt and Benetos 2018) or its easy online (web/app) access, or a combination of the two which warrants further study investigation in the future. However, data gathered from our dedicated monitoring website in the final community study (Chapter 5) further confirmed that the SER protocol was successfully adopted by the intervention group; based on engagement time spent on the website/app (1.5 hours on average per volunteer over the 6 weeks) which was reflected on the success of the primary outcomes of the final study (improvements in body composition, reduced energy intake and improved eating mindfulness).

Finally, the instructional nature of the SER protocol and the fact that it was easily adopted in the community, improve its potential of easy incorporation (and dissemination) into national lifestyle change guidelines for overweight or obese people. As such, slow eating rate is mentioned as part of the latest, 2018 NICE guidelines as one of the recommended lifestyle changes for adults who are overweight and obese, without though any specific details on how to achieve slow eating. Given the omnipresence of the internet and its adoption by a wide
range of the population (see Chapter 1) our user-friendly video protocol could be easily incorporated into a website link within the NICE guidelines, which the public could utilise and further understand how to slow their eating in a simple way.

6.3.2 Monitoring eating rate in a clinical setting

As part of the second developmental study (Study B) we tested the use of QTM software and movement analysis to automatically detect ER in an attempt to make ER detection less cumbersome and time-efficient than the gold standard (at the time) of counts by an observer (Ekuni et al., 2012). The use of the QTM software and its motion detection technology showed poor reproducibility and did not test well against counts by observers; we therefore did not pursue this ER automatic detection mechanism. Although manual counts by an observer (via video playback) is a reliable ER detection technique in use since the 70’s (see chapter 1, section 1.4) it is cumbersome as it requires long time to process (by at least 2 different observers followed by an internal validation of chew counts) and use of additional members of staff, we therefore pursued the creation of an automatic ER detection system via our collaboration with the robotics department. In study D, AlexNet was a successful automatic ER detection system (80% accuracy) as it allows faster and more efficient ER video data processing. The novel software of AlexNet was also used to successfully analyse data from the final study clinical days (visits 1,2,3 and 4, see Chapter 5) and represents a promising automatic detection system for future ER studies. Furthermore, as AlexNet can detect data from any video as long as the face, mouth and jaw area are visible, it has the potential of being integrated in automated ER screening tools where the public can simply consume a meal whilst recorded (by their phone/laptop/computer’s camera) and then given feedback on whether their eating style is a risk to their health or not (linear or decelerated eating style). If a dangerous eating style was to be detected, users could then be prompt to a web link with the video SER protocol which they could use in order to reduce their health risk, especially if they belong to the overweight/obese category.
6.3.3 Additions under development to the SER protocol

Although the preliminary version of Winkens et al (2018) MEQ questionnaire did not seem as a valid proxy for measuring ER (Study E), its final and published version provided valuable information on subscales of mindful eating. In the community intervention study, the intervention group significantly improved their mindfulness score in all four subscales by following the SER protocol for 6 weeks, adding a novelty element to the study.

Developmental study B, showed that the addition of earplugs in a SER protocol, although novel, did not show a statistically significant change to subjective perceived satiety (Koidis et al 2018). Given the increasing global popularity of headphones with noise-cancelling ones having the highest sales in the UK (©Statista 2018) further studies of the headphone-induced occlusion effect on appetite in a larger and more varied population are warrant.
6.4 Does slow eating rate facilitate weight loss?

Following the 6-week community intervention, the intervention completers group as compared to the completers-control group showed a significant change in: 1) body composition parameters; weight (p = 0.006), BMI (p=0.006), body fat % (p = 0.026) and visceral fat (p= 0.007), 2) a significant reduction in total fat (g) p = 0.026 and total carbohydrates (p = 0.041) and a trend towards reduced energy intake (p=0.086), 3) increased mindful eating [ increased Sensory (p=0.037), Awareness (p=0.044) and Body cues (p=0.33) and a trend for improved distraction (p=0.099, no trend post ITT analysis)]. However, the intervention completers-group compared to the control-completers group showed no significant change in subjective appetite, hormones and metabolites and in ER [chews/minute (p=0.55) or meal duration (p = 0.12)].

As ER did not significantly change over the intervention period, and consequently the well documented mechanism of slower ER and reduced appetite (Robinson et al., 2014, Krop et al., 2018) did not cause the improvements observed in body composition parameters. However, as there were significant changes in mindfulness, it seems that the SER protocol utilises psychological mechanisms and not physiological ones. Such mechanisms have been proposed by Ferriday et al (2015) suggesting that slower eating might affect episodic memory (making the memory of the meal eaten slower more vivid) as well as the study by Arch et al (2016) where brief mindfulness induced by a set of instructions led to lower consumption of “junk” food. In agreement with the potential psychological effect of the SER protocol is also our developmental study B, where addition of earplugs to the SER protocol did not significantly affect meal duration nor enhance satiety although there was a positive effect on the SER-In group.

The predictive model of BMI change (Chapter 5) is a novelty, that although applies only to the population studied, it can be further tested in larger population cohorts and validated.
Given the current socioeconomic burden of the obesity epidemic (Finucane et al., 2011) predictive models like the one produced in this study could:

- Inform weight loss interventions, suggesting that participants will respond better to regular but small input instead of large input less regularly (i.e. weekly brief consultations sessions of 20 minutes instead of monthly hourly sessions)

- Inform future web/app-based interventions in structuring the user interface with small regular inputs (i.e. through regular notifications/messages) in order to achieve maximum results

- Inform the frequency of the monitoring of the intervention in the community to avoid overwhelming the participants and ensuring adherence to the protocol. In our study, twice/week automatic email contact was not reported as cumbersome or disruptive by any of the participants in the intervention group.

- Predictive models can also help clinicians/healthcare practises detect/correct underperformance of a client during their weight loss journey. i.e. if a group of individuals visiting a weight management practise are not achieving their predicted change in BMI, health care practitioners can take early action and investigate potential solutions.
6.5 Improvements/Limitations

Our final study (chapter 5) suggests that improved measures of mindfulness might have a direct impact on energy intake though future study designs should allow better community monitoring of energy intake so the underlying mechanism (i.e. healthier food choices, smaller portions, less meals consumed) can be investigated. Such investigation could be pursued by adding an electronic diet diary to the website/app monitoring tool (ability to take daily logs and pictures), therefore allowing participants to report their intake in an easier way.

An important limitation in the final study (chapter 5) is the lack of investigation of the effects the SER could have on satiety peptides. As discussed in the introduction, previous studies have shown that slow eating rate acutely modulates the release of satiety peptides such as GLP-1 and PYY, an effect which might have been missed in this study.

Given the study’s limitations (small number of participants, underreporting in the 3-Day food diaries) further studies on a broader and more diverse population are warrant. A longer-term study with a 6 and 12 month follow up would inform us of the longer-term effects of the SER protocol and its sustainability as a weight loss tool for the overweight public. Furthermore, incorporating indirect calorimetry in the study design of future SER studies, will allow for more accurate calculation of the participant’s energy expenditure (A PAL of 1.4 was assumed in this study) and consequently more accurate estimates of the accuracy of energy intake reported.

The lack of change in ER in the final study highlights the importance of establishing specific ER cut offs in chews/minute and meal duration, in order to better inform future ER studies. Furthermore, future ER studies should establish if/how much habitual ER can change or deviate from a genetically pre-disposed level (Llwellyn et al., 2008) so its underlying mechanisms (physiological, psychological) can be better understood.

The custom-made ER monitoring software AlexNet showed promising results as it was reliable, efficient and non-invasive and due to its simple functionality (video playback feed) it can be used in large data ER studies with minimal equipment (video camera and AlexNet software). In future, and through further collaborative studies with the mechanical engineering department the software could become more robust by feeding it larger volume of data and “teaching” it to detect mouthfuls, swallows as well as automatic detection of energy intake (via video-imaging, see section 1.4 in Chapter 1) as well as automated feedback designed to help people slow their eating speed and meal duration [via screen messages like
the Mandometer (Forde et al 2010) or device vibrations (Hermans et al., 2017)]. Furthermore, integrating appetite rating score (VAS) in the community-monitoring software, so daily variability of subjective appetite rating, during long term interventions can be monitored too, would be an interesting investigation.

6.6 Summary

The growing obesity epidemic and the lack of long term sustainable results from traditional dietary approaches require new weight management methodologies, focusing on behavioural approaches and in-sync with technological advancements available to the general public. In this project a protocol focusing on parameters of slow eating was developed, tested and enhanced with a technological arm. The developed SER protocol showed novel findings as a weight management tool in the free-living overweight population through its effects on body composition and mindfulness. This project has also highlighted areas of ER requiring further investigation (heritability, cut offs) and has showcased the potential of the SER protocol to be further developed in future ER studies and be integrated as an added electronic tool in weight management interventions in the community.
Bibliography


Blundell JE (2006) Perspective on the central control of appetite. Obesity (Silver Spring, Md.) 14: 160S-3S.


Bolhuis DP, Lakemond CM, de Wijk RA, Luning PA, de Graaf C (2013) Consumption with large sip sizes increases food intake and leads to underestimation of the amount consumed. PloS one 8: e53288.


Ferriday D, Bosworth ML, Godinot N, Martin N, Forde CG, Van Den Heuvel E, Appleton SL, Mercer Moss FJ, Rogers PJ, Brunstrom JM (2016) Variation in the oral processing of
everyday meals is associated with fullness and meal size; a potential nudge to reduce energy intake? Nutrients 8: 315.


Forde CG. (2018) From perception to ingestion; the role of sensory properties in energy selection, eating behaviour and food intake. Food Qual Prefer 66, 171-7


National Institute for Health and Care Excellence (NICE) (2018) Lifestyle changes for adults who are overweight or obese. Available at: https://pathways.nice.org.uk/pathways/obesity


Appendices

Appendix 1: Mandometer

The Mandometer was developed at the Section of Applied Neuroendocrinology and Mandometer Clinic, Karolinska Institutet, Stockholm, Sweden. It is a portable weighing scale connected to a small computer that can generate a graph representing food removal from the plate, with weight of food (grams) on the y axis and time (minutes) on the x axis. The user puts a measured portion of food determined by a therapist on the scale and the computer records and displays, in real time graphics, the weight loss from the plate as the user eats: time zero on the graph effectively displays total portion size. Removing food from the plate generates a gradually developing line on a screen that can be compared and matched to a preset eating line displaying the speed at which the therapist wants the user to eat. Deviation from the training line by eating too quickly or slowly elicits a spoken request from the Mandometer to slow down or eat faster. At regular intervals, a rating scale appears on the screen and the user rates their level of fullness: from 0 (no satiety) to 100 (maximum satiety). That rating appears as a dot on the screen, yielding a “development of satiety” curve and allowing comparison of the development of fullness to a “normal” fullness curve again preset on screen. During training the user gradually adopts a more normal pattern of eating and satiety by following these training lines and curves. A short video showing Mandometer training is available at www.someguys.se/clients/mandolean/mandometer_popup.html (Ford et al., 2010).
Appendix 2: Visual Analogue Scale: Food and Opinion Questionnaire

Study Day_______________            Time__________
Participant code_________________________________ Date___/___/___

Visual Analogue Scale

Answer the questions by placing a vertical mark through the line for each question. Mark the line according to how you feel at this moment. Regard both the ends of the lines as indicating the most extreme sensations you have ever felt.

How drowsy do you feel?
Drowsy | Alert

How tense do you feel?
Tense | Relaxed

How happy do you feel?
Happy | Sad

How friendly do you feel?
Friendly | Angry
How uncertain do you feel?

Uncertain | Confident

How clear-headed do you feel?

Muddled | Clear-headed

How interested do you feel?

Interested | Disinterested

How hungry do you feel?

I am not hungry at all | I have never been more hungry

How much do you think you can eat?

Nothing at all | A lot

How full do you feel?

Not at all full | Extremely full
How thirsty do you feel?

| Not at all thirsty | Extremely |

Would you like to eat something sweet?

| No, not at all much | Yes, very |

Would you like to eat something salty?

| No, not at all much | Yes, very |

Would you like to eat something savoury?

| No, not at all | Yes, very much |

Would you like to eat something fatty?

| No, | Yes |
Appendix 3: Three Factor Eating Questionnaire

Participant code_____________________________ Date ___/___/___

Please answer the following questions as carefully and honestly as possible.

Read each question and simply fill in the column, which best applies to you.

<table>
<thead>
<tr>
<th>Question</th>
<th>Never</th>
<th>Seldom</th>
<th>Sometimes</th>
<th>Often</th>
<th>Very often</th>
<th>Not relevant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. If you have put on weight, do you eat less than you usually do?</td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>2. Do you have a desire to eat when you are irritated?</td>
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<tr>
<td>3. If food tastes good to you, do you eat more than you usually do?</td>
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<tr>
<td>4. Do you try to eat less at meal times than you would like to eat?</td>
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<tr>
<td>5. Do you have a desire to eat when you have nothing to do?</td>
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<tr>
<td>6. Do you have a desire to eat when you are depressed or discouraged?</td>
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<tr>
<td>7. If food smells and looks good, do you eat more than you usually eat?</td>
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<tr>
<td>8. How often do you refuse food or drink offered because you are concerned about your weight?</td>
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<tr>
<td>9. Do you have a desire to eat when you are feeling lonely?</td>
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<tr>
<td>10. If you see or smell something delicious, do you have a desire to eat it?</td>
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<tr>
<td>11. Do you watch exactly what you eat?</td>
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<tr>
<td>12. Do you have a desire to eat when somebody lets you down?</td>
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<tr>
<td>13. If you have something delicious to eat, do you eat it straight away?</td>
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<tr>
<td>14. Do you deliberately eat foods that are slimming?</td>
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<tr>
<td>15. Do you have a desire to eat when you are cross?</td>
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<td>16. Do you have a desire to eat when you are approaching something unpleasant to happen?</td>
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<tr>
<td>17. If you walk past the baker do you have a desire to buy something delicious?</td>
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<td></td>
<td>Question</td>
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<tr>
<td>18.</td>
<td>When you have eaten too much, do you eat less than usual the following days?</td>
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<tr>
<td>19.</td>
<td>Do you get a desire to eat when you are anxious, worried or tense?</td>
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<td>20.</td>
<td>If you walk past a snack bar or café, do you have a desire to buy something delicious?</td>
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<tr>
<td>21.</td>
<td>Do you deliberately eat less in order not to become heavier?</td>
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<td>22.</td>
<td>Do you have a desire to eat when things are going against you, or things have gone wrong?</td>
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<td>23.</td>
<td>If you see others eating, do you have also the desire to eat?</td>
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<tr>
<td>24.</td>
<td>How often do you try not to eat between meals because you are watching your weight?</td>
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<td>25.</td>
<td>Do you have a desire to eat when you are frightened?</td>
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<tr>
<td>26.</td>
<td>Can you resist eating delicious food?</td>
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<tr>
<td>27.</td>
<td>How often in the evening do you try not to eat because you are watching your weight?</td>
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<tr>
<td>28.</td>
<td>Do you have a desire to eat when you are disappointed?</td>
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<tr>
<td>29.</td>
<td>Do you eat more than usual when you see other eating?</td>
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<tr>
<td>30.</td>
<td>Do you take your weight into account when you eat?</td>
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<tr>
<td>31.</td>
<td>Do you have a desire to eat when you are emotionally upset?</td>
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<tr>
<td>32.</td>
<td>When preparing a meal are you inclined to eat something?</td>
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<tr>
<td>33.</td>
<td>Do you have a desire to eat when you are bored or restless?</td>
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</tbody>
</table>
Appendix 4: Items of the Dutch Eating Behaviour Questionnaire (Van Strien et al., 1986)

1. If you have put on weight, do you eat less than you usually do?
2. Do you have a desire to eat when you are irritated?
3. If food tastes good to you, do you eat more than you usually do?
4. Do you try to eat less at meal times than you would like to eat?
5. Do you have a desire to eat when you have nothing to do?
6. Do you have a desire to eat when you are depressed or discouraged?
7. If food smells and looks good, do you eat more than you usually eat?
8. How often do you refuse food or drink offered because you are concerned about your weight?
9. Do you have a desire to eat when you are feeling lonely?
10. If you see or smell something delicious, do you have a desire to eat it?
11. Do you watch exactly what you eat?
12. Do you have a desire to eat when somebody lets you down?
13. If you have something delicious to eat, do you eat it straight away?
14. Do you deliberately eat foods that are slimming?
15. Do you have a desire to eat when you are cross?
16. Do you have a desire to eat when you are approaching something unpleasant to happen?
17. If you walk past the baker do you have a desire to buy something delicious?
18. When you have eaten too much, do you eat less than usual the following days?
19. Do you get a desire to eat when you are anxious, worried or tense?
20. If you walk past a snack bar or café, do you have a desire to buy something delicious?
21. Do you deliberately eat less in order not to become heavier?
22. Do you have a desire to eat when things are going against you, or things have gone wrong?
23. If you see others eating, do you have also the desire to eat?
24. How often do you try not to eat between meals because you are watching your weight?
25. Do you have a desire to eat when you are frightened?
26. Can you resist eating delicious food?
27. How often in the evening do you try not to eat because you are watching your weight?
28. Do you have a desire to eat when you are disappointed?
29. Do you eat more than usual when you see others eating?
30. Do you take your weight into account when you eat?
31. Do you have a desire to eat when you are emotionally upset?
32. When preparing a meal are you inclined to eat something?
33. Do you have a desire to eat when you are bored or restless?
Appendix 5: General Health Questionnaire

General Health Questionnaire

Date ..................................  Reference number: ............

PERSONAL DETAILS

Title: ...................................  Family name: .........................

Initials: .................................  Address: .................................

........................................

........................................

........................................

Telephone:  Day ......................  Evening .........................

Weight (kg): .........................  Height (m): ......................

DOB: ......../....../.....  Age: .........................(18 – 60)

GENERAL HEALTH

<table>
<thead>
<tr>
<th>Condition/Disorder</th>
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<th>NO</th>
</tr>
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<tbody>
<tr>
<td>Psychiatric Disorders</td>
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<tr>
<td>Drug/Alcohol dependence</td>
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<tr>
<td>Epilepsy</td>
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<tr>
<td>Sleep Disorders e.g.</td>
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<tr>
<td>Insomnia</td>
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<tr>
<td>Endocrine Disorders (inc.</td>
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<tr>
<td>Diabetes</td>
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<tr>
<td>Cardiovascular Disease</td>
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<tr>
<td>Blood Pressure-Hypertension</td>
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<tr>
<td>Renal Disease</td>
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<tr>
<td>Hepatic Disease</td>
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<tr>
<td>Gastrointestinal Disease</td>
<td></td>
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</tbody>
</table>
Any food allergies    YES/NO

IF YES:
Food/s
............................................................................................................................

GENERAL QUESTIONS

Nicotine (tick the relevant box)  Smoker  □ How many a day………
□ Non-smoker

Average caffeine consumption/day
How many cups/day:

Tea        …………

Coffee……

Cola ………

Average alcohol consumption/week    …………………

Pint of beer is 2 units
Glass of wine (125ml)1.5 units

Typical breakfast (cereal/toast-tea, coffee)
............................................................................................................................
Appendix 6: Items and subscales of the Mindful Eating Questionnaire
(Winkens et al., 2018)

Sensory Eating

1. I notice flavours and textures when I am eating my food.
2. I notice how my food looks.
3. I notice the smells and aromas of food.

Awareness

4. It is easy for me to concentrate on what I eat.
5. I snack without being aware that I am eating.
7. I eat something without really being aware of it.

Body Cues

8. I trust my body to tell me when to eat.
9. I trust my body to tell me what to eat.
10. I trust my body to tell me how much to eat.
11. I trust my body to tell me when to stop eating.

Distraction

12. My thoughts tend to wonder while I am eating.
13. I think about things I need to do while I am eating.
Appendix 7: Ethics Committee approval (EC/2014/102/FHMS)

Mr Filip Koidis
School of Biosciences and Medicine
FHMS
29 September 2014

Dear Mr Koidis

UEC ref: EC/2014/102/FHMS

Study Title: Development of a robust eating rate protocol. Assessment of the use of headphones on protocol effectiveness.

On behalf of the Ethics Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the submitted protocol and supporting documentation.

Date of confirmation of ethical opinion: 29 September 2014.

The final list of documents reviewed by the Committee is as follows:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Email from researcher responding to queries in email of 12 Sep 2014</td>
<td></td>
<td>18 Sep 2014</td>
</tr>
<tr>
<td>Signed covering letter from researcher responding to Committee's queries in letter of 05 Aug 2014</td>
<td>2</td>
<td>12 Aug 2014</td>
</tr>
<tr>
<td>Protocol Cover Sheet</td>
<td>1</td>
<td>Filename: 12 Aug 2014</td>
</tr>
<tr>
<td>Summary</td>
<td>1</td>
<td>16 Jul 2014</td>
</tr>
<tr>
<td>Protocol- tracked copy</td>
<td>2</td>
<td>Submitted: 18 Sep 2014</td>
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<tr>
<td>Appendix 1: Advertising Poster (Technical Development)- tracked copy</td>
<td>2</td>
<td>12 Aug 2014</td>
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<tr>
<td>Appendix 2: Advertising Poster (Pilot Study)- tracked copy</td>
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<td>12 Aug 2014</td>
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<tr>
<td>Appendix 3: Consent Form (Technical Development)</td>
<td>2</td>
<td>12 Aug 2014</td>
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<tr>
<td>Appendix 4: Participant Information Sheet (Technical Development) tracked copy</td>
<td>2</td>
<td>12 Aug 2014</td>
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<tr>
<td>Appendix 5: Consent Form (Pilot Study)</td>
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<td>12 Aug 2014</td>
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<td>Appendix 6: Participant Information Sheet (Pilot Study)- tracked copy</td>
<td>2</td>
<td>12 Aug 2014</td>
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<tr>
<td>Appendix 7: General Health Questionnaire</td>
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<td>12 Aug 2014</td>
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<tr>
<td>Appendix 8: Three Factor Eating Questionnaire</td>
<td>1</td>
<td>12 Aug 2014</td>
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<tr>
<td>Appendix 9: Visual Analogue Scale: Food and Options Questionnaire</td>
<td>2</td>
<td>12 Aug 2014</td>
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<tr>
<td>Risk Assessment</td>
<td>1</td>
<td>16 Jul 2014</td>
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</table>
This opinion is given on the understanding that you will comply with the University’s Ethical Principles & Procedures for Teaching and Research.

If the project includes distribution of a survey or questionnaire to members of the University community, researchers are asked to include a statement advising that the project has been reviewed by the University’s Ethics Committee.

If you wish to make any amendments to your protocol please address your request to the Secretary of the Ethics Committee and attach any revised documentation.

The Committee will need to be notified of adverse reactions suffered by research participants, and if the study is terminated earlier than expected with reasons. Please be advised that the Ethics Committee is able to audit research to ensure that researchers are abiding by the University requirements and guidelines.

You are asked to note that a further submission to the Ethics Committee will be required in the event that the study is not completed within five years of the above date.

Please inform me when the research has been completed.

Yours sincerely

Dr Sophie Wehrens

Research Integrity and Governance Officer, Research & Enterprise Support

cc. Dr Shelagh Hampton, School of Biosciences and Medicine, FHMS
Appendix 8: Consent Form

Consent Form

I, the undersigned, voluntarily agree to take part in the study on “Assessment of the use of headphones on protocol effectiveness and impact on satiety using a robust slow eating rate protocol.”

I have read and understood the Information Sheet provided. I have been given a full explanation by the investigators of the nature, purpose, location and likely duration of the study, and of what I will be expected to do. I have been advised about any discomfort and possible ill effects on my health and well-being which may result. I have been given the opportunity to ask questions on all aspects of the study and have understood the advice and information given as a result.

I agree to comply with any instruction given to me during the study and to co-operate fully with the investigators. I shall inform them immediately if I suffer any deterioration of any kind in my health or well-being, or experience any unexpected or unusual symptoms.

I consent to my personal data, as outlined in the accompanying information sheet, being used for this study and other research. I understand that all personal data relating to volunteers is held and processed in the strictest confidence, and in accordance with the Data Protection Act (1998).

I understand that I am free to withdraw from the study at any time without needing to justify my decision and without prejudice.

I acknowledge that in consideration for completing the study I shall receive the sum of £60. I recognise that the sum would be less, and at the discretion of the Principal Investigator, if I withdraw before completion of the study.

I give permission for photographs and videos of tests in which I feature to be used in:

- Peer reviewed journal and conferences publications
- Publicity material (brochures and departmental webpages)
I confirm that I have read and understood the above and freely consent to participating in this study. I have been given adequate time to consider my participation and agree to comply with the instructions and restrictions of the study.

Name of volunteer (BLOCK CAPITALS) ......................................................

Signed ......................................................

Date ......................................................
Appendix 9: Participant information sheet (Study B)

Participant Information Sheet

Assessment of the use of headphones on protocol effectiveness and their impact on satiety.

Introduction

Dr Shelagh Hampton, Dr Michelle Gibbs and Dr Aliah Shaheen supervise this research project. I am Filip Koidis PhD student in the Faculty of Health & Medical Sciences and we would like to invite you to take part in a research project. I am developing a methodology of slow eating rate that will ultimately be used as an easy weight management tool that may in part contribute towards lowering disease risk.

We would like to invite you to take part in a research project. Before you decide you need to understand why the research is being done and what it will involve for you. Please take the time to read the following information carefully. Talk to others about the study if you wish.

What is the purpose of the study?

This study seeks to test the effect of an easy to use low cost method of slowing eating speed. This technique has the potential to improve weight and may reduce the risk of developing diabetes. The aim of this research would be to provide robust information to practitioners and consumer guidance to encourage change in eating rate behaviour for improved health and quality of life.
**Why have I been invited to take part in the study?**

We are looking for healthy volunteers aged between 18-70 years. You can be a student – undergraduate or postgraduate or a member of staff at the University of Surrey, or a member of the general public.

**Do I have to take part?**

No, you do not have to participate. There will be no adverse consequences if you decide not to participate. You can withdraw at any time without giving a reason.

**What will my involvement require?**

If you are happy to take part we will ask you to attend all three sessions. The first session will last approximately 30 minutes in order to assess you normal eating rate. You will be provided with a standard meal (spinach and ricotta pasta) and asked to consume it at your habitual pace, whilst being video recorded (mouth, jaw and throat areas only). Four very small monitors will be placed on your: hand, throat, chin and upper lip so that your movement whilst eating can be accessed via the QTM 2.0 software. The monitors used in this study will only reflect the movement of your hand, jaw, throat and mouth and the cameras will only depict your mouth and neck area.

If you do not wish to be video then you cannot take part in the study. This video data will be anonymised.

If you still fit the criteria you will be asked to attend two further sessions which will be randomised for each participant. These two sessions will be with and without headphones and you will be given clear instruction on how to eat the meal. Each session will last a maximum of three hours. All sessions will be held in Movement Analysis Lab DK0007, Centre for Vision, Speech and Signal Processing, University of Surrey for the first 30 minutes after which you will be taken to the CIU (AX00) where you will complete the study. All information obtained will be restricted to the investigators only and will be kept strictly confidential.
What will I have to do?

If you would like to take part please make sure to follow the instructions stated by the Investigators. You will be asked to fast from 20:00h the previous evening prior to each study phase and adhere to your normal physical activity and diet patterns twenty four hours prior to each study day. Strenuous physical activity should be avoided and no caffeine containing products should be consumed. You will, then be provided with breakfast and asked to consume it between 07:30h and 08:30h on the study in your own home. You will be requested to arrive at the clinical facility between12:00h - 14:00h and between13:00h-15:00h a lunch-meal (spinach and ricotta pasta) will be provided. Only one person can be video at a time therefore we will discuss with you allocated of a time slot prior to each meal. We expect that on each occasion the time slot would be similar. You will be video-recorded with high definition cameras using the QTM2.0 software, so that the investigators can measure different variables required for the study. You will also be asked assess appetite using Electronic Pro-Diary food visual analogue scales at regular intervals for directly prior to and after consuming the meal for the next three hours. Also your overall eating rate manipulation experience will be documented. At the end of each study phase you will need to fill out a tick-sheet questionnaire.

Where will this study take place?

The study will be conducted in the Movement Analysis Lab DK0007, Centre for Vision, Speech and Signal Processing, University of Surrey and Clinical Investigation Unit (CIU AX00) at the University of Surrey, School of Biosciences and Medicine. You will be asked to attend the Movement Lab/CIU three times, the first time for maximum one hour and the remaining two times between 12:30-1300h for 3 hours.

Remuneration

You will be reimbursed for you time and inconvenience with a maximum payment of £60 for completion of the study. Should you withdraw before completion of the study you will receive a pro-rata payment at the discretion of the investigators.
What are the possible disadvantages or risks of taking part?

There are few minor risks when taking part to this study. Discomfort during slow eating and a minor risk of food poisoning which is unlikely since food is prepared fresh by trained personnel. You will be asked about food allergies to avoid any allergic responses.

What are the possible benefits of taking part?

There will be no direct benefit obtained from this study apart from the expenses that will be paid to you. We also hope that your participation could assist us in future research and lead to some interesting data that can be investigated in future.

What happens when the research study stops?

When the research study stops, we hope to publish the results in peer-reviewed journals. We can notify you of the results of our study via email if you wish so.

What if there is a problem?

Any complaint or concern about any aspect of the way you have been dealt with during the course of the study will be addressed; please contact { Dr. Shelagh Hampton, Tel: 01483 689732, Email:s.hampton@surrey.ac.uk, Dr. Michelle Gibbs, Tel: 01483 682532, Email: m.gibbs@surrey.ac.uk, Filip Koidis, Tel: 07531932965, Email: fk00016@surrey.ac.uk }
The information collected will be stored at the University of Surrey. Regulatory authorities to check that the study is being carried out correctly may inspect this. Your name, however, will not be disclosed outside of the research team. Records held will be identified only by code numbers and are kept in a secure place in the FHMS for 10 years at the University of Surrey. The results from the study may be used for publication but all results will be kept strictly anonymous. All data used will be within the terms of the Data Protection Act 1998.

**Contact details of researcher and, where appropriate supervisor?**

Dr S M Hampton  
Faculty of Health and Medical Sciences,  
University of Surrey  
Guildford  
GU2 7XH  
01483 689732  
e-mail: s.hampton@surrey.ac.uk

Mr F Koidis (Student)  
Faculty of Health and Medical Sciences,  
University of Surrey  
Guildford  
GU2 7XH  
07531932965  
e-mail: fk00016@surrey.ac.uk

Dr M Gibbs  
Faculty of Health and Medical Sciences,  
University of Surrey  
Guildford  
GU2 7XH  
01483 682532  
e-mail: m.gibbs@surrey.ac.uk

**Who is organising and funding the research?**
The research is part of Filip Koidis PhD research studies taking place in the Faculty of Health and Medical sciences.

Who has reviewed the project?

The study has been reviewed and received a favourable Ethical Opinion (FEO) from the University of Surrey Ethics Committee.

Thank you for taking the time to read this Information Sheet.
**Appendix 10: Slow eating rate protocol**

- Before you start eating, take a comfortable position that you usually eat in and try to maintain that position until you finish your meal.

- Take one piece of pasta at a time.

- After each mouthful place your fork down on the table.

- Chew each mouthful 30 times before you swallow for the first time. Then chew 10 more times the food that remains in your mouth.

- If you lose count of chews, pause for 10 seconds, swallow and then take your next mouthful.

- You are only allowed 250ml of water, please drink only in-between mouthfuls, unless if necessary.

- Eat until you are comfortably full. The remaining of the pasta will be given to you to take away in a plastic container.

- Please make a note of how many times you missed chew counting here_______
Appendix 11: Ethics Approval UEC/2016/020/FHMS

06 July 2016

Dear Mr Koidis

UEC ref: UEC/2016/020/FHMS

Study Title: Investigation of the short and longer term effects of a slow eating rate protocol in overweight people, in a community intervention study

On behalf of the Ethics Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the submitted protocol and supporting documentation.

Date of confirmation of ethical opinion: 06 July 2016

The final list of documents reviewed by the Committee is as follows:
<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover letter from researcher in response to comments from UEC and RIGO, sent 26 May 2016</td>
<td></td>
<td>26 Jun 2016</td>
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<tr>
<td>Cover letter from researcher in response to comments from UEC and RIGO, sent 12 Apr 2016</td>
<td></td>
<td>18 May 2016</td>
</tr>
<tr>
<td>Ethics Application Form - tracked copy</td>
<td>2</td>
<td>05 May 2016</td>
</tr>
<tr>
<td>Protocol - tracked copy</td>
<td>3</td>
<td>27 Jun 2016</td>
</tr>
<tr>
<td>Appendix 1: Participant Information Sheet, Group A - tracked copy</td>
<td>3</td>
<td>27 Jun 2016</td>
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<tr>
<td>Appendix 2: Participant Information Sheet, Group B - tracked copy</td>
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<td>27 Jun 2016</td>
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<tr>
<td>Appendix 3: Consent Form, Main study - tracked copy</td>
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<td>27 Jun 2016</td>
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<tr>
<td>Appendix 4: Consent Form, Subgroup - tracked copy</td>
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<td>27 Jun 2016</td>
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<td>Appendix 5: General Health Questionnaire</td>
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<td>Appendix 6: Dutch Eating Behaviour Questionnaire</td>
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<td>Appendix 7: Advertising poster - tracked copy</td>
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<td>05 May 2016</td>
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<td>Appendix 8: Volunteer Instructions Sheet</td>
<td>3</td>
<td>27 Jun 2016</td>
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<td>Appendix 9: Visual Analogue Scales: Food and Opinions Questionnaire</td>
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<td>Appendix 10: Mindful Eating Questionnaire</td>
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<td>Appendix 11: Clinical Research Insurance Proforma</td>
<td>1</td>
<td>26 Feb 2016</td>
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<tr>
<td>Appendix 13: Pilot study paper publication 2014</td>
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<td>Appendix 14: Developmental study abstract publication</td>
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<tr>
<td>Letter confirming HTA compliance</td>
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<td>24 Jun 2016</td>
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<tr>
<td>Risk Assessment</td>
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<td>Feb 2016</td>
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<tr>
<td>Clinical Trials Insurance Certificate</td>
<td>1</td>
<td>16 Jul 2016</td>
</tr>
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</table>

This opinion is given on the understanding that you will comply with the University’s Ethical Principles & Procedures for Teaching and Research.

If the project includes distribution of a survey or questionnaire to members of the University community, researchers are asked to include a statement advising that the project has been reviewed by the University’s Ethics Committee.

If you wish to make any amendments to your protocol please address your request to the Secretary of the Ethics Committee and attach any revised documentation.

The Committee will need to be notified of adverse reactions suffered by research participants, and if the study is terminated earlier than expected with reasons. Please be advised that the Ethics Committee is able to audit research to ensure that researchers are abiding by the University requirements and guidelines.
You are asked to note that a further submission to the Ethics Committee will be required in the event that the study is not completed within five years of the above date.

Please inform me when the research has been completed.

Yours sincerely

Miss Rebecca Green
Research Integrity and Governance Co-ordinator, Research & Enterprise Support

Copy to.
Dr Kathryn Hart, School of Biosciences and Medicine, FHMS.
Appendix 12: Participant information sheet (Study E)

Participant information sheet

Introduction

We would like to invite you to take part in a research project. Before you decide you need to understand why the research is being done and what it will involve for you. Please take the time to read the following information carefully and ask questions about anything you do not understand. Talk to others about the study if you wish.

What is the purpose of the study?

This study aims to test whether a new short questionnaire assessing mindful eating is related to eating rate (how fast or slow a person usually eats). This will help us develop tools for use in future studies and may help in the design of tools to help people manage their weight.

Why have I been invited to take part in the study?

You have been invited to take part in this study because you are a healthy adult living in the Surrey area.

To be eligible to take part in the study, you must meet the following criteria:

- BMI greater than 15.9kg/m^2 (calculated by dividing your weight in kg by your height in metres squared – see http://www.bbc.co.uk/health/tools/bmi_calculator/bmi.shtml)
- Aged between 18 and 70 years
- Willing to fill out health and demographic questionnaires
- Willing to be filmed [mouth and jaw area] for the purposes of the study
- Able to give verbal and written consent
- Have a full set of dentures and able to eat normally
- Not allergic to any of the test meal ingredients (vegetarian stuffed pasta)

We aim to recruit up to 100 participants will take part in this study.

Do I have to take part?

No, you do not have to participate. There will be no adverse consequences in terms of your legal rights if you decide not to participate or withdraw at a later stage. You can withdraw your participation at any time. You can request for your data to be withdrawn until 30th April 2018 without giving a reason and without prejudice.

If you withdraw from the study identifiable data already collected will be retained if you allow us to.Anonymous data already collected will be used (because we cannot trace the latter information back to you). No further data would be collected or any other research procedures would be carried out on or in relation to you.

What will my involvement require?
If you agree to take part, we will then ask you to sign a consent form. If you do decide to take part you will be given this information sheet to keep and a copy of your signed consent form. The research will last for up to 6 months but your involvement would only be for one study visit lasting approximately 30 minutes.

What will I have to do?

An appointment will be made for you to attend the Clinical Investigation Unit at the University of Surrey in Guildford for one lunch time visit.

On the day before your visit you will be asked to avoid caffeine, alcohol or strenuous exercise as this make the measurements of body-fat and body water more accurate. On the study day itself you will be asked to consume your normal breakfast at least 4 hours before your visit time, e.g. by 8am for a 12 noon appointment and then to refrain from eating or drinking anything other than water until your visit.

On arrival at the unit we will measure your weight and body-fat percentage using a stand-on electrical bioimpedance machine (TANITA). This can be done in light clothing but requires bare feet. Your height will be measured with a wall mounted height measure and we will calculate your BMI for you (weight to height ratio). We will measure your waist and hip measurements (over light clothing) using a tape measure and give you a copy of your measurements to take home.

You will be asked to complete a short questionnaire asking about your eating behaviours and general health – this will be available in paper or electronic form as you prefer. Finally we will ask you to rate on a small handheld computer how hungry you feel.

You will then be taken to an individual booth and served a test meal consisting of spinach and ricotta stuffed pasta. We will ask you to eat this at your normal eating rate without any distractions (no book or mobile phone) and you will be filmed whilst you eat with one camera [front view]. Once you have finished the meal in your own time the recordings will be stopped and you will be free to leave the unit. If you wish to be entered into a free prize draw (for a £50 shopping voucher) we will ask you to leave your name and contact details. These will be used solely for the purpose of contacting the winner at the end of the study and will be destroyed as soon as the prize has been awarded.

What will happen to data that I provide?

All data derived from the questionnaires and body measurements will be anonymised (identifiable information like names removed) and entered into a study spreadsheet for analysis. This will be stored separately from any email addresses collected for the purposes of the prize draw. The videotapes will be analysed both automatically, using a computer programme, and manually by members of the research team to count the numbers of chews, mouthfuls and total eating time. This data will be added to the anonymous spreadsheet.

Research data are stored securely for at least 10 years following their last access and project data (related to the administration of the project, e.g. your consent form) for at least 6 years in line with the University of Surrey policies.

Personal data will be handled in accordance with the UK Data Protection Act (1998). With your consent, to make the most of your participation and support efficient advancements in science, any anonymised data may be used for future research. We cannot tell you at this moment in
time what this research will entail or what analyses will be carried out but we can assure you that all appropriate legal, ethical and other approvals will be in place. For practical reasons your consent will not be sought again unless you indicate you wish us to do this. Your data will not be used for commercial purposes.

**What are the possible disadvantages or risks of taking part?**

There are no disadvantages or risks of taking part in the study, other than the time required for the visit itself.

**What are the possible benefits of taking part?**

You will receive a free lunch, and copy of your body measurements. If you wish we will enter you into a free prize draw to win a £50 shopping voucher, which will be drawn at random at the end of the study.

**What happens when the research study stops?**

Nothing further will be required of you once your study visit is complete. The study itself is planned to run until April 2018 at which point all the data will be collated and analysed.

**What if there is a problem?**

Any complaint or concern about any aspect of the way you have been dealt with during the course of the study will be addressed; please contact Principal Investigators Lucy Georgiou lg00301@surrey.ac.uk or Dimitra Theodoraki dt00127@surrey.ac.uk in the first instance or their Supervisor Dr Kathryn Hart, on 01483 686438, k.hart@surrey.ac.uk. You may also contact Prof Susan Lanham-New, Head of Nutritional Sciences (s.lanham-new@surrey.ac.uk) who is independent of this study.

The University of Surrey holds insurance policies, which apply to this study. If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should follow the instructions given above.

**Will my taking part in the study be kept confidential?**

Yes. Your details will be held in complete confidence and we will follow ethical and legal practice in relation to all study procedures. Personal data [name, contact details, audio/video recordings] will be handled in accordance with the (UK) Data Protection Act 1998 so that unauthorised individuals will not have access to them.

Your personal data will be accessed, processed and securely destroyed by members of the research team including principal and co-investigators, student researchers and their supervisors. In order to check that this research is carried out in line with the law and good research practice, monitoring and auditing can be carried out by independent authorised individuals. Data collected during the study
may be looked at by authorised individuals from the University of Surrey. All will have a duty of confidentiality to you as a participant and we will do our best to meet this duty. We will anonymise any documents or records that are sent from the University of Surrey, so that you cannot be identified from them.

The data you provide will be anonymised and your personal data will be stored securely (separately from those anonymised data). You will not be identified in any reports/publications resulting from this research and those reading them will not know who has contributed to it.

Full contact details of research team

Principal Investigators: Lucy Georgiou lg00301@surrey.ac.uk or Dimitra Theodoraki dt00127@surrey.ac.uk

PhD student: Filip Koidis, f.koidis@surrey.ac.uk

Supervisor: Dr Kathryn Hart, 01483 686438, k.hart@surrey.ac.uk

Who is organising and funding the research?

This research is organised and funded by the University of Surrey.

Who has reviewed the project?

This research has been looked at by an independent group of people, called an Ethics Committee, to protect your interests. This study has been reviewed by and received a favourable ethical opinion from the Faculty of Health and Medical Sciences Ethics Committee.

Thank you for taking the time to read this Information Sheet.