Appetite and Metabolic Responses to Acute and Moderate-Term Dairy Snack Consumption in Young People

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ABSTRACT

In adolescents, an emergent body of evidence supports the hypothesis that milk-based dairy foods elicit anti-obesity properties, providing a modest protective effect against adiposity. To date, efforts to establish the underlying relationship between dairy and adiposity have identified several putative mechanisms. The least well studied of these mechanisms in adolescents is that of milk-based dairy consumption on appetite and metabolism, both of which exert the potential to impact on energy regulation and thus body composition. Accordingly, the overall aim of this thesis was to explore the impact of milk-based dairy consumption on subsequent appetite, feeding behaviour and resting metabolism in healthy young people. To accomplish this, four experimental studies were conducted. The initial experimental chapter of this thesis (chapter two) set out to establish the potential of an alternative methodological approach to quantify appetite- and metabolism-related peptide concentrations for use with paediatric populations. In this sense, chapter two examined the agreement and between-day test-retest reproducibility of several appetite-related peptides between fingertip-capillary and antecubital-venous blood sampling. The second experimental chapter (chapter three) compared dairy consumption patterns among a children (9-11 y) and adolescents (15-18 y), and was primarily designed to establish dairy food popularity (types, frequencies and amounts) and identify potential populations (sex & age) to target within the intervention-based sections of this thesis. The third (chapter four) and fourth (chapter five) experimental chapters of this thesis subsequently employed the findings of chapter two and three to determine the acute- (1-d) and moderate-term (28-d) influence of mid-morning milk consumption on feeding behaviour, metabolic and appetite-related responses in adolescent males (15-18 y).

For the first time, chapter two revealed that at rest fingertip-capillary blood sampling offers an appropriate methodological and reproducible approach to systematically assess concentrations of appetite- and metabolism-related peptides. In this sense, analysis revealed no evidence of systematic or proportional bias between antecubital-venous (mean ± $_{SD}$, 90.1 ± 23.8 pg·mL) and fingertip-

capillary (90.0 ± 37.2 pg·mL) time-averaged AUC estimates of plasma glucagon, GLP-$1_{7\text{-}36}$ (mean ± $_{SD}$, 8.6 ± 3.4 pg·mL vs. 9.1 ± 3.0 pg·mL, respectively) and leptin (mean ± $_{SD}$, 664.5 ± 350.3 pg·mL vs. 741.0 ± 375.2 pg·mL, respectively), representing good agreement for glucagon and modest agreement for GLP-$1_{7\text{-}36}$ and leptin. For insulin, although no evidence of systematic bias between antecubital-venous (mean ± $_{SD}$, 302.4 ± 154.7 pmol·L) and fingertip-capillary (mean ± $_{SD}$, 236.2 ± 113.0 pmol·L) blood sampling was observed, proportional bias was evident at higher concentrations illustrating poor agreement. No systematic bias existed between visits for any fingertip-capillary-derived peptide ($p \geq 0.05$ for all). Between-day reproducibility of plasma glucagon was strong (CV_r = 8.2%). Plasma GLP-$1_{7\text{-}36}$ and leptin demonstrated modest reproducibility (CV_r = 22.7 and 25.0%, respectively). Again, insulin exhibited the greatest variability (CV_r = 36.0%) indicating a large degree of random error between visits. Knowledge concerning the between-day test-retest typical error for plasma glucagon (8.2%) was subsequently applied to facilitate study design in the intervention parts of the thesis, where sample size estimates were based on these findings.

Findings of the second experimental chapter revealed that milk was the most favourable dairy food consumed among children and adolescents, consumed by 91% of participants. In addition, the results revealed a main effect for sex on overall milk consumption ($F_{1,71}$ = 7.07, p = 0.010) and daily milk portions ($F_{1,71}$ = 6.79, p = 0.011), indicating that independent of age, boys consumed greater amounts of milk compared to girls. Regardless of this, although no statistical evidence was sought that milk-based dairy food consumption differed significantly between middle-childhood and adolescence, for boys and adolescent males, a downward trend of total daily dairy food consumption with increasing age was noted whereas patterns of milk and dairy food consumption remained widely stable among girls and female adolescents. As dietary habits shaped throughout adolescence may ultimately track into adulthood, continual milk and dairy food avoidance could be disadvantageous, particularly among adolescents, leading to lasting nutritional and health-related implications. For this reason, it was deemed necessary to target the intervention-

based studies on adolescent males utilising milk as the main test food. The observations arising from chapter four indicated, that in an acute setting, milk consumption influences short-term feeding behaviour, reducing energy intake at an *ad libitum* pasta meal compared to an isoenergetic and isovolumetric fruit-juice in adolescent males (mean difference ± 90% CI; -596.4 kJ: 90% CI: -105.7, -1087.1). Additionally, milk consumption elicited an increased postprandial (90-180 min) glucagon response (mean difference ± 90% CI; 16.8 pg·mL; 90% CI: 27.5, 6.1) and led to an increase in energy expenditure (mean difference ± 90% CI; 109.2 kJ; 90% CI: 197.2, 21.6). Following yogurt consumption, time-averaged AUC (90-180 min) estimates of GLP-$1_{7\text{-}36}$ were increased (mean difference ± 90% CI; 1.2 pg·mL; 90% CI 2.3, 0.2) and blood glucose lower (mean difference ± 90% CI; -0.4 mmol·L; -0.1, -0.7) relative to the fruit-juice, but failed to impact on feeding behaviour. In chapter five, daily milk supplementation (28-d) impacted favourably on feeding behaviour under free-living conditions, reducing energy intake relative to baseline observations (mean difference ± 90% CI; 1882.8 kJ; 90% CI: 2706.0, 1059.6), whereas the opposite was apparent for fruit-juice. Relative to baseline observations, concentrations of insulin displayed a greater time-averaged AUC (0-90 min) following daily milk consumption (mean difference ± 90% CI; -79.3 pmol·L; 90% CI: -29.5, -129.3). This was also evident for 90-180 min time-averaged AUC (mean difference ± 90% CI; -32.4 pmol·L; 90% CI: -5.3, -59.6). Consistent with the insulinotropic effect of milk, endpoint time-averaged AUC (90-180 min) estimates of blood glucose were lower relative to baseline observations (mean difference ± 90% CI; 0.3 mmol·L; 90% CI: 0.4, 0.1). Milk supplementation, however, failed to impact on resting metabolism. In contrast, daily fruit-juice consumption resulted in increased energy intake at the *ad libitum* pasta meal. Nonetheless, we observed no capacity for fruit-juice supplementation to influence measures of subjective appetite, appetite- and metabolism-related peptides or postprandial metabolism relative to baseline observations. Overall, these studies suggest that acute- and moderate-term milk consumption impacts favourably on feeding behaviour, and it is reasonable to suggest this is facilitated through integrated metabolic and endocrine responses.

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CHAPTER FIVE

LIST OF ABBREVIATIONS

The following abbreviations have been used throughout this thesis. Terms coupled with their unabridged terms have been defined upon first appearance in the thesis.

Abbreviation	Unabridged Term
AgRP	Agouti-Related Peptide
ARC	Arcuate Nucleus
AUC	Area Under the Curve
BMI	Body Mass Index
$CaCO_3$	Calcium Carbonate
CART	Cocaine and Amphetamine Regulated Transcript
CI	Confidence Interval
CKK	Cholecystokinin
CLA	Conjugated Linioleic Acid
CNS	Central Nervous System
CV	Coefficient of Variation
DEC	Decreased
DIT	Diet induced thermogenesis
DPP-IV	Dipeptidyl Peptidase-IV
EDTA	Ethylenediamintetraacetic Acid
FAS	Fatty Acid Synthase
FM	Fat Mass
FMM	Fat Free Mass
FO	Fat Oxidation

GLP-1	Glucagon-Like Peptide-1
HDL	High-Density Lipoprotein
HSE	Health Survey for England
INC	Increased
LDL	Low-Density Lipoprotein
MCFA	Medium Chain Fatty Acids
NDNS	National Diet and Nutrition Survey
NPY	Neuropeptide Y
OXM	Oxyntomodulin
POMC	Proopiomelanocortin
PTH	Parathyroid Hormone
PVN	Paraventricular Nucleus
PYY	Peptide Tyrosine Tyrosine
VAS	Visual Analogue Scale
UK	United Kingdom
US	United States
1,25 $(OH)_2D_3$	$_{1,25}$ Dihydroxyvitamin D_3
$[Ca^{2+}]i$	Intracellular Calcium

PREFACE

Academic peer-reviewed publications arising from this thesis

Green, B. P., Gonzalez, J. T., Thomas, K., Stevenson, E., & Rumbold, P. L. S. (2014). Agreement between fingertip-capillary and antecubital-venous appetite-related peptides. *Endocrine Connections, 3*(4), 233-242. 10.1530/ec-14-0110.

Green, B. P., Turner, L., Stevenson, E., & Rumbold, P. L. S. (2015). Short communication: Patterns of dairy consumption in free-living children and adolescents. *Journal of Dairy Science*. 10.3168/jds.2014-9161

Green, B. P., Stevenson, E., & Rumbold, P. L. S. (*In preperation*). Appetitive and metabolic responses to acute- and moderate-term milk consumption in adolescent males.

Academic peer-reviewed conference proceedings arising from this thesis

Green, B. P, Gonzalez, J., Thomas, K., Dodd-Reynolds, C., Bryans, J., Stevenson, E., & Rumbold, P. (2013). Agreement of capillary-obtained acylated ghrelin, active GLP-1, glucagon, insulin and leptin with their venous equivalents. *British Journal of Sports Medicine, 47*(17), e4.

Green, B. P., Gonzalez, J., Thomas, K., Bryans, J., Stevenson, E., & Rumbold, P. (2015). Reproducibility of appetite- and metabolism-related peptides following fingertip-capillary blood sampling. *Appetite, 87*(0), 379.

Green, B. P., Turner, L. A., Bryans, J., Stevenson, E., & Rumbold, P. L. S. (2015). Milk and dairy food consumption; a comparison between children and adolescents. *Proceedings of the Nutrition Society, 74*(OCE1)

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AUTHORS DECLARATION

I, Benjamin Paul Green, declare that the body of work contained in this thesis comprises all my own work and has not been submitted for any other award. I confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Any ethical clearance for the research presented in this thesis has been approved. Approval has been sought and granted by Northumbria University Ethics Committee.

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Date:

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CHAPTER ONE

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.0 Introduction

This chapter aims to provide an overview of the literature concerning milk-based dairy food consumption, appetite, feeding behaviour and metabolism in children and adolescents. The initial sections provide a brief depiction of the estimated prevalence of overweight and obesity among children and adolescents, physiological determinants contributing to weight gain and the importance of studying the adolescent period. This initial section closes by identifying approaches available to accurately assess body composition in children and adolescents. This will be followed with a portrayal of milk-based dairy foods, and an overview concerning consumption patterns from 1997 through 2011-12 among children and adolescents in the United Kingdom (UK). To facilitate this, evidence from nationally representative surveillance programmes including the Health Survey for England (HSE) and the National Diet and Nutrition Survey (NDNS) have been examined. This opening section concludes by highlighting several methodological limitations associated with the NDNS, which subsequently confound comparison between earlier reports and identifies avenues that warrant further investigation.

The subsequent sections of this chapter provide a review of the literature concerning the relationship between milk-based dairy consumption and body mass, and the suggestion that dairy and adiposity may be aetiologically related. To support this, the purported mechanisms underlying this link will be highlighted. In particular, focus will be centred on metabolism, appetite and feeding behaviour. To facilitate this, an overview of the homeostatic and non-homeostatic regulation of appetite and feeding behaviour will be given. Throughout these sections, methodological approaches and issues associated with the assessment of appetite and feeding behaviour in children and adolescents will be considered. Where possible, this review will focus on child and adolescent populations, however, where there is no applicable literature, data from adult groups will be reviewed. Finally, this chapter will conclude by outlining the scope of the present thesis.

1.1 Childhood and adolescent obesity

Overweight and obesity can be characterised as an abnormal accumulation of adipose tissue, resulting in an increased body mass greater than the limits of physical requirement (Sikaris, 2004). Overweight and obesity have commonly been assessed using estimates of body mass index (BMI), calculated according to body mass (kg) divided by stature squared (m^2). For adults, overweight and obesity is universally accepted as possessing a BMI of $\geq 25kg \cdot m^2$ and $\geq 30kg \cdot m^2$, respectively (World Health Organisation., 2013). For children and adolescents, weight status is defined using BMI classifications however these are interpreted according to sex specific percentiles based on the 1990 United Kingdom (UK) reference population (Cole, Freeman, & Preece, 1995). Children and adolescents between the 85^{th} and 94^{th} percentile are classified as overweight, whereas children and adolescents beyond the 95^{th} percentile are classified as being obese.

In England, the prevalence of overweight and obesity among children and adolescents has increased markedly over the last two decades (Ryley, 2013). Figures obtained from the 2013 HSE indicate that 29.5% of children and adolescents (2-15 y) are presently classified as being either overweight ($\geq 85^{th}$ centile) or obese ($\geq 95^{th}$ centile) (Cole et al., 1995; Ryley, 2013). Thus, in 2013, approximately three in 10 boys and girls were categorised as being either overweight or obese. Overall, the estimated prevalence of obesity among boys (2-15 y) has increased from 11.1% in 1995 to 15.7% in 2013. Across the same time period, the estimated prevalence of obesity has risen from 12.2% to 14.7% for girls (2-15 y). Rates of overweight and obesity among children and adolescents peaked in 2004 (34.3%), and have slowly declined thereafter. In recent years, the proportion of children and adolescents in England who are classified as either overweight or obese has been lower and may therefore suggest that the prevalence of obesity is decreasing. Indeed, figures reported from the HSE (2013) are consistent with evidence from other Westernised countries (Reilly, 2012) . In this sense, secular trends of obesity among children and adolescents are in agreement with nationally representative surveillance data from Switzerland (Aeberli, Ammann, Knabenhans, Molinari, & Zimmermann, 2010), France (Lioret et al., 2009; Salanave, Peneau, Rolland-Cachera,

Hercberg, & Castetbon, 2009) and the United States (US) (Ogden, 2008). Nonetheless, childhood and adolescent overweight and obesity continue to be of great concern.

1.1.1 Determinants contributing to weight gain (and the influence of milk as a snack on energy balance)

The exact aetiology of obesity is a complex matter. Accumulating evidence has confirmed numerous genetic, biological, behavioural and environmental factors interact synergistically to the development of obesity. Indeed, this complexity is reflected throughout the comprehensive obesity map (Foresight, 2007), consisting of 108 variables and 304 causal connections. Central to this model, however, is the concept of energy balance, a principle based on the first law of thermodynamics whereby energy can neither be created nor destroyed. For a balance to be achieved, the amount of energy consumed (food and drink) must equal the total output of heat and mechanical work (energy expenditure) (Frayn, 2010). Any deviation from this equilibrium will result in weight gain or loss. Consequently, in today's obeseogenic environment where energy dense diets and sedentary lifestyles (Popkin, 2001) have rapidly become the accepted norm, it is generally acknowledged that weight gain is fundamentally triggered following an caloric imbalance (Moinuddin, Collins, Kramer, & Leehey, 2012). The rise in the prevalence of overweight and obesity subsequently reflects a strong desire to consume food in a volume greater than that required, which may be unsurprising considering the abundance of food availability. Habitual dietary patterns that affect energy intake are therefore fundamental components of energy balance, and thus weight status (Chaput & Tremblay, 2009; Willett & Leibel, 2002).

In recent years, snacking has grown in popularity. Snacking may be defined an episode of food consumption (including all food and beverage items) that takes place outside the context of typical main meals (Chapelot, 2011), and is often considered as a determinant to the development of overweight and obesity in children and adolescents (Mattes, Shikany, Kaiser, & Allison, 2011). For

children and adolescents, sugar-sweetened beverages, fruit-juice drinks and milks (plain and flavoured) represent several common snack items consumed between main meals (Duffey et al., 2012). Sugar-sweetened beverages hold a negligible nutritive value and a recent systematic review (Woodward-Lopez, Kao, & Ritchie, 2011) has concluded that high rates of consumption are linked with increased obesity in children and adolescents, while the opposite is apparent for milk-based dairy food consumption (Dror, 2014). In an early investigation, Ludwig and colleagues (2001) conducted a 19 month prospective study and demonstrated a converse relationship between sugar-sweetened beverage consumption and the risk of overweight and obesity in children (11-12 y). Indeed, the findings of Ludwig and colleagues (2001) have since been replicated in children and adolescents (6-14 y) (Cantoral et al., 2015; Pan et al., 2014). Authors of these studies observed a larger rate of weight gain with greater sugar sweetened beverage consumption, and concluded this was primarily because participants failed to compensate by reducing caloric intake at subsequent main meals (Ludwig et al., 2001). The role of snacking in the development of overweight and obesity may, therefore, be due to a disruption to the normal homeostatic control of appetite and feeding behaviour, consequently leading to an overconsumption of calories and thus weight gain (Chapelot, 2011). In this sense, beverages may exert a lower satiating capacity than their solid counterparts (DiMeglio & Mattes, 2000). Furthermore, high-fructose corn syrup (a caloric sweetener) contained in common sugar sweetened beverages, may contribute to an overconsumption of calories and thus weight gain through actions on lipogenesis and appetite- and metabolism-related peptide activity (Bray, Nielsen, & Popkin, 2004). In the body, fructose fails to stimulate insulin secretion and favours *de novo* lipogenesis (Bray et al., 2004). This may be because the β cell in the pancreas and the brain lack the fructose transporter, Glut-5 (Curry, 1989). In the literature, it is well known that insulin release can modulate feeding behaviour and increase leptin secretion (Saad et al., 1998), albeit with a time-lag of some hours. Consequently, a reduced concentration of insulin following fructose ingestion would be associated with lower circulating

leptin. As leptin also acts to modulate feeding behaviour, reduced circulating leptin induced by fructose would likely heighten food intake (Bray et al., 2004).

It is interesting to note that as sugar-sweetened beverage consumption has increased, a concomitant reduction in milk-based dairy food consumption is noticeable. Indeed this may suggest sugar-sweetened beverages displace milk-based dairy food consumption in the diet of children and adolescents (Harnack, Stang, & Story, 1999; Mrdjenovic & Levitsky, 2003). Consuming snack items of a high nutritive value or reducing sugar-sweetened beverage consumption may help prevent the onset of weight gain and obesity, particularly in children and adolescents (James, Thomas, Cavan, & Kerr, 2004). Relative to sugar-sweetened beverages and fruit-juice drinks, milk-based dairy foods are recognised as holding a high nutritive value and have a unique potential to exert favourable effects on elements of energy balance (energy intake and energy expenditure). In this sense, milk-based dairy foods contain a host of components and bioactive constituents that act individually, and probably synergistically, to impart beneficial effects on body weight regulation through favourable actions on feeding behaviour and energy expenditure (Aziz, Anderson, & Saarela, 2007). These effects may be attributed to high-quality proteins (whey and casein, and their products of digestion) that are known to stimulate concentrations of plasma appetite-regulating peptides (Anderson & Moore, 2004; Bowen, Noakes, & Clifton, 2006; Luhovyy, Akhavan, & Anderson, 2007; Schneeman, Burton-Freeman, & Davis, 2003), inhibit gastric emptying and subsequently reduce energy intake (Dougkas, Minihane, Givens, Reynolds, & Yaqoob, 2012; Lluch et al., 2010). In addition to proteins, medium-chain triglycerides, conjugated linoleic acid and lactose may also be implicated in the role of milk and dairy foods on reducing energy intake (Aziz et al., 2007). Furthermore, milk-based dairy food consumption may result in an increased rate of energy expenditure in comparison to isocaloric sugar-sweetened beverages (St-Onge et al., 2004). This increased rate of energy expenditure may be modulated by two different mechanisms. Firstly, evidence suggests that certain constituents contained within milk-based dairy foods give rise to

concentrations of plasma appetite- and metabolism-related peptides. For example, whey and casein proteins provide an abundance of amino acids, which subsequently causes a rise in plasma amino acid concentration (Luhovyy et al., 2007). The release of plasma amino acids (namely valine, alanine, isoleucine and leucine) mediates insulin and glucagon secretion (Schmid, Schusdziarra, Schulte-Frohlinde, Maier, & Classen, 1989; van Loon, Saris, Verhagen, & Wagenmakers, 2000b). Indeed, there is scientific literature proposing glucagon elicits properties that may stimulate energy expenditure and thermogenesis (Heppner et al., 2010; Marroquí et al., 2014). In addition, protein consumption elicits a greater effect on diet induced thermogenesis (20-35% of energy consumed) compared to calorie matched intakes of carbohydrate (5-15% of energy consumed) or fat (0-3% of energy consumed) (Halton & Hu, 2004). Thus, considering milk-based dairy foods naturally contain large amounts of protein compared with sugar-sweetened beverages a greater effect on diet-induced thermogenesis may be unsurprising. Taken together, it appears that milk-based dairy foods and their properties may work synergistically impacting appetite, feeding behaviour and metabolism in an attempt to maintain energy homeostasis. However, little is known concerning the influence of milk and dairy food consumption (as a snack) on the physiological mechanisms controlling energy regulation, particularly in children and adolescents, and thus warrants further investigation.

1.1.2 Why is the adolescent period of importance?

Childhood and the teenage years are significant stages in human life. Adolescence is the critical transitional period between childhood and the onset of adulthood (13-19 y), characterised by growing independence and marked physical development (Alberga, Sigal, Goldfield, Prud' homme, & Kenny, 2012). Excess adiposity during childhood and adolescence is a significant precursor to the development of many immediate and long-term health implications including cardiovascular disease, hypertension, dyslipidaemia, and insulin resistance (Ogden, Yanovski, Carroll, & Flegal, 2007; Wang, McPherson, Marsh, Gortmaker, & Brown, 2011). Moreover, childhood and adolescent

obesity is associated with obesity in adulthood (Freedman, Mei, Srinivasan, Berenson & Dietz, 2007; Reilly & Kelly, 2011), contributing further to the severity of the aforementioned complications. The likelihood of childhood obesity continuing through to adulthood rises from 20% at the age of 4 y to approximately 80% throughout the adolescent period (Kvaavik, Tell, & Klepp, 2003). Consequently, the incidence of overweight and obesity is associated with profound economic burdens. Unfortunately, the cost of overweight and obesity solely for children and adolescents is not available. However, estimates suggest the annual economic cost of overweight and obesity-related ill health to the National Health Service at approximately £5.1 billion from paediatrics through to adults (Scarborough et al., 2011).

In conjunction with this, the adolescent phase typically foresees various behavioural changes in attitudes toward dietary and physical activity habits. Dietary and physical activity habits shaped throughout this phase may ultimately carry forth and track into adulthood (Lake, Mathers, Rugg-Gunn, & Adamson, 2006). Various features of the adolescent period including growing independence (Rossow & Rise, 1994), shifting daily routine and environmental and/or peer group influence contribute to feeding behaviour and consequently nutritional status (Lake et al., 2004). Thus, the period of adolescence is well acknowledged to play an influential role in the development and persistence of obesity into adulthood. It is therefore of importance to monitor and track dietary trends to help identify the role these modifiable behaviours play on the risk of adolescent adiposity. Furthermore, these modifications warrant attention as persistence of dietary practices deemed unhealthy may incur nutritional and health-related implications including: cardiovascular disease, hypertension, dyslipidemia, and insulin resistance in adolescence and adulthood (Ogden et al., 2007; Wang et al., 2011).

1.1.3 Methodological assessment of body composition in children and adolescents

The collection of anthropometric measures is of practical importance for the estimation of body composition. The capability to precisely quantify body composition is important because of the recognised link between increased levels of body fat and numerous health implications (Biaggi et al., 1999; Maughan, 1993). In clinical and research practice, a number of routinely used predictive techniques are available to researchers when collecting anthropometric measures to determine body composition, each with its own merits and shortcomings. To date, Cadaver analysis (body composition method utilising a freshly deceased human corpse, dissecting and determining the percentage fat in each body part) represents the gold standard approach for body composition analysis (Ellis, 2000), however renders itself inappropriate for use *in vivo*. Analysis *in vivo* predicts body composition from measurements of body properties, rather than measuring it directly (Wells & Fewtrell, 2006). Consequently, to ascertain an accurate estimate of body composition it is important to seek methodological approaches that distinguish between fat mass (FM) and fat free mass (FFM) (Ellis, 2000). As previously alluded to, overweight and obesity in children and adolescents have commonly been assessed utilising estimates of BMI. Used as an estimate of fatness among individuals, BMI is a non-invasive, cost effective technique, and correlates well with percentage body fat in children and adolescents (Chan, Leung, Lam, Peng & Metreweli, 1998; Pietrobelli, 1998). Nonetheless, this approach fails to distinguish between FM and FFM (Wells et al., 2006).

Approaches available to assess FM and FFM in children and adolescents, and thus body composition include dual-energy X-ray absorptiometry, hydrodensitometry and air displacement plethysmography. Hydrodensitometry, pioneered by Behnke (1942), represents one of the most commonly applied (classified as the gold standard) densitometric methods, and includes weighing a participants mass on land and again under water. The principle of hydrodensitometry provides an accurate method for the measurement of body density from which the percentages of FM and FFM can be determined using standard equations. There are many methodological considerations

associated with hydrodensitometry that often limit its application. In this sense, hydrodensitometry is often considered unpleasant and/or difficult, time- and cost-consuming and consequently precludes itself from use within vulnerable populations. This is primarily due to the practical issues associated with the hydrodensitometry procedure in young people. Fully immersing young people underwater presents increased ethical consideration, which are further exasperated, as participants are required to perform a forced maximal exhalation prior to immersion to obtain a measure of pulmonary residual volume. This is a difficult process for young people (Claessens, Beunen, & Malina, 2000), which consequently can result in errors of 2.5% (Deurenberg, Pieters, & Hautvast, 1990) to ~4% (Forsyth, Plyley, & Shephard, 1988) when estimating percentage body fat. Alternatively, whole body air displacement plethysmography (Dempster & Aitkens, 1995) offers an appropriate methodological tool to estimate body composition. The automated approach of whole body air displacement plethysmography poses numerous advantages including ability to distinguish FM and FFM (Wells et al., 2006), simplistic application, comfort and is a non-invasive technique that is suitable for use among young people (Wagner & Heyward, 1999). The whole measurement procedure using the BOD POD lasts approximately 8-10 minutes, unlike hydrostatic weighing which can take 30 minutes and more (Claros, Hull, & Fields, 2005). Consequently, this method of body composition analysis is appealing to researchers who work with young populations. Only one company to date manufactures the machinery to conduct whole body air displacement plethysmography, a dual chambered plethysmograph with the trade name 'BOD POD'. When a participant is placed (sitting) inside the air-tight plethysmograph measurement chamber (Life Measurement, Inc, Concord, CA), a volume of air is displaced equivalent to the participants body volume. From this, an indirect measure can be predicted by detracting the volume of air remaining inside the compartment when the participant is inside from the volume of air in the chamber when it is unfilled. While the density of fat is indeed relatively constant, that of FFM varies according to its composition. Therefore, to obtain an accurate estimate of body composition it is of crucial importance to stringently control for methodological and biological factors. For example, hydration

status of the participant has been shown to impact on the estimation of FFM (underestimated) and FM (overestimated) (Heiss et al., 2009; Le Carvennec et al., 2007; Vukovich & Peeters, 2003).

The application of air displacement plethysmography in children and adolescents has increasingly been implemented to determine body composition (Carnier et al., 2010; Demerath et al., 2002; Gately et al., 2003; Lockner, Heyward, Baumgartner, & Jenkins, 2000; Moon et al., 2008; Radley et al., 2003). Furthermore, air displacement plethysmography has successfully been implemented in paediatric (Rumbold, St Clair Gibson, Allsop, Stevenson, & Dodd-Reynolds, 2011; Rumbold et al., 2013) and adolescent appetite-related investigations (Carnier et al., 2010). In relation to the validity, precision and accuracy of the BOD POD compared with reference techniques (hydrodensitometry and dual-energy X-ray absorptiometry) relatively few studies have been conducted in young people and adults. Evidence from the available scientific literature pertaining to this issue, suggests the validity of this approach in young people is ambiguous. In this sense, BOD POD assessments of body fat were reportedly higher compared to hydrostatic weighing, though not significantly different (0.6-1.7%) (Dewit, Fuller, Fewtrell, Elia & Wells, 200; Nuenz et al., 1999; Wells, 2000). Despite these differences, research conducted by Fields and Goran (2000) and Lockner and colleagues (2000) described R^2 values of 0.72 and 0.87, identifying that the BOD POD explained 72%-87% of the variance in hydrostatic weighing, respectively (Fields & Goran, 2000; Lockner et al., 2000). Furthermore, evidence from Fields and Goran (2000) showed a good standard error of the estimate (3.3%). In the original study of Fields and Goran (2000), the cited authors investigated the validity of the air displacement plethysmography technique by regressing fat mass calculated by the BOD POD with fat mass from dual-energy X-ray absorptiometry, as opposed to percentage body fat. Consequently, it was concluded that the BOD POD is the only technique that can accurately, precisely, and without bias estimate fat mass in 9-14 year old children (Fields & Goran, 2000). Similarly, when estimates of percentage body fat are regressed against dual-energy X-ray absorptiometry and a 5-compartment model, favourable results

are identified. The BOD POD has been identified to explain between 70% - 96% of the variance in dual-energy X-ray absorptiometry and a 5-compartment model with good standard errors of estimates ranging from 1.55–4.10% (Fields et al., 2000; Lockner et al., 2000; Nunez et al., 1999) , which according to Lohman (1992), indicate good and very good agreement. In a later study by Field and colleagues (2002) it was concluded that that the BOD POD and hydrodensitometry agree within 1% of body fat in children, adolescents and adults (Fields, Goran & McCrory, 2002). Furthermore, Gately et al. (2003) examined the precision of body composition estimates from air displacement plethysmography, dual-energy X-ray absorptiometry and total body water compared to a criterion 4-compartmental model in overweight and obese children and adolescents. The authors' reported that air displacement plethysmography was the most accurate approach to assess body composition in overweight and obese children and adolescents compared with a 4-compartmental model (Gately et al., 2003). To summarise, it appears that in children and adolescents air displacement plethysmography may exert a similar rate of accuracy compared with other densitometry techniques. Because the methodological approach of the BOD POD is non-invasive and not logistically challenging, it is subsequently an attractive method to provide an means of body composition analysis for individuals aged 4 y and onwards. However, this remains true only when biological and methodological influences are kept consistent.

1.2 Milk-based dairy foods

For millennia, the inclusion of milk-based dairy foods as a component of a healthy balanced diet has been recognised extensively. The phrase dairy is used as a blanket term to label milk-based foodstuff that originate from the mammary gland of mammals. This foodstuff (encompassing products such as milk, yogurt, and cheese, amongst others) contains numerous biological constituents and represents a functional food. Consequently milk-based foodstuff exert the potential to impact on human health (Fiorito, Mitchell, Smiciklas-Wright, & Birch, 2006). Briefly, milk

represents an emulsified colloidal substance comprising fat globules dispersed through an aqueous solution which contains an extensive assortment of nutrients including vital minerals, macro- and micro-nutrients (Kliem & Givens, 2011). Accordingly, milk-based dairy foods represent a nutrient dense foodstuff, and consumption can improve the overall nutritional quality of the diet (Fiorito, Mitchell, et al., 2006).

The composition of milk is approximately 87% water and 13% solids. Lactose, the primary carbohydrate portion of milk, is comprised of glucose and galactose. Lactose is broken down in the intestine into molecules of D-glucose and D-galactose by the enzyme lactase during lactose hydrolysis. Lactose, a disaccharide, acts like a low glycaemic food (GI ~ 43) eliciting a short-lived rise in plasma glucose concentration (Gannon, Nuttall, Krezowski, Billington, & Parker, 1986). The lipid component of milk and milk-based dairy foods contribute numerous properties including the provision of fat-soluble micro-nutrients, essential fatty acids in addition to influencing flavour, texture and appearance (Parodi, 2004). Triacylglycerols represent the majority of lipid contained in milk (~ 97%), with the remainder composed of phospholipids (~ 1%), diacyl-glycerol (~ 2%), cholesterol and fat-soluble micro-nutrients (Haug & Harstad, 2007). Of the lipid content within milk, roughly 64% is composed of saturated fatty acids, with a considerable amount (~26%) from monounsaturated fatty acids and a lesser contribution from trans and polyunsaturated fatty acids (both ~ 3%) (MacGibbon & Taylor, 2006). Additionally, milk-based dairy foods provide high-quality proteins, namely casein and whey. Casein and whey constitute approximately 82% and 18% of the total protein found in milk and provide an abundance of essential amino acids (Wilkinson et al., 2007). From a physiological perspective, milk proteins are distinguishable according to their digestion and contribution to protein synthesis. Whey protein consumption produces a large and early spike in concentrations of plasma amino acids, primarily due to the fact that this protein is acid-soluble, rapidly digested and absorbed (Pennings et al., 2011). Moreover, casein is not acid-soluble (Boirie et al., 1997; Boutrou et al., 2013). On impact with the stomach (an acidic

environment), consumption delays gastric emptying and absorption due to clot formulation (Boirie et al., 1997; Boutrou et al., 2013). Consequently, casein and whey are commonly referred to as 'slow' and 'fast' proteins respectively, primarily according to their effect on plasma amino acid concentrations (Boirie et al., 1997). In this sense, the consumption of whey protein causes a large (yet short-lived) rise in plasma amino acid concentration (Luhovyy et al., 2007). Peak concentrations may be observed 40 min to 2 h following consumption, and returns to baseline values after 3 to 4 h (Luhovyy et al., 2007). Concentrations of plasma amino acids yield a lower and more sustained presence following casein consumption (~ 7 h), supporting the concept of a 'slow' protein which is attributed to the reduced rate of gastric emptying (Boirie et al., 1997).

In addition, milk-based dairy foods contain vital micro-nutrients (provided only through the diet) that contribute to dietary quality, bone health, and overall nutritional status. With the exception of vitamin C (which is broken down during pasteurisation), milk is a source of all vitamins (Tunick & Van Hekken, 2014). Calcium and phosphorous, crucial to healthy growth and development and other biological processes, are the most prominent minerals present in dairy. In addition, milk and other milk-based dairy foods make significant contributions to intakes of other major minerals. To highlight this, **Table 1.0** displays the nutritional composition of commonly consumed milk-based dairy foods in the UK. Investigations concerning children and adolescents illustrate that consumption of milk-based foods bolsters calcium intakes and nutritional status, without significantly impacting on daily calorie consumption, fat intake or anthropometric measures (Fayet, Ridges, Wright, & Petocz, 2013). Consequently, sufficient intakes of milk and dairy food are therefore encouraged, particularly during childhood and adolescence, promoting improved dietary quality, bone health, and overall nutritional status (Fiorito, Mitchell, et al., 2006).

Indeed, continual dairy food avoidance may incur detrimental nutritional and health-related implications, particularly among adolescents, leaving populations vulnerable to micronutrient deficiencies and thus lasting health implications (Nicklas, 2003).

Table 1.0 Nutritional composition of common milk-based dairy foods

Per 100 g	Whole-milk	Semi-Skimmed milk	Skimmed milk	Flavoured milk	Yogurt	Low-fat yogurt	Cheddar Cheese
Energy (kJ)	274	195	144	270	333	237	1725
Protein (g)	3.3	3.5	3.5	3.6	5.7	4.8	25.4
Carbohydrate (g)	4.6	4.7	4.8	9.6	7.8	7.4	0.1
of which sugars (g)	4.6	4.7	4.8	8.9	7.8	7.1	0.1
Fat (g)	3.9	1.7	0.3	1.5	3	1	34.9
of which saturates	2.5	1.1	0.1	1	1.7	0.7	21.7
monounsaturates	1	0.4	0.1	0.3	0.9	0.2	9.4
polyunsaturates	0.1	Trace	Trace	0.1	0.2	Trace	1.1
trans fatty acids	0.1	0.1	Trace	Trace	N/A	Trace	1.4
Calcium (mg)	118	120	125	120	200	162	739
Riboflavin (mg)	0.23	0.24	0.22	0.17	0.27	0.22	0.39
Vitamin B6 (mg)	0.06	0.06	0.06	0.03	0.1	0.01	0.15
Vitamin B12 (μg)	0.6	0.9	0.8	0.1	0.2	0.3	2.4
Vitamin C (mg)	2	2	1	Trace	1	1	Trace
Retinol (μg)	30	19	1	20	28	8	364
Carotene (μg)	19	9	Trace	8	21	Trace	141
Sodium (mg)	43	43	44	52	80	63	723
Potassium (mg)	155	156	162	168	280	228	75
Magnesium (mg)	11	11	11	12	19	16	29
Phosphorus (mg)	93	94	96	102	170	143	505
Zinc (mg)	0.4	0.4	0.5	0.4	0.7	0.6	4.1
Manganese (mg)	Trace	Trace	Trace	Trace	Trace	Trace	Trace
Iodine (μg)	31	30	30	N/A	[63]	34	30

Note: N/A = values not available for this food; [n] = values have been estimated; Trace = nutrient is present in less than 0.1g per 100g. Adapted from the Dairy Council, UK. Available at: http://www.milk.co.uk/publications/default.aspx

1.2.1 Trends of milk-based dairy consumption in children and adolescents

To date, the NDNS (Bates et al., 2014) remains the only surveillance programme in the UK providing a nationally representative assessment concerning dietary habits of individuals, aged 1.5 y and older, living in private households within the UK. Knowledge concerning dairy consumption in the UK is therefore limited. In Westernised societies, current recommendations for milk-based dairy food intake generally encourage two to three servings daily (Gidding et al., 2006), however, suggestions concerning appropriate serving sizes are not provided in the UK. Statistics obtained from the most recent NDNS indicate that in 2011-12, the consumption of milk and dairy foods was approximately 30% greater in children (4-10 y: 275 $g \cdot d^{-1}$) compared with their adolescent counterparts (11-18 y: 197 $g \cdot d^{-1}$) (Bates et al., 2014). Furthermore, among male and female adolescents, per capita milk-based dairy food consumption has fallen by 14.1% in comparison to reported intakes in 1997. To highlight the temporal and age related trends in milk-based dairy food consumption from 1997 through to 2011-12, total intakes are presented in **Table 1.1** for children 4-10 y and adolescents 11-18 y. From **Table 1.1** presented below it is clear that milk-based dairy food consumption has remained widely stable across boys and girls (4-10 y), yet has steadily declined in adolescents (11-18 y). The overall decline in milk and milk-based dairy food consumption of adolescent males and females, however, is consistent with earlier findings of US children and adolescents (Cavadini, Siega-Riz, & Popkin, 2000; Morton & Guthrie, 1998). Furthermore, an increasing time trend of low-fat milk consumption (particularly in children 4-10 y) is in accordance with findings reported in German children (Alexy & Kersting, 2003).

The determinants facilitating the abovementioned trends are poorly understood. The choices individuals make around foods determine which nutrients are consumed, however consumers do not choose their foods exclusively for the nutrients they provide (Pollard, Kirk, & Cade, 2002). Over the past few years our food environment has become increasingly obeseogenic, complimented by an increasing diversity in the availability of highly palatable foods (Egecioglu et

al., 2011). Consequently, we have witnessed a decline in the consumption of staple foodstuff (for example cereals and milk-based dairy foods), and an increased consumption of more palatable foods including meat, fish, sugar and vegetable fats (Kearney, 2010; Shapouri & Rosen, 2007). Eating behaviour is thus complex and an understanding of the impact of factors affecting food choice is vital given the priority for population dietary change (Pollard et al., 2002). Although the cited figures cannot be used to elucidate motivations for differences in dairy food consumption, evidence from social science begins to reveal factors that may influence consumer behaviour and subsequent consumption patterns. These include social, economic and cultural factors (F. Johnson & Wardle, 2014). In addition, some factors are likely to exert influence through more proximal determinants of behaviour such as attitudes, personal ideologies about health-related benefits, individual lifestyle and perceived barriers (F. Johnson, Pratt, & Wardle, 2011; Wardle & Steptoe, 2003). In this sense, numerous observations indicate that consumers often perceive dairy consumption with increased body mass and adverse health effects (Nolan-Clark, Neale, Probst, Charlton, & Tapsell, 2011; Wham & Worsley, 2001). Research suggests female adolescents decrease dairy food consumption due to concerns regarding weight gain and the notion that dairy foods are fattening (Gulliver & Horwath, 2001; Neumark-Sztainer, Story, Dixon, Resnick, & Blum, 1997). Indeed, dairy foods contain saturated fats, and historical views concerning elevated plasma cholesterol following consumption have been linked to increased cardiovascular risk (Soerensen, Thorning, Astrup, Kristensen, & Lorenzen, 2014; Tunick et al., 2014). Taken together, the belief that dairy foods are fattening and consumption should be limited may have contributed to the assumption that dairy is a factor in obesity (Elwood, Pickering, Givens, & Gallacher, 2010), and represent important barriers to increasing consumption. Somewhat paradoxically, trends concerning the incidence of overweight and obesity are concurrent with declining levels of dairy consumption, which may suggest that dairy foods confer a direct or indirect protective effect against adiposity and consequently warrants further investigation (Huang & McCrory, 2005).

Table 1.1 Milk-based dairy food consumption for children (4-10 y) and adolescents (11-18 y) from 1997 through to 2011-12

	1997		2008		2009/10		2010/2011		2011/12	
Age (y)	**4-10**	**11-18**	**4-10**	**11-18**	**4-10**	**11-18**	**4-10**	**11-18**	**4-10**	**11-18**
Milk and Milk Products (g·d)		*								
Whole-milk (3.8% fat)	112	54	77	44	95	38	89	37	86	33
Semi-skimmed milk (1.8% fat)	83	109	106	93	102	89	105	89	105	100
1% fat milk	NG	NG	NG	NG	1	3	1	2	1	2
Skimmed milk (0.5% fat)	4	8	4	4	7	4	6	5	6	6
Other milk and cream	11	12			16	16	16	16	16	16
Cheese	9	11	12	11	10	11	10	11	10	11
Yogurt, fromage frais and other dairy desserts	33	22	33	22	35	20	36	20	39	19
Ice-cream	13	11	16	10	13	9	14	9	13	8
Total (g·d)	**265**	**227**	**248**	**184**	**279**	**190**	**276**	**189**	**275**	**197**

* Values recalculated to represent four days: Total quantities of food consumed (grams) per day. NG; not given. Table adapted from NDNS (Bates et al., 2014)

Originally established in 1992, leading on from the 1986-87 Dietary and Nutrition Survey of British Adults, the NDNS in general formerly focused on specific population groups. These first few cross-sectional dietary surveys analysed the types and amounts of food consumed by pre-school children aged 1.5 through 4.5 y (Gregory , Collins, Davies, Hughes, & Clarke, 1995), young people aged 4 through 18 y (Gregory et al., 2000), adults aged 19 through 64 y (Henderson, Gregory, & Swan, 2003) and older adults aged 65 y and above (Finch et al., 1998). To date, the most recent series of dietary surveys was introduced in 2008 (the rolling programme), and comprises dietary habits of individuals aged 1.5 y and older. A notable limitation concerning the series of NDNS relates to the fact that methodological approaches used to collect dietary information have varied across these cross-sectional assessments, which consequently limits comparability between preceding surveys. For example, dietary habits of young people (Gregory et al., 2000) and adults (Henderson et al., 2003) were obtained from 7 day self-reported weighed food records. In addition, the NDNS of pre-school children (Gregory et al., 1995) utilised a parental 4 day weighed food record approach, which was believed to oversample weekend days. Following the formulation of the rolling programme, self-reported dietary habits of individuals aged 1.5 y and older have also adopted a 4-day food record approach, yet consumption patterns are estimated and not weighed. The majority of the methodological approaches used to collect dietary information have typically relied on retrospective dietary assessment methods, which pose complications of misreporting error and are therefore not entirely robust. As evaluations are confounded by methodological disparities, trends concerning milk-based dairy food consumption may therefore represent methodological inadequacies rather than changes in habitual dietary practises. Furthermore, the use of wide-ranging age groupings, 4-10 y and 11-18 y for example, make it difficult to differentiate between consumption patterns in middle-childhood and adolescence.

1.3 The relationship between milk-based dairy consumption and body mass

The initial suggestion that dairy consumption may confer a protective effect against weight gain and attenuate weight loss arose over 30 years ago (McCarron, Morris, Henry, & Stanton, 1984) from an influential investigation initially designed to study the relationship between nutrient intakes and blood pressure. McCarron and colleagues examined data from 10,372 American adults (18-74 y) and determined that greater rates of calcium consumption were inversely associated with BMI (McCarron et al., 1984). These findings were later replicated in 2000 from data obtained from the National Health and Examination Survey (Zemel, Shi, Greer, Dirienzo & Zemel, 2000). Zemel and colleagues (2000) collated data from 1988 through to 1994 to determine the association between dairy food consumption and/or dietary calcium intake on body composition (Zemel et al., 2000). The authors' noted that the risk of being classified in the highest quartile of body fat was inversely associated with dairy food and calcium consumption. Zemel and colleagues (2000) noted that in obese African-Americans increasing daily calcium intake from ~400 to 1000 mg/d for 1 y resulted in a 4.9 kg decrease in body fat (Zemel et al., 2000). It is relevant to highlight these findings remained after controlling for energy intake, age, ethnicity and physical activity level. It was indeed the aforementioned studies that provided the platform for investigating the role of dairy, and in particular calcium, consumption and weight management in children and adolescents.

An accumulating body of literature has since arisen corroborating a role for milk-based dairy foods and/or calcium consumption and weight management in children and adolescents. Evidence obtained from cross-sectional and prospective investigations have demonstrated an inverse relationship (Abreu et al., 2013; Barba, Troiano, Russo, Venezia, & Siani, 2005; Bradlee, Singer, Qureshi, & Moore, 2010; Carruth & Skinner, 2001; L. L. Moore, Singer, Qureshi, & Bradlee, 2008; Olivares et al., 2004) or a neutral effect between milk-based dairy consumption and adiposity (Almon, Patterson, Nilsson, Engfeldt, & Sjostrom, 2010; Fiorito, Ventura, Mitchell, Smiciklas-Wright, & Birch, 2006; Huh, Rifas-Shiman, Rich-Edwards, Taveras, & Gillman, 2010;

Keller, Kirzner, Pietrobelli, St-Onge, & Faith, 2009; Phillips et al., 2003). Barba and colleagues (2005) were the first group to indicate that milk consumption was significantly inversely associated with BMI in children (3-11 y). In this study, 884 children completed a 1 y dietary frequency questionnaire (parents of the children completed the questionnaire) evaluating lifestyle and dietary habits. The authors' illustrated, after controlling for confounding variables, the frequency of milk consumption in relation to age- and sex-specific BMI z-scores was significantly inversely related. Interestingly, observations remained significant for whole-milk consumption, yet not skimmed milk. In a larger study comprising more than 10,000 children (5-11 y) and adolescents (12-16 y), Moore et al. (2008) examined the link between milk-based dairy consumption and body fat among individuals taking part in a national dietary survey (NHANES). During the survey, milk-based dairy food consumption was estimated through the use of 24 h dietary recalls, and analysis of covariance to control for potential confounding factors (age, sex, socio-economic status, ethnicity, stature and television viewing) were conducted. Analysis revealed, for adolescents only, that milk-based dairy consumption was inversely associated with anthropometric measures of body fat.

Although, the findings from the aforementioned studies provide promise with regard to milk-based dairy food consumption and adiposity it is relevant to highlight these findings have been drawn from observational research. Findings from observational research cannot infer a cause and effect relationship (Mann, 2003). To distinguish a causal relationship, randomised controlled trials centred on a control and/or placebo are believed to be the gold standard experimental approach (Sullivan, 2011). To date, a small number of well controlled randomised investigations have been conducted to evaluate the effect of milk-based dairy food consumption on body mass and composition of children and adolescents (Albala et al., 2008; G. M. Chan, Hoffman, & McMurry, 1995; Ghayour-Mobarhan et al., 2009; Merrilees et al., 2000; St-Onge, Goree, & Gower, 2009), and all have reported a neutral effect of milk-based dairy food consumption on indices of adiposity. While there is a dearth of literature from well controlled studies, a recent meta-analysis comprising

information from observation and intervention studies in pre-school, school-age children and adolescents indicated that the consumption of milk-based dairy foods provides a neutral effect against adiposity during early and middle-childhood and a modestly protective effect in adolescence (Dror, 2014). Consequently, interest concerning the association between adiposity and the consumption of dairy foods has driven investigators to examine the impact of milk-based dairy food on factors influencing energy balance. To date, efforts to establish the underlying relationship between the consumption of dairy and adiposity have not been clearly elucidated in young people, however, several plausible mechanisms have been identified. A diagrammatic representation of these mechanisms is presented in **Figure 1.0**.

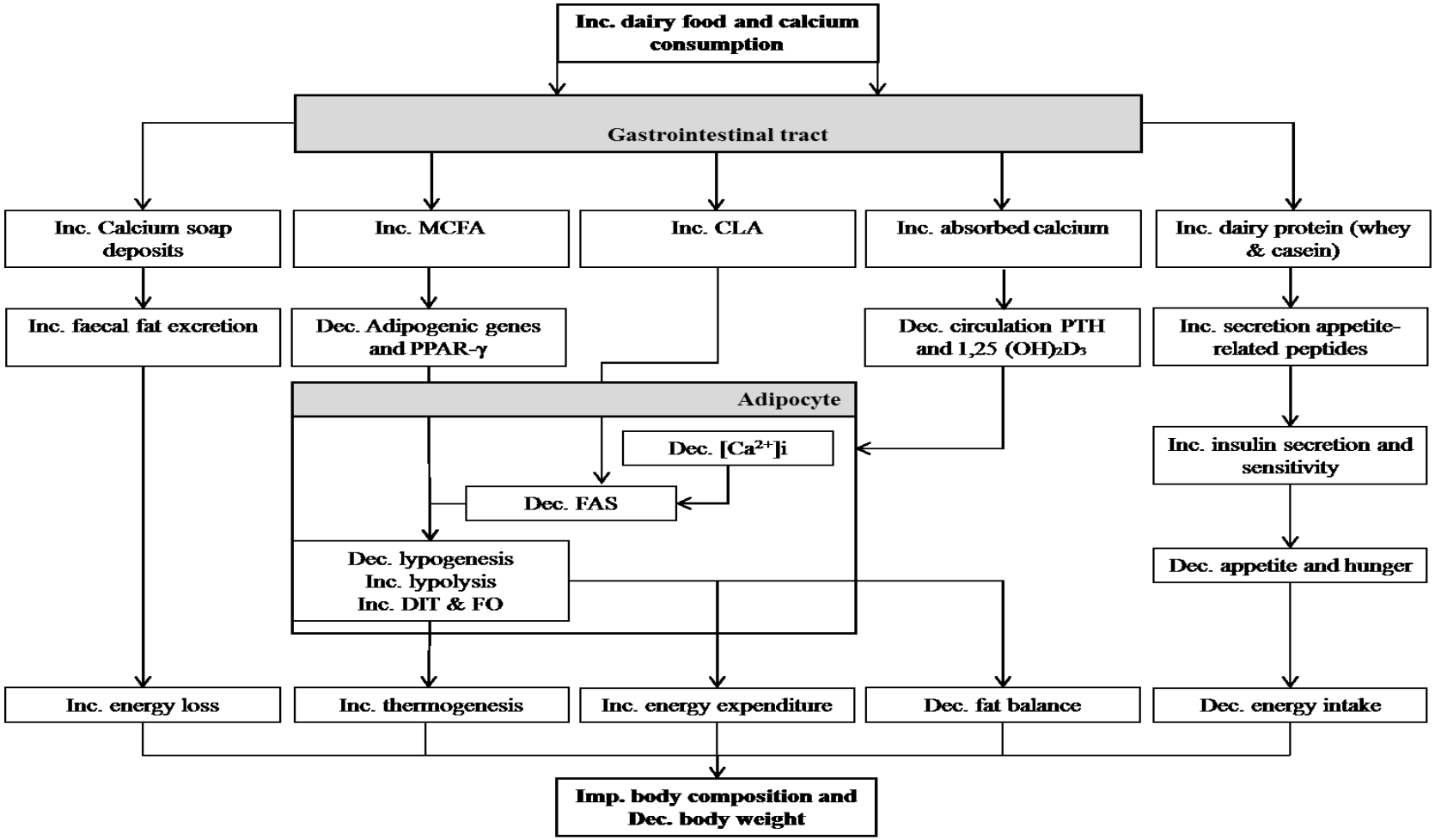

Figure 1.0: Mechanisms underlying the link between milk-based dairy food consumption and adiposity. Inc = increased; Dec = decreased; MCFA = medium chain fatty acids; CLA = conjugated linoleic acid; PTH = parathyroid hormone; 1,25 $(OH)_2D_3$ = $_{1,25}$ dihydroxyvitamin D_3; $[Ca^{2+}]I$ = intracellular calcium; FAS = fatty acid synthase; DIT = diet induced thermogenesis; FO = fat oxidation PPAR-γ = peroxisome proliferator activated receptor-g. Adapted from (Dougkas et al., 2011).

1.3.1 Mechanisms underlying the impact of milk-based dairy consumption on body mass regulation

The possible mechanisms underlying the relationship between milk-based dairy food consumption and the regulation of energy balance have not been clearly revealed. Research involving adult cohorts indicates the consumption of dairy and their constituents including calcium, medium-chain triglycerides and conjugated linioleic acid to calcium may inhibit lipid accretion and influence adipocyte lipid metabolism (Dougkas et al., 2012). In this sense, a collection of studies support the notion that high dietary calcium and/or dairy food consumption increases postprandial fat oxidation (Melanson, Donahoo, Dong, Ida & Zemel, 2005; Van Loan, 2009), and faecal fat excretion (Bendsen, Hother, Jensen, Lorenzen, & Astrup, 2008). In humans, an augmented rate of fat oxidation is one mechanism that has been suggested to facilitate the influence of dietary calcium and/or dairy products on body fatness. It is known that low dietary calcium intake increases blood concentrations of calcitropic hormones (parathyroid hormone and $_{1,25}$ dihydroxyvitamin D_3 [active metabolite of vitamin D]) (Soares, Murhadi, Kurpad, Chan She Ping-Delfos, & Piers, 2012). Both parathyroid hormone and $_{1,25}$ dihydroxyvitamin D_3 increase the concentration of intracellular calcium in human adipocyte. Increasing adipocyte intracellular calcium appears to promote triglyceride storage in human adipocytes mediating a co-ordinated control of lipogenesis and lipolysis (Zemel et al., 2000). The cited authors hypothesised that greater dairy consumption (and thus increased dietary calcium) may suppress circulating concentrations of parathyroid hormone and $_{1,25}$ dihydroxyvitamin D_3. It is therefore probable that reduced circulating concentrations of calcitropic hormones thereby reduce intracellular calcium concentration, lipid storage and thus enhance lipolysis.

Consistent with this hypothesis, Melanson and colleagues (2003) were the first to report a link between dietary calcium intake and fat oxidation in humans. In this study 35 young healthy adults (21 males, 14 females) stayed in a whole room calorimeter for a 24 h period. During this time, participants were permitted to select their food preferences (e.g. some participants avoided

dairy products) and consumed a diet designed to achieve energy balance, estimated from FFM. Acutely measured dietary calcium intake during the 24 h period was positively correlated with fat oxidation ($r = 0.38$, $p = 0.03$) and inversely correlated with 24 h respiratory quotient ($r = 0.36$, $p = 0.04$) (Melanson et al., 2003). Habitual dietary calcium consumption (as determined from 4-day self-reported weighed food records), however, was not significantly correlated to fat oxidation or respiratory quotient. It must be noted, however, that this study was of a cross-sectional design, and therefore findings do not show directly that calcium promotes fat metabolism. Melanson and colleagues (2005) later compared the impact of high and low dietary calcium intake (through dairy products) on macronutrient oxidation (Melanson et al., 2005). In a randomised crossover design, overweight men and women (10 males, 9 females) took part in four 7 day experimental protocols. The protocols involved either 7 days of high (~1400 mg·d) or low (~500 mg·d) dietary calcium intake. This was achieved through the use of whole dairy foods. The diets issued throughout the experimental periods were matched for energy and macronutrient content, yet differed only in the amount of dietary calcium. Each participant completed each protocol twice, once under conditions of energy balance and once under an acute 600 kcal energy deficit. On day seven of each experimental protocol, participants were studied for 24 h in a whole room calorimeter. Under energy balance conditions, there was no effect of diet treatment on respiratory quotient or 24 h macronutrient oxidation. Under energy deficit conditions, however, 24 h fat metabolism was significantly increased (28% greater) following the high dietary calcium diet (Melanson et al., 2005. Therefore, one might conclude that calcium intake without caloric restriction would not influence fat metabolism. Indeed, most recent work (under energy balance conditions) has failed to replicate increased fat metabolism at rest and during exercise with acute and chronic (14 day) calcium supplementation (Gonzalez, Green, Campbell, Rumbold & Stevenson, 2014; Gonzalez, Rumbold & Stevenson, 2013).

At the time of writing, only two investigations (one acute-term and one moderate-term study) have attempted to establish such mechanisms within a child and adolescent population (Apolzan et al., 2006; Weaver et al., 2011), and the results are equivocal. In overweight adolescent boys (13-15 y) and girls (12-14 y), Weaver and colleagues (2011) sought to evaluate moderate-term (3-week) calcium carbonate ($CaCO_3$) or dairy calcium consumption on modulation of energy metabolism. The aforementioned age categories represent the epochs of peak calcium accretion for males and females, respectively, and correspond to a comparable stage of pubertal development, therefore matching genders for biological maturity. Exercised in a randomised crossover fashion as part of a summer research camp, which was rigorously controlled, 25 girls and 17 boys participated in two 3-week metabolic balance sessions that contained two amounts of calcium; 650 mg·d during the control period and 1300 mg mg·d (one half of participants achieved this through $CaCO_3$ supplementation and the other through dairy foods) during the intervention period. During the supplementation periods participants followed a strictly controlled diet, and observations for energy expenditure, substrate oxidation and energy balance were conducted. The experimental data from the cited study demonstrated no capacity of $CaCO_3$ or dairy calcium to alter energy balance under controlled conditions relative to baseline observations (Weaver et al., 2011), despite significant increases in postprandial serum parathyroid hormone suppression. These results may therefore suggest that, under conditions when energy intake is controlled and not reduced for weight loss purposes, dietary calcium and dairy consumption does not influence energy balance. In addition, Apolzan et al. (2006) evaluated energy expenditure ($kcal \cdot min^{-1}$) for 240 min after a low calcium non-dairy control, supplemental calcium or a dairy-based product in (n = 42) overweight adolescent males and females (12-15 y). They observed a greater rate of energy expenditure (value not given) following the consumption of the dairy-based product compared with the low calcium non-dairy control, but only in adolescent males. No differences were recorded following supplemental calcium ingestion, which may suggest additional constituents housed within milk-based dairy foods

act to impact on metabolism. Consequently, this warrants the need for further investigation among this age group where the use of whole milk-based dairy foods may be more appropriate.

1.3.1.1 Milk-based dairy consumption and appetite

Accumulating evidence suggests that an additional mechanism of action through which dairy may confer an anti-obesity effect is through favourable actions on feeding behaviour (Aziz et al., 2007). Evidence obtained from adult studies suggest that numerous constituents housed within milk-based dairy foods may facilitate body mass regulation through dietary and appetite regulation and are associated with increased plasma concentrations of appetite-regulating peptides (Anderson et al., 2004; Bowen et al., 2006; Luhovyy et al., 2007; Schneeman et al., 2003), inhibiting gastric emptying and subsequently reducing energy intake (Dougkas et al., 2012; Lluch et al., 2010). In this sense, several hormonal peptides of gastrointestinal, pancreatic and adipose tissue origin have been shown to mediate appetite and energy intake responses, and the effects of these hormones may be potentiated by the consumption of milk-based dairy foods. Research concerning the consumption of milk-based dairy foods on appetite and postprandial hormonal response among children and adolescents is however sparse, particularly within the free-living environment. Nonetheless, from the available scientific literature it appears that acute consumption of milk-based dairy foods in children and adolescents is also associated with reduced energy intake (Birch, McPhee, Bryant, & Johnson, 1993; Mehrabani et al., 2014; Vien et al., 2014; Zandstra, Mathey, Graaf, & van Staveren, 2000), and increased concentrations of appetite-related peptides (Vien et al., 2014). Before the relationship between milk-based dairy food consumption on appetite and feeding behaviour is discussed further, it is prudent to provide an overview of appetite regulation, feeding behaviour and the methodological considerations associated with their assessment in children and adolescents.

1.4 Regulation of appetite and feeding behaviour

Appetite comprises numerous regulatory processes associated with the initiation and termination of eating, selection and amount of food consumed. The phenomenon of appetite encompasses a variety of feeling states directly related to feeding behaviour including hunger, satiation and satiety. Hunger signifies an individual's drive to find and consume food (King, Tremblay, & Blundell, 1997), whereas satiation is commonly characterised as the perception of satisfaction and consequently the cessation of eating thus influencing the amount of energy consumed at eating occasions (Benelam, 2009). Satiety, on the contrary, is characterised as the perception of fullness whereby further food consumption is inhibited pending the onset of hunger (Benelam, 2009). The regulation of appetite and feeding behaviour depend on the detection and integration of signals emulating nutritional status and their interaction with signals associated with food palatability and gastrointestinal handling in addition to circadian, social, emotional, habitual and other situational influences (Woods, Lutz, Geary & Langhans, 2006). Consequently, appetite and the regulation of feeding behaviour are sophisticated processes, regulated through homeostatic and non-homeostatic influences (Berthoud, 2004). Appetite, as such, is therefore modulated within a psychobiological environment.

1.4.1 Homeostatic regulation of appetite and feeding behaviour

From a physiological perspective, the central nervous system (CNS) plays a central role in the mechanistic control of energy regulation through complex interactions between nutrients, hormones, neuropeptides and numerous brain regions (Lenard & Berthoud, 2008). In general, the hypothalamus of the brain exerts a principal role in energy regulation, eliciting divergent actions on appetite and feeding behaviour. Specifically, the arcuate nucleus (ARC) within the hypothalamus of the brain senses signals arising from the periphery of numerous endocrine organs, which act on two separate neuronal populations (Harrold, Dovey, Blundell, & Halford, 2012). These neuronal populations can be distinguished according to their influence on appetite and feeding behaviour

(Wynne, Stanley, McGowan, & Bloom, 2005). The orexigenic agouti-related peptide (AgRP) and neuropeptide Y (NPY) are coexpressed by one population (Druce & Bloom, 2006). The remaining population releases cocaine and amphetamine regulated transcript (CART) and proopiomelanocortin (POMC), both of which elicit anorexigenic actions (Druce et al., 2006). In addition, accumulating evidence has confirmed the homeostatic control of appetite and feeding behaviour to be heavily, yet not exclusively, governed by multiple hormonal peptides within the peripheral circulation of gastrointestinal, pancreatic and adipose tissue origin (Lancha, Frühbeck, & Gómez-Ambrosi, 2012). These peptides act to influence the physiological mechanisms and neuronal populations controlling energy intake and expenditure, communicating acute nutritional status and chronic energy availability to the CNS. In this sense, expressing receptors for several of the hormonal and neuropeptides, the ARC is able to access the peripheral circulation and assimilate hormonal peptides to promote orexigenic (stimulates appetite) and anorexigenic (suppresses appetite) behaviours (Neary, Goldstone, & Bloom, 2004). Two types of hormonal peptides exist in the circulation, specifically episodic (short-term) and tonic (long-term) peptides, and are subsequently characterised according to their kinetic activity (Blundell et al., 2006). Episodic and tonic peptides characteristically arise following food consumption and communicate with the hypothalamic region of the brain, eliciting divergent actions on appetite, feeding behaviour and metabolism (Huda, Wilding, & Pinkney, 2006). In humans, hormonal peptides such as glucagon-like peptide-1 (GLP-1) (Verdich et al., 2001), glucagon (Chan et al., 1984), insulin (Air, Benoit, Blake Smith, Clegg, & Woods, 2002), leptin (Schwartz, Woods, Porte, Seeley, & Baskin, 2000), peptide tyrosine tyrosine (PYY) (Batterham et al., 2002), oxyntomodulin (OXM) (Wynne et al., 2006), and cholecystokinin (CKK) (Pi-Sunyer, Kissileff, Thornton, & Smith, 1982) have been implicated in the regulation of appetite and feeding behaviour. In this literature review, however, further focus will only concern four hormonal peptides that are currently receiving increased attention and consequently inclusion within this thesis (GLP-1, glucagon, insulin and leptin).

1.4.1.1 Episodic hormonal peptides

Episodic hormonal peptides including GLP-1, glucagon, among others, act to signal short-term nutritional state and are rhythmically synchronised with eating occasions (Blundell et al., 2010) or fasting. These peptides express anorectic properties encouraging meal termination, satiation and attenuate inter-meal hunger (Halford & Harrold, 2008). Glucagon-like peptide-1, a 30 amino acid peptide, is primarily produced by the mucosal L-cells of the intestine (small and large), alpha cells of the islets of Langerhans and by neurons within the nucleus of the tractus solitarius of the brainstem (Crespo, 2014). Glucagon-like peptide-1 exists in two biologically active forms, namely GLP-$1_{7\text{-}37}$ and GLP-$1_{7\text{-}36}$ (Ørskov, Rabenhøj, Wettergren, Kofod, & Holst, 1994), with the latter representing the major circulating form in human plasma (20% and 80% of GLP-1 secretion, respectively) (Orskov et al., 1986). The primary function of GLP-1 is to optimise nutrient disposition and attenuate postprandial glycaemia and expression of amino acid concentrations following food consumption. Plasma concentrations of GLP-1 are low when fasted (Holst, 2007). The appearance of GLP-1 occurs in the circulation following food consumption in proportion to energy content (Huda et al., 2006), and functions as a potent incretin potentiating the release of insulin, in a glucose dependent manner (Kreymann, Ghatei, Williams, & Bloom, 1987; P. E. MacDonald et al., 2002). Secretion of GLP-1 is mediated according to carbohydrate and fat consumption, although proteins and amino acids likewise exert an effect (Elliott et al., 1993; Herrmann et al., 1995). The secretion of GLP-1 elicits a biphasic response, with the first peak observed within the first 15-30 min of the postprandial period. The second peak happens some hours after. Recent evidence also suggests that levels rise in anticipation of a meal (Dailey, Stingl, & Moran, 2012). In the circulation, GLP-1 holds a plasma half-life of approximately 1-2 min (Baggio & Drucker, 2007). The ubiquitous plasma enzyme dipeptidyl peptidase-IV (DPP-IV), of which is expressed on the capillary walls, acts to rapidly degrade, inactivate and remove GLP-1 from hepatic circulation (Baggio et al., 2007). Of note, milk components (namely casein and whey protein hydrolysates) appear to display a natural DPP-IV inhibitory effect, exerting the potential to

increase the half-life of GLP-1 (Lacroix & Li-Chan, 2012; Nongonierma & FitzGerald, 2013; Tulipano, Sibilia, Caroli, & Cocchi, 2011). Several acute human intravenous infusion studies have regularly reported a role of GLP-1 in reducing food intake and appetite. In a meta-analysis (comprising 115 participants) concerning the effect of GLP-$1_{7\text{-}36}$ on subsequent *ad libitum* feeding behaviour in humans, the authors' (Verdich et al., 2001) provided evidence that GLP-1 produces a dose dependent reduction in energy intake in lean and overweight participants (Verdich et al., 2001). Circulating concentrations of total plasma GLP-1 were associated with, albeit rather weakly, subjective sensations of hunger (r = -0.26, p = 0.09), fullness (r = 0.38, $p < 0.05$), and prospective food consumption (r = -0.43, $p < 0.01$), which the authors' acknowledged may be attributed to a slower rate of gastric emptying (Verdich et al., 2001). Indeed, the slower rate of gastric emptying and intestinal motility may contribute to the ileal break mechanism, a gastrointestinal feedback mechanism that functions to optimise digestion and absorption (Cummings & Overduin, 2007). The ileal break can be defined as a distal to proximal feedback mechanism to control transit of a meal through the gastrointestinal tract in order to optimise nutrient digestion and absorption (Van Citters & Lin, 1999). At present, there is not a clear consensus on the physiological factors mediating the actions of the ileal break. In general, nutrients in the small intestine influence gut function as well as satiety and food intake via activation of neural afferents or the release of gut peptides (Read, French, & Cunningham, 1994). Of the gut peptides, proglucagon-derived peptides and neurotensin have initially been proposed as hormonal mediators of the ileal break (Larsen & Holst, 2005).

Glucagon, a 29 amino acid peptide, is secreted by the α-cells of the pancreatic islets (Woods et al., 2006) and was discovered in 1923 (Kimball & Murlin, 1923). The catabolic role of plasma glucagon in glucose homeostasis is well recognised, working as a counter-regulatory peptide opposing the actions of insulin (Aronoff, Berkowitz, Shreiner, & Want, 2004). Consequently, glucose is the greatest determinant of glucagon secretion, but its secretion is also influenced by amino acids present in meals (Marroquí et al., 2014). The consumption of a meal

causes an abrupt, yet short-lived release of glucagon (de Jong, Strubbe, & Steffens, 1977). When fasted or in circumstances of rapid glucose use, plasma glucagon acts to maintain euglycaemia and thus prevents hypoglycaemia (Woods et al., 2006). In this sense, the infusion or ingestion of glucose in humans and animal models elicits a suppression of glucagon release, while attenuated glucose is associated with higher concentrations of glucagon (Ohneda, Aguilar-Parada, Eisentraut, & Unger, 1969). In the circulation, plasma glucagon holds a half-life of approximately 5 min (Goodman, 2009). In humans, an increased concentration of glucagon has been shown to potently increase satiety and acutely reduce feeding behaviour (Flint et al., 2007; Parker et al., 2013; Penick, Hinkle & Paulsen, 1961; Woods et al., 2006). Furthermore, there is evidence suggesting that glucagon exerts properties that may stimulate energy expenditure and lipolysis in the adipose tissue (Heppner et al., 2010; Marroquí et al., 2014). The first indication that glucagon may elicit anorectic actions and influence feeding behaviour came from early human studies (Penick et al., 1961), which illustrated glucagon administration diminish subjective hunger and decrease energy intake. In the body, glucagon may be recognised by peripheral vagal nerves that relay information to satiety control regions of the hypothalamus (Marroquí et al., 2014). Taken together, GLP-1 and glucagon appear to influence physiological mechanisms controlling energy regulation, eliciting divergent actions on appetite, feeding behaviour and metabolism.

1.4.1.2 Tonic hormonal peptides

In contrast to the abovementioned episodic peptides, tonic peptides such as insulin and leptin indicate long-term energy balance (Duca & Covasa, 2012; Stephen C. Woods, 2005) and signal chronic nutritional state. Tonic peptides circulate at values proportionate to stored lipids in the fasted state (Huda et al., 2006) and help regulate body mass and energy homeostasis. Leptin, an adipocyte peptide comprising 167 amino acids, is synthesised in white adipose tissue and is the protein product of the obese gene (Park & Ahima, 2015). Leptin plays a central role in the

regulation of feeding behaviour, energy expenditure, metabolism, and body mass. In weight stable individuals, plasma concentrations of leptin circulate at values proportionate to indices of lipids in the fasted state (Considine et al., 1996; Huda et al., 2006) and help regulate body mass and energy homeostasis. In this sense 95% of circulating leptin originates from adipose tissue, but is also secreted by the stomach and pituitary gland. Acting as a governing signal, mirroring the stores of adipose tissue, leptin impedes NPY and stimulates POMC/CART neurons (Park et al., 2015). This occurs in the ARC nucleus of the hypothalamus and acts to reduce energy intake and increase energy expenditure (Park et al., 2015). Circulating levels of leptin are low during times of food restriction, and this is reversed following re-feeding (Flier, 2004). Leptin exerts a diurnal and pulsatile rhythm, with peak levels observed in the evening (Park et al., 2015). It is well accepted that leptin inhibits pancreatic β-cell insulin secretion (Marroquí et al., 2012). Central nervous system and peripheral administration of leptin to the rhesus monkey results in inhibition of food intake and decreased body mass (Tang-Christensen, Havel, Jacobs, Larsen, & Cameron, 1999). Leptin acts on neurons in the ARC nucleus to stimulate anorexigenic neurons and inhibit orexigenic neurons. Under conditions of deficiency (or resistance), leptin promotes the desire to consume energy and thus body mass gain (Knight, Hannan, Greenberg, & Friedman, 2010). The principle role of leptin is therefore to reduce occurrences of hyperphagia (excessive hunger or increased appetite) (Friedman, 2009). In the rodent model, administration of leptin induces a potent reduction in adipose mass, and is brought about through actions on feeding behaviour but also through actions on the adipose tissue. In this sense, leptin inhibits fat absorption and stimulates lipolysis.

The hormonal peptide insulin comprises 51 amino acids. In the circulation, the principle role of insulin is to act to lower concentrations of plasma glucose through increased glucose uptake (Woods & Porte, 1983), and this response is facilitated through a GLP-1 reduction in glucagon secretion (Lim et al., 2009). Food consumption rapidly gives rise to increased concentrations of insulin, a hormone synthesised in the β-cells of the pancreatic islets of Langerhans (Polonsky,

Given, & Van Cauter, 1988), and subsequently acts to limit energy intake. In particular, glucose is the main regulator of insulin (Thorens, 1995), however, amino acids also provide a stimulus for insulin secretion (van Loon, Saris, Verhagen, & Wagenmakers, 2000a). In a similar fashion to that of leptin, levels of insulin circulate at concentrations proportionate to indices of lipids in the fasted state (Baskin et al., 1999). Normal weight individuals therefore present lower levels of insulin compared with their overweight and/or obese counterparts. Weight loss prompts a reduced secretion of insulin, subsequently lowering the level of insulin that reaches receptors within the hypothalamic region of the brain. Insulin is cleared quickly from the circulation with a half-life of 4-6 min (Goodman, 2009). As insulin in the brain acts as an anorexigenic signal suppressing food intake, feeding behaviour is thus increased in an attempt to restore body mass (Baskin et al., 1999). Equally, weight gain causes insulin to rise and impacts on feeding behaviour until the weight is lost (Baskin et al., 1999). It is interesting to highlight that the consumption of milk-based dairy foods elicits an insulinotropic effect (Nilsson, Stenberg, Frid, Holst, & Björck, 2004; Östman, Liljeberg Elmståhl, & Björck, 2001), and this may be one avenue in which milk-based dairy food consumption effects subsequent appetite and feeding behaviour.

In humans, insulin also exhibits episodic characteristics and has subsequently been implicated in the modulation of short-term satiety and feeding behaviour (Hallschmid, Higgs, Thienel, Ott, & Lehnert, 2012). Circulating levels of plasma leptin also relay acute fluctuations in nutritional status. Within hours, reduced concentrations of insulin and leptin are present following fasting (Neary et al., 2004), weight loss or in a state of negative energy balance (Maffei et al., 1995) and promote energy conservation and the desire to find food (Flier, 2004). Together, episodic and tonic hormonal peptides work synergistically directly impacting on appetite and feeding behaviour, in an attempt to maintain energy homeostasis.

1.4.2 Non-homeostatic regulation of appetite and feeding behaviour

In addition to the homeostatic regulation of appetite and feeding behaviour, the non-homeostatic regulation of appetite and feeding behaviour may be influenced by the presence of external motivations existing in the physical environment (Berthoud, 2004, 2006). These external motivations exert powerful effects on appetite and feeding behaviour and include social circumstance, psychological, and environmental stimuli (De Castro, 1996). Additionally, appetite and feeding behaviour is also under hedonic control (Saper, Chou, & Elmquist, 2002), triggered by the sensory desire and perceived reward to consume palatable food (Lowe & Levine, 2005) which indeed may override the normal homeostatic control of appetite and feeding behaviour (Martins, Morgan, & Truby, 2008). A large proportion of human appetite and feeding behaviour may therefore be driven by the hedonic desire to consume palatable food, and not the physiological requirement for energy (Lutter & Nestler, 2009). This may, however, be unsurprising considering the obeseogenic environment in which we live and the readily accessible highly palatable food. In the context of palatability, participant perception of taste, smell, texture and reward contribute to sensory aspect of appetite and feeding behaviour (Chambers, McCrickerd, & Yeomans, 2015). These non-homeostatic features are formed primarily in cortico-limbic structures such as the prefrontal cortex, amygdala (forebrain) and ventral striatum (Berthoud, 2006). The awareness that appetite and feeding behaviour is regulated by more than simply the homeostatic factors arose over two decades ago (Blundell, Rogers & Hill, 1987). In this sense, the Satiety Cascade, which was gestated by Blundell and colleagues (1987) suggests that even in advance of food arriving in the gastrointestinal tract (cephalic phase), signals of cognitive and sensory origin caused by the appearance, smell and orosensory experience of food act synergistically to modulate feeding behaviour (Chambers et al., 2015). Homeostatic and non-homeostatic controls therefore work in a synergistic manner to regulate appetite and feeding behaviour.

1.5 Methodological considerations associated with appetite and feeding behaviour assessment

When working with child and adolescent populations there are various considerations that must be accounted for. The methodological approaches deemed most appropriate for the study of appetite and feeding behaviour assessment will differ according to the objective of the surveillance, the type of data required, available resources and the population of interest (Gibbons, Finlayson, Dalton, Caudwell, & Blundell, 2014). In children and adolescents, it is of great importance to adopt methodological approaches that are non-invasive and exert a low level of participant burden. It is therefore relevant to discuss appropriate techniques available to researchers to quantify appetite and feeding in child and adolescent populations.

1.5.1 Subjective appetite assessment in children and adolescents

Accurately quantifying human appetite and feeding behaviour is of great importance when conducting investigations exploring energy regulation. Hunger, satiation and satiety possess both subjective (learned) and objective (physiological) components. Consequently, there are a variety of methodological approaches that may be employed to quantify these constructs. Tracking changes in measures of subjective appetite provides important information in relation to the structure of the effects of feeding events, for instance the effect of diet composition on feeding behaviour or the effects of physiological variables on the appetite control system. Measures of subjective hunger, satiation and satiety are generally assessed following the implementation of psychometric scales (Stubbs et al., 2000). These participant centred scales provide a representation of the intensity of subjective sensation at a given time. In clinical and research settings, visual analogue scales [(VAS) typically taking the form of 100-150 mm horizontal lines] (Hill & Blundell, 1982) or likert scales can be implemented to give a quantitative measure of appetite in children and adolescents. For VAS and likert scales, at either end of the scale diametrically opposed feelings of extremity are labelled. Issues surrounding the use these scales in younger cohorts include extreme responses, whereby

children do not have the capacity to translate sensations of appetite through the use of visual analogue scales. Nonetheless, such issues can be abolished following thorough instruction and practical familiarisation (Hanet, Salah, & Lluch, 2010). Furthermore, for likert scales (an interval scale) it is assumed that, for example, a response of four represents a perceived feeling twice as strong as a response of two (Wewers & Lowe, 1990). Although this may be true, no scientific literature exists confirming this or the precision and accuracy of the use of likert scales for children and adolescents, consequently making it challenging to identify the role of likert scales in appetite-related research. Based on this, for VAS, one would assume that a response of 80 mm represents a perceived feeling twice as strong as a response of 40 mm (Wewers et al., 1990). Indeed, this seems plausible considering the perception of hunger is likely to vary both quantitatively and qualitatively amongst people. The level of hunger that prompts feeding will differ between persons in a given condition and will vary within an individual in different circumstances. This may therefore suggest that measures of subjective appetite in children and adolescents are best quantified in within-subject, repeated-measures designs utilising VAS.

Questions commonly asked through VAS address hunger [‘how hungry do you feel?’ anchored with ‘*not hungry at all*’ (0 mm) and ‘*very very hungry*’ (100 mm)], gut fullness [‘how full do you feel?’ anchored with ‘*not full at all*’ (0 mm) and ‘*very very full*’ (100 mm)], prospective food consumption [‘how much do you think you can eat?’ anchored with ‘*nothing at all*’ (0 mm) and ‘*a lot*’ (100 mm), and satisfaction [‘how satisfied do you feel?’ anchored with ‘*I am completely empty’* (0 mm) and *‘I cannot eat another bite’* (100 mm)] (Gibbons et al., 2014). Respondents are requested to place a vertical mark between the diametric phrases corresponding to the intensity of their feelings at the time of administration. Visual analogue scales represent a simplistic and convenient approach to administer, and have the advantage of being quick and easy to use, simple to interpret, presented in a standardised format that can be compared under a variety of different experimental manipulations (Wewers et al., 1990). The implementation of VAS to quantify

sensations of hunger, satiation and satiety have been employed in studies concerning older children and adolescents across a variety of experimental conditions (Bellissimo, Thomas, Goode, & Anderson, 2007; M. S. Moore, Dodd, Welsman, & Armstrong, 2004; Rumbold, St Clair Gibson, Allsop, et al., 2011; Rumbold et al., 2013; Thivel et al., 2012), and represent a valid and reproducible tool in relation to feeding behaviour (Flint, Raben, Blundell & Astrup, 2000; Parker et al., 2004).

1.5.2 Assessing energy intake and feeding behaviour in children and adolescents

It is relevant to highlight that appetite and feeding behaviour are generally assessed alongside one another, providing valuable insights concerning the mechanisms impacting on appetite and feeding behaviour (Stubbs, Ferres & Horgan, 2000). Accurately quantifying feeding behaviour in children and adolescents is of great importance when conducting investigations exploring energy regulation, especially in the free-living environment. At present, there is no agreement concerning the best approach of assessing energy intake and feeding behaviour in children and adolescents (Livingstone, Robson & Wallace, 2004; McPherson, Hoelscher, Alexander, Scanlon & Serdula, 2000). The most suitable approach to dietary surveillance will be influenced by the objective of the observation, information required, population of interest and the resources available to conduct dietary intake assessment (Black, 2001; Rockett & Colditz, 1997). There are a variety of methodological approaches that may be employed to quantify energy intake, each inherent with its own advantages and shortcomings. Some of the commonly utilised techniques to determine energy intake and feeding behaviour among children and adolescents in the free-living environment include food frequency questionnaires, self-reported food records (weighed or estimated), and 24 h dietary recall (Dodd, 2007).

1.5.2.1 Self-reported food records

Prospective approaches such as self-reported weighed food records are acknowledged as the gold-standard approach to assess energy intake and feeding behaviour in both adults and young people (Ashley & Bovee, 2003), and have the potential for providing quantitatively accurate information on food consumed during the recording period (Gibson, 2005). In this method, the participant is requested to give full comprehensive recordings of all food and beverage intake (in the form they are consumed along with any leftovers) at the time of consumption. In order to facilitate this, and subsequent dietary analysis, participants are required to confirm methods of preparation and cooking, names of branded products and condiment use. Self-reported weighed food diaries are often collected for 7 days, but this may often be less and indeed may be more (Livingstone & Robson, 2000). To ensure a high degree of accuracy it is necessary that participants are trained in the level of detail required (Coulston, Boushey & Ferruzzi, 2013; Livingstone et al., 2000). Self-reported weighed food diaries therefore elicit considerable subject burden. Attenuating the level of subject burden is therefore of crucial importance to prevent any lack motivation and issues concerning compliance (livingstone et al., 2000). In this sense, the duration of assessment should be tailored to the level of information required (Nelson, Black, Morris, & Cole, 1989). In an early investigation, Nelson and colleagues (1989) provided a comprehensive representation of the within- and between-subject variation in nutrient intake for British children (5-17 y), adults and the elderly. From this, the authors' were able to provide an estimate concerning the duration of assessment necessary to derive accurate estimates of energy, macro- and micro-nutrients with a given level of accuracy ($r \geq 0.9$). If the primary objective of the observation period is to elucidate intakes of particular nutrients, for example dietary calcium, the data emerging from this study suggest an observation period of 4 days is sufficient in children and adolescents (5-17 y) (Nelson et al., 1989). It has also been suggested that young peoples habitual feeding behaviour is largely determined on the environment and nature of foods available at both home and in the school environment (Taylor, Evers & McKenna, 2005). For this reason, it is probable that that habitual feeding behaviour will

differ between weekdays and weekend days considering they differ structurally (Rothausen et al., 2012), and this may ultimately influence nutrient intake. Indeed variability in feeding behaviour, and thus dietary quality, has been recorded in young people on weekdays compared with weekend days, whereby dietary quality was lower on weekend days compared to weekdays (Bjelland et al., 2011; Cullen & Lara, 2002). This finding was also replicated in a more recent study. Svensson and colleagues (Svensson et al., 2014) compared the consumption of total sugars, foods and beverages rich in added sugar and energy intake in young peoples diets on weekdays (Monday to Thursday), Fridays and weekend days in 9497 children (2-9 y). Results from this investigation highlighted that there were no differences in energy intake between weekdays and weekends, however, total sugars ($p < 0.001$) and of foods and beverages rich in added sugar ($p < 0.001$) were increased on weekend days compared with weekdays (Svensson et al., 2014). Concluding remarks from the cited authors' and literature concerning dietary assessment methodologies reference the importance of collecting dietary intake data over both weekdays and weekend days in an attempt to provide a more in-depth representation of habitual dietary behaviors (Svensson et al., 2014).

Since there is no objective measure of energy intake (Rutishauser & Black, 2002) the accuracy of energy intake assessment techniques are validated against an external criterion, known as a reference technique, such as doubly labelled water (DLW) (Bratteby, Sandhagen, Fan, Enghardt, & Samuelson, 1998). Comparisons of DLW derived energy expenditure can be compared to reported food intake to determine the accuracy of energy intake assessment techniques (Bandini, Schoeller, Cyr, & Dietz, 1990). With regards to the validity, precision and accuracy of self-reported food records compared with to DLW, few validation studies in children and adolescents have been conducted (Bratteby et al., 1998; Livingstone et al., 1992). Compared with DLW, all of these studies observed an increased level of underreporting with increasing age. Livingstone and colleagues (1992) were the first study to examine the validity of self-reported weighed food records for assessing energy intake and feeding behaviour in young people (7-18 y). Over seven

consecutive days, 29 non-obese participants self-reported energy intake using weighed food records. Energy expenditure, as quantified using DLW, was simultaneously assessed. For younger participants (7-9 y), parents were requested to record dietary behaviours. On a group level, compared with DLW, Livingstone and colleagues (1992) reported that self-reported weighed food records offer a valid approach to quantify free-living feeding behaviour. Agreement was greatest among younger participants, however, there was a tendency for underreporting of energy intake with increasing age, and this was particularly prevalent among adolescents (12-18 y). In this sense, the mean (±SD) energy intakes expressed as a percentage of energy expenditure (to provide an indication of reporting accuracy) yielded highly favourable results for the 7 and 9-year-old age group (91.7 ± 13.8 and 101.2 ± 18.8%, respectively), whereas for adolescents (12, 15 and 18 y) a trend for underreporting of energy intake was recorded with increasing age (84.7 ± 12.4, 68.0 ± 20.5 and 77.4 ± 20.2%, respectively).

Similar findings using an identical methodological approach were observed by Bratteby and colleagues (1998) in adolescents (15 y). In this study, the authors' reported that energy intake was underreported by approximately 20% in males and females when represented as a percentage of total energy expenditure (78.3 ± 16.4%). Taken together, the results of the aforementioned investigations suggest that adolescents underreport energy intake and feeding behaviour, and the likelihood of this occurring grows with increasing age. Based on these observations, if the objective of the observation is to determine total energy intake self-reported food records may not be appropriate. Indeed, the use of self-reported dietary assessment techniques has recently come under scrutiny (Dhurandhar et al., 2014), where alternative approaches to dietary assessment may prevail. Additionally, it is of great importance when collecting dietary intake data to consider observation periods that encompass both weekdays and weekend days in an attempt to provide a more in-depth representation of habitual dietary behaviours.

1.5.2.2 24 h dietary recalls

Retrospective approaches to assessing energy intake and feeding behaviour such as the 24 h dietary recall involve participants estimating all food and beverage intake for the previous 24 h (Ashley et al., 2003). Information concerning dietary intakes are collected by trained research staff during short consultations, and can be conducted wherever suited (Johnson, 2002). In general, the 24 h dietary recall can follow a two- (Ashley et al., 2003) or multiple-pass approach (Rutishauser & Black, 2002). Both approaches seek to establish feeding behaviours. In the former approach (two-pass), participants are requested to recollect all eating episodes of the preceding day, highlighting the main food and drink items consumed. Research staff subsequently encourage participants to further elaborate on information relating to brand names, condiment use, forgotten food or drink items, portion size and food handling. The latter approach (multiple-pass) follows an almost identical fashion, however, contains a third phase whereby completed dietary recalls are reviewed in an attempt to highlight any further missing items or eating occasions. In comparison to prospective assessments of energy intake and feeding behaviour, the 24 h dietary recall are simple to administer, elicit low participant burden and quick to carry out (Hill, Rogers & Blundell, 1995).

Greger and Etnyre (1978), assessed researcher observed energy intake and feeding behaviour compared with 24 h dietary recalls in adolescent females (12.5-14.5 y). Adolescent females were enrolled on a 30-day metabolic study and completed frequent 24 h dietary recalls throughout this period. The authors' observed no significant differences in estimated energy intake and feeding behaviour following 24 h dietary recalls when compared with observed energy intake, and subsequently concluded that this dietary assessment approach represented an accurate estimate of energy intake and feeding behaviour for use with adolescents (Greger et al., 1978). In addition, the author's noted that the accuracy of 24 h dietary recalls was improved when performed on multiple occassions (Greger et al., 1978). With regards to the validity of the 24 h dietary recall in children and adolescents, few studies have been conducted (Greger et al., 1978; Lindquist, Cummings, & Goran, 2000). Lindquist and colleagues (2000) compared energy intake as assessed

via 24 h dietary recall (over two weekdays and one weekend day) with total energy expenditure in children (6.5-12 y) as obtained by DLW. On a group level, the dietary recall approach was established as an effective estimate of energy intake and feeding behaviour, presenting only a minimal bias (0.04 MJ·d), however, validity was uncertain on an individual basis (Lindquist et al., 2000). Taken together, the results of the aforementioned studies suggest that the 24 h dietary approach to quantify energy intake and feeding behaviour is a valid approach for use with children and adolescents. Nonetheless, on an individual basis, the validity of this methodological approach is uncertain and warrants further investigation, particularly considering the available evidence was observed over a decade ago.

1.5.2.3 Combined use of energy intake and feeding behaviour assessment techniques

One way to enhance the accuracy or self-reported dietary intake may be to combine more than one measure of feeding behaviour (Livingstone et al., 2000). In this sense, studies that have combined energy intake measurement techniques have typically employed one retrospective method and one prospective method (Trabulsi & Schoeller, 2001). The potential use of self-reported weighed food records combined with 24 h recall interviews to assess energy intake has been demonstrated as a valid approach to quantify energy and feeding behaviour in adolescent (Rumbold, St Clair Gibson, Stevenson, & Dodd-Reynolds, 2011) and child studies (Lytle et al., 1993). The former rigorously controlled investigation by Rumbold and colleagues (2011) explored the accuracy of a combined weighed self-report food record and 24 h dietary recall approach to quantify energy intake and feeding behaviour in a group of thirteen adolescents (14-16 y) in a laboratory and free-living environment. During the study days, participants were allowed to consume provided food items *ad libitum*, but were required to weigh and record all food and beverage items consumed. All food and beverage items had previously been pre-measured by the investigators. Investigators subsequently recorded participant food and beverage consumption, in an attempt to quantify the agreement

between researcher observed and participant self-reported energy intake and feeding behaviour (Rumbold et al., 2011). Although there was a slight tendency to over report (4.2%) energy intake, the authors' concluded that the use of self-reported weighed food records combined with 24 h recall interviews is an effective methodological approach for use with adolescents when quantifying energy intake and feeding behaviour.

1.5.3 Quantifying appetite- and metabolism-related peptides

In clinical and research practice, quantitative measures of appetite-related peptides have commonly been assessed utilising venepuncture or antecubital-venous catheterisation. The methodological approach of venous blood sampling is characterised with a great degree of complexity, presenting increased ethical concern. Consequently, this renders blood collection difficult and to some extent prevents work in vulnerable populations (subject factors), certain exercise and field settings (situational factors), where alternative methodological approaches to blood sampling may prevail. Indeed, this may explain the dearth of literature concerning appetite-related peptide expression in investigations exploring energy regulation. Nonetheless, several approaches are available from which to measure appetite-related peptides. In this sense, methods including saliva or capillary samples may provide an alternative approach that may help overcome such issues associated with venous blood collection is that of fingertip capillary blood sampling.

The nature of saliva sampling offers a feasible alternative to quantify measures of appetite-related peptides, however, contamination from eating episodes is likely to impact on concentrations especially following nutritional interventions. In addition, there is no concrete scientific evidence to support the use of saliva samples to quantify appetite-related peptides. For this reason, it would be advantageous for researchers to explore different avenues concerning appetite-related peptide expression. In particular, the agreement between antecubital-venous and fingertip-capillary blood samples should be determined. An ability to collect appetite-related peptide data following

fingertip-capillary blood sampling would potentially offer an alternative method for quantifying appetite- and metabolism-related responses in children and adolescent populations. To date, only three studies have employed fingertip-capillary blood sampling for quantification of appetite- and metabolism-related peptides including leptin, insulin and GLP-$1_{7\text{-}36}$ (Balaguera-Cortes, Wallman, Fairchild, & Guelfi, 2011; Cani et al., 2009; Sim, Wallman, Fairchild, & Guelfi, 2014). Data from these studies demonstrated fingertip-capillary-derived measures were representative of values reported from previous research employing antecubital-venous sampling, and begin to provide affirmation for the use of this approach in energy-related research. Nonetheless, No evidence exists confirming fingertip-capillary-derived measures of appetite- and metabolism-related peptides are reproducible, and accurately reflect concentrations in comparison to their antecubital-venous equivalents. The capability to compare hormonal appetite data following fingertip-capillary and antecubital-venous blood sampling may encourage further research in the appetite domain. This would allow for more comprehensive measures to be obtained in populations where research is currently lacking (e.g. paediatrics, adolescents, and elderly).

1.6 Milk-based dairy consumption, appetite and feeding behaviour in children and adolescents

In light of the link between milk-based dairy food consumption and adiposity, one putative mechanism that may contribute to weight maintenance and/or weight loss may elicit through actions on appetite and energy intake regulation. In children and adolescents, the short-term effect of milk-based dairy consumption on measures appetite and feeding behaviour has been investigated by a limited number of studies. At present, there are only four studies exploring acute appetite and energy intake responses following milk-based dairy food consumption (Birch et al., 1993; Mehrabani et al., 2014; Vien et al., 2014; Zandstra et al., 2000). From the available scientific literature, all of these studies reported significant reductions in energy intake at *ad libitum*

assessments, suggesting that milk-based dairy consumption influences feeding behaviour in an acute setting.

Birch and colleagues (1993) fed 24 pre-school children (age: 33-47 months) one of three ice-cream preloads or a baseline control (Cheerios and apple juice, 334 kJ) as a mid-morning snack. Each child participated in all conditions. The three fixed weight (113 g) ice-cream preloads were matched for protein content (4 g), yet varied according to energy, fat and carbohydrate content. Accordingly, the three preloads were as follows: fat-free ice-cream (740 kJ, 0 g fat, 40 g carbohydrate); medium fat ice-cream (953 kJ, 12 g fat, 26 g carbohydrate); and high fat ice-cream (1150 kJ, 18 g fat, 24 g carbohydrate). The ice-cream preloads were offered at 09:30 am, and consumed within a 15 min period. Ninety min to 2 hours following, the children were presented with an *ad libitum* lunch. Compared with the energy intake during the baseline condition (1295 kJ), the authors identified that all preloads significant suppressed energy intake at the *ad libitum* lunch ($p < 0.01$). Following the three snack preloads the children consumed comparable amounts of food: 1040, 1077 and 1064 kJ following the fat-free, medium fat and high fat conditions, respectively (Birch et al., 1993). Considering the identical protein content and energy intake suppression of the ice-cream preloads, these findings may highlight the importance of protein in terms of satiating capacity. Indeed, protein has been touted as a more satiating macronutrient compared to fat (Astrup, 2005).

In a similar fashion, 30 children (age: 4-6 y) consumed one of four strawberry yogurt preloads or a completed a no-preload condition, during their regular classroom snack period (Zandstra et al., 2000). Each child participated in all conditions. Comparable with that of Birch et al. (1993) the composition of the preloads provided varied in energy and macronutrient content (low fat, low carbohydrate and low energy; high fat, medium energy; high carbohydrate, medium energy; high fat, high carbohydrate and high energy). The yogurt pre-loads were offered at 09:45 am on the school campus grounds followed 75 mins later with a self-selected *ad-libitum* lunch buffet. In

comparison with the no-preload condition, all children consumed less energy (p <0.05) at the self-selected *ad-libitum* lunch buffet meal after the high fat, high carbohydrate and high energy preload. The findings also indicated that the children consumed more (p <0.05) energy following the high carbohydrate, medium energy yogurt compared to the no-preload condition (Zandstra et al., 2000).

More recently, researchers in Iran have conducted an investigation to determine the effect of low-fat milk consumption on energy intake at an *ad libitum* lunch meal compared with isovolumetric servings of apple juice or water (Mehrabani et al., 2014). Recruited from an elementary school, 34 obese boys (age: 10-12 y) completed three preload conditions in a randomised three-way crossover design. The three preloads were matched for volume (240 mL), but not for energy or macronutrient content. Following an overnight fast, the boys were issued with a standardised fixed-energy breakfast along with one of the test preloads at 07:00 am. Further food and fluid (except water) consumption were prohibited until lunch, which was offered *ad libitum* 300 min later. Overall energy intake from breakfast until completion of the *ad libitum* lunch meal was significantly lower (6209 kJ, p < 0.05) when the low-fat milk preload was issued with breakfast, compared with apple juice (6456 kJ) and water (6719 kJ). It may be unsurprising that the consumption of low-fat milk led to a significant suppression in energy intake at the *ad libitum* lunch meal considering the differing protein content of the drinks (low-fat milk: 8.5g; apple juice: 0.34g; water 0g), and time-lag between breakfast and lunch (5 h). This research group also evaluated energy intake for 48 h following the intervention. Nonetheless, energy intake as recorded by weighed food records across this period did not differ significantly between trials, which may suggest that a dairy-induced suppression of appetite may be short-lived. It is interesting to note, however, that an earlier reduction in overall energy intake following low-fat milk did not provoke an overcompensation in feeding behaviour in the days following.

Taken together, the findings from the abovementioned studies appear to suggest that milk-based dairy consumption influences feeding behaviour in an acute setting, but may be a short-lived

phenomenon in children and adolescents. Whether the mechanism of this control is a result of the macronutrient composition (i.e. protein content) of the dairy products is unclear, but certainly warrants further investigation. It remains difficult to discuss the findings of the aforementioned studies, considering the preloads differed according to volume and energetic content. Furthermore, no quantitative measures of subjective appetite and/or appetite- and metabolism-related peptides were included which may have provided valuable insights concerning the mechanisms impacting on appetite and feeding behaviour. To date, only one investigation (comprising two experiments) has sought to establish the effect of dairy food consumption on appetite and feeding behaviour in children and adolescents (9-14 y), where subjective appetite and appetite-related peptides were measured (Vien et al., 2014). In both experiments, preloads (*experiment 1*: 1% fat chocolate milk, 2% fat milk, 1.5% fat yogurt drink, fruit punch or a water drink; *experiment 2*: 2% fat milk or a fruit punch) were provided 60 min preceding and during an *ad libitum* pizza meal. All preloads were matched for volume (250 mL) and energy content (130 kcal, 543.9 kJ). The first experiment comprised measures of subjective appetite, whereas the second experiment included measures subjective appetite together with appetite-related peptides (serum glucose, insulin and plasma GLP-1 and peptide YY). In the first experiment, Vein et al. (2014) illustrated reduced energy intake ($p < 0.01$) at a pizza meal offered 60 min following chocolate milk and yogurt consumption compared to a water drink. Consistent with a reduction in energy intake, subjective appetite (combined appetite score) was significantly lower following 2% fat milk consumption compared with the yogurt drink only ($p < 0.01$). No additional effects were observed concerning energy intake following the consumption of 2% fat milk and fruit punch or on subjective measures of appetite after 1% fat chocolate milk, 1.5% fat yogurt drink, fruit punch or water. In the second experiment, identical procedures were followed, however antecubital-venous concentrations of serum glucose and insulin and plasma GLP-1 and peptide YY were collected. Compared with the fruit punch preload, milk consumption resulted in a significantly greater GLP-1 area under the curve (AUC) ($p < 0.03$).

Nonetheless, *ad libitum* energy intake, insulin and glucose AUC were comparable between trials (Vien et al., 2014).

Considering the putative mechanisms of milk-based dairy consumption on appetite and feeding behaviour, no studies are available which have explored the impact of moderate-term dairy consumption on both appetite regulation and feeding behaviour in adolescents. Few studies have investigated the effect of longer-term milk consumption on energy intake in pre-school children (Hägg, Jacobson, Nordlund, & Rössner, 1998; Wilson, 1991, 1994, 1999) (**Table 1.2**), and those that have are methodologically flawed and failed to quantify measures of subjective appetite and appetite- and metabolism-related peptides expression. Consequently, this renders discussion of the mechanisms influence appetite and feeding behaviour challenging. In overweight and obese adults, during energy-restricted weight-loss interventions, a dietary pattern high in dairy and calcium for 3-months (Jones et al., 2013) or supplementation with milk for 6-months relative to an isoenergetic placebo (Gilbert et al., 2011) attenuated appetite regulation and feeding behaviour. Both interventions resulted in similar weight loss, however, milk-based dairy food consumption attenuated the orexigenic effect of weight loss, brought about by suppressed subjective sensations of appetite (for example hunger) and increases in plasma GLP-1 and PYY (Gilbert et al., 2011; Jones et al., 2013). Taken together, supplementation with milk appears to favourably impact on appetite, appetite- and metabolism-related and feeding behaviour. Nonetheless, it remains difficult to comment on the potential impact of milk supplementation for energy regulation in children and adolescents. In this context, understanding the relationship milk-based dairy consumption, feeding behaviour and appetite regulation is of great importance.

Table 1.2 Longer-term studies exploring the effects of milk-based dairy consumption on energy intake in children

Study	Participants	Milk-based dairy food	Study protocol	Energy intake	Subjective appetite, and appetite-and metabolism-related peptides
Wilson (1991)	Pre-school children 7 girls, 12 boys (1.6-3.3 y), 10 girls, 11 boys (3.3 y-4.6 y)	Milk	Pre-set lunch provided on Tuesdays and Thursdays (8 weeks). Half the meals were served with plain low fat milk (2%, 606 kJ/cup) and the other half with sucrose-sweetened chocolate milk (794 kJ/cup). No limit on amount of lunch or beverage consumed.	25% more energy consumed when chocolate milk served with a meal compared to plain milk. However, significantly more chocolate milk consumed in comparison to plain milk	Measures of subjective appetite or appetite- and metabolism-related peptides were not quantified
Wilson (1994)	Pre-school children (1.5-5.5 y) n = 24	Milk	Meals offered at home 2 days per week for 6 weeks. Four meals provided three times over the 6 weeks. Each meal provided once with each drink, *ad libitum* (8oz of plain milk – 606 kJ; sucrose-sweetened chocolate milk – 983 kJ kcal, aspartame-sweetened chocolate milk – 623 kJ)	Similar findings to Wilson (1991)	
Hägg (1998)	Pre-school children (4-6 y) n = 36	Milk	Water or milk preloads served alternately with three food dishes (12 weeks), following a standardised breakfast. Milk or water was provided *ad libitum.*	An additional 17% of energy was consumed when milk was provided with meals.	
Wilson (1999)	Pre-school children (1.5-5.5 y) n = 135	Milk	Four different lunches, provided six times over 12 weeks, served with three different beverages (plain milk, sucrose-sweetened chocolate milk, aspartame-sweetened chocolate milk). No limit on amount of beverage consumed. 3 hours following lunch, provided with mid-afternoon snack.	Similar findings to Wilson (1991; 1994) and Hägg et al. (1998), in addition the children who consumed more milk at lunch did not down regulate their energy intake at the mid-afternoon snack.	

1.7 Summary

Despite a growing body of scientific literature concerning the effect of milk-based dairy consumption on appetite and feeding behaviour in adults, there is a large scope for future research, specifically in child and adolescent populations. In children and adolescents, it appears that milk-based dairy food consumption influences feeding behaviour in an acute setting, however, it remains difficult to comprehensively discuss the physiological mechanisms impacting on appetite and feeding behaviour considering only one investigation has included subjective measures of appetite and appetite- and metabolism-related peptides in adolescents. Additionally, considering the putative mechanisms of milk consumption on appetite, feeding behaviour and metabolism, no studies are available which have explored the impact of moderate- to long-term milk-based dairy consumption on both appetite regulation and feeding behaviour in adolescents. In particular, researchers examining the influence of milk-based dairy food consumption on appetite and feeding behaviour in child and adolescent populations should seek to establish measures of subjective appetite and appetite- and metabolism-related peptide expression to provide further insight to the physiological mechanisms influencing appetite and feeding behaviour. Understanding the role of milk-based dairy food consumption on the regulation of appetite, feeding behaviour and metabolism among adolescents is necessary, especially concerning the link between milk-based dairy food consumption and adiposity. One concern is whether the methodological approach to measure appetite-related peptide expression represents an ethically sound technique for use within child and adolescent populations. Establishing an alternative approach when quantifying appetite-related peptide expression (maintaining the strengths of existing measures but address their limitations) may prove valuable and facilitate comparisons between studies of varying methods and populations, as this problem has not been assessed directly.

1.8 Thesis purpose and aims

Accordingly, the overall aim of this thesis was to establish the appetite and metabolic responses to acute and moderate-term dairy snack consumption in young people. To achieve this, the present thesis comprises four experimental chapters. The initial experimental chapter of this thesis (chapter two) set out to establish the potential of an alternative methodological approach to quantify appetite- and metabolism-related peptide expression for use with paediatric populations. For this, the agreement of GLP-$1_{7\text{-}36}$, glucagon, insulin and leptin between fingertip-capillary and antecubital-venous blood sampling was assessed. In addition, this chapter explored the between day test-retest reproducibility of the aforementioned peptides in a resting state utilising fingertip-capillary blood. The second experimental chapter (chapter three) compared patterns of dairy food consumption among a sample of children (9-11 y) and adolescents (15-18 y) from the North-East of England. In particular, this study was designed to establish dairy food popularity (types, frequencies and amounts) and identify potential populations (sex & age) to target within the intervention-based sections of this thesis. Establishing habitual dietary practices (in particular dairy food consumption) and alternative approaches to quantify appetite- and metabolism-related peptide expression would help facilitate the methodological design of intervention-based studies, ensuring the overreaching aim of this thesis was met, but also providing further insight to the physiological mechanisms influencing appetite and feeding behaviour. In this sense, the third (chapter four) and fourth (chapter five) experimental chapters of this thesis subsequently employed the findings of chapter two and three to determine the acute- and moderate-term influence of milk-based dairy consumption on subjective measures of appetite, appetite-related peptide expression, feeding behaviour and metabolism. Finally, the sixth chapter of this thesis will collate and discuss the findings of chapter's two to five. Throughout this chapter the implications and practical relevance of study findings will be discussed and potential future directions of research are proposed.

CHAPTER TWO

EVALUATION OF TWO BLOOD SAMPLING TECHNIQUES TO QUANTIFY APPETITE- AND METABOLISM-RELATED PEPTIDES: A METHOD-COMPARISON STUDY

2.0 Introduction

In clinical and research practice, quantitative measures of appetite- and metabolism-related peptides have commonly been assessed utilising venepuncture or antecubital-venous catheterisation, which represents an important limitation for vulnerable populations. The methodological approach of venepuncture or antecubital-venous catheterisation is characterised with a great degree of complexity, and is often problematic to implement in certain exercise and field settings. Furthermore, obtaining intravenous access renders blood collection difficult and to a certain extent inhibits work with vulnerable populations (for example children and adolescents) as a consequence of increased ethical concern, where alternative techniques to blood sampling may be more appropriate. Indeed, this may explain the severe dearth of scientific literature in children and adolescents concerning appetite- and metabolism-related peptide expression in energy regulation studies. For this reason, it seems prudent to explore the potential effectiveness of alternative sampling techniques where measurement of appetite- and metabolism-related peptides is feasible. In this sense, methods including saliva sampling or fingertip-capillary blood sampling may provide an alternative approach to help overcome such issues associated with venous blood collection. Nonetheless, due to the risk of contamination from eating episodes impacting on concentrations of appetite- and metabolism-related peptides within nutritional interventions prevent this technique from being utilised. Fingertip-capillary blood sampling offers an additional approach to venous sampling and may help overcome issues associated with venepuncture or antecubital-venous catheterisation. Furthermore, the nature of fingertip-capillary blood sampling poses numerous advantages including simplistic application, reduced ethical consideration and volume of blood required for analysis (Dayre McNally, Matheson, Sankaran, & Rosenberg, 2008).

Multiple hormonal peptides pertinent to the regulation of energy homeostasis are produced and secreted into the circulation in response to the ingestion of a meal. Emanating from the periphery of the gastrointestinal tract, pancreas and adipose tissue these peptides act to influence the physiological mechanisms controlling energy regulation, eliciting divergent actions on feeding

behaviour and metabolism (Lancha et al., 2012). In humans, hormonal peptides such as glucagon-like peptide-1 (GLP-1) (Verdich et al., 2001), glucagon (Chan et al., 1984), insulin (Air et al., 2002) and leptin (Schwartz et al., 2000), among others, represent several commonly measured metabolic variables documented as key effectors targeting energy intake and expenditure. Accurate quantification of these peptides is therefore essential when exploring hormonal responses in studies concerning appetite, feeding behaviour and metabolism. To date, only three studies have employed fingertip-capillary blood sampling for quantification of appetite- and metabolism-related peptides including leptin, insulin and GLP-$1_{7\text{-}36}$ (Balaguera-Cortes et al., 2011; Cani et al., 2009; Sim et al., 2014). Data from these studies demonstrated fingertip-capillary-derived measures were representative of values reported from research employing antecubital-venous sampling, and begin to provide affirmation for the use of this approach in energy-related research. No evidence exists, however, confirming fingertip-capillary-derived measures of appetite- and metabolism-related peptides are reproducible, and accurately reflect concentrations in comparison to their antecubital-venous equivalents, a limitation which was acknowledged in the conclusions of the above cited articles. Indeed, we have echoed the necessity for validation of appetite- and metabolism-related peptides between antecubital-venous and fingertip-capillary blood sampling to facilitate future paediatric energy regulation work (Rumbold et al., 2013).

The test-retest reproducibility of these peptides is therefore essential, making certain that an intervention(s) or variable(s) is responsible for any observed differences and are not brought about by random variability or measurement error (Atkinson & Nevill, 1998; Hopkins, 2000). Incidences of random variability or measurement error are often difficult to explain but could arise due to biological or technical error. In this sense, technical error may directly be attributable to methodological shortcomings including blood collection techniques and sample handling, whereas biological error may reflect situational and subject specific factors such as specimen collection and discrepancies in the measurement protocol. Quantifying agreement and reproducibility between

methods would facilitate appropriate comparisons between studies using venous and capillary blood sampling. This is particularly important given that blood obtained from different sampling locations (e.g. antecubital-venous and fingertip-capillary blood) is characteristically dissimilar. Capillary blood, for example, encompasses an assortment of blood from venules, arterioles, intracellular and interstitial fluid, and of course the capillaries. The quantity of arterialised blood is more pronounced in capillary blood than that of antecubital-venous blood, and is therefore thought to be more reflective of arterial blood (Merton, Jones, Lee, Johnston, & Holt, 2000).

Accordingly, the aims of the present study were twofold. Firstly, the study was designed to examine the agreement of GLP-$1_{7\text{-}36}$, glucagon, insulin and leptin between fingertip-capillary and antecubital-venous blood sampling in a resting state (part 1). Secondly, the present study assessed the between day test-retest reproducibility (part 2) of the aforementioned peptides in a resting state utilising fingertip-capillary blood. As alluded to previously and throughout the literature review, the nature of antecubital-venous blood sampling presents increased ethical concern for use in children and adolescents. Given there should not be any underpinning physiological difference influencing study results between population groups, the results of this study are presented for healthy adults.

2.1 Materials and methods

2.1.1 Participants

In total, nineteen healthy adult participants (15 males and 4 females) from the staff and student population of Northumbria University at Newcastle-upon-Tyne participated in part 1. Eighteen healthy adult participants (9 males and 9 females) participated in part 2 of this study. A total of six participants participated in both part 1 and 2. Participant details are provided in **Table 2.0**. Participants were informed of the purpose, procedures and potential risks of the study and written informed consent was obtained from all volunteers prior to data collection. The study was conducted according to the guidelines laid down in the 2013 Declaration of Helsinki (WMA, 2013),

and all procedures involving human participants were approved by the Faculty of Health and Life Sciences Ethics Committee of the University of Northumbria.

Table 2.0. Participant characteristics according to study participation

	Part 1 (n = 19)		**Part 2 (n = 18)**	
	Mean	**(SD)**	**Mean**	**(SD)**
Age (y)	24.1	(5.7)	23.1	(3.5)
Mass (kg)	73.7	(10.9)	69.3	(12.7)
Stature (m)	1.8	(0.1)	1.7	(0.1)
BMI (kg/m^2)	23.6	(2.1)	23.1	(2.7)

Abbreviations: BMI, body mass index

2.1.2 Study design

In part 1, participants attended the laboratory on a single occasion where simultaneous samples of fingertip-capillary and antecubital-venous blood were collected to assess the agreement between measures of appetite- and metabolism-related peptides. Samples were obtained in the fasting and postprandial state for the determination of plasma GLP-1_{7-36}, glucagon, insulin and leptin. For part 2, participants attended the laboratory on two separate occasions, separated by 7 days, where fingertip-capillary blood was sampled to determine the reproducibility of the aforementioned peptides. Participants reported to the clinical testing laboratory at designated times between 0700

and 0900 h, following a 12 h overnight fast. The time participants attended the laboratory was documented and kept consistent between successive trials (part 2 only). Participants were instructed to refrain from the consumption of caffeine and alcohol (≥ 12 h) and strenuous physical activity (≥ 24 h) preceding data collection. Upon waking and until arrival at the clinical testing laboratory consumption of water was only permitted. Participants were requested to record, document and replicate morning water consumption for subsequent trials. Following baseline (t = 0) blood samples, participants were issued with a standardised cereal and milk breakfast. Further samples of antecubital-venous (part 1 only) and fingertip-capillary blood were collected at 30, 60, 90 and 120 min during the postprandial period (**Figure 2.0**). Additional food and beverage consumption was prohibited until test termination, apart from water that was offered *ad libitum*. In-trial *ad libitum* fluid consumption (if any) was documented and matched for subsequent trials. Throughout test periods, participants remained sedentary in an environment free from food cues.

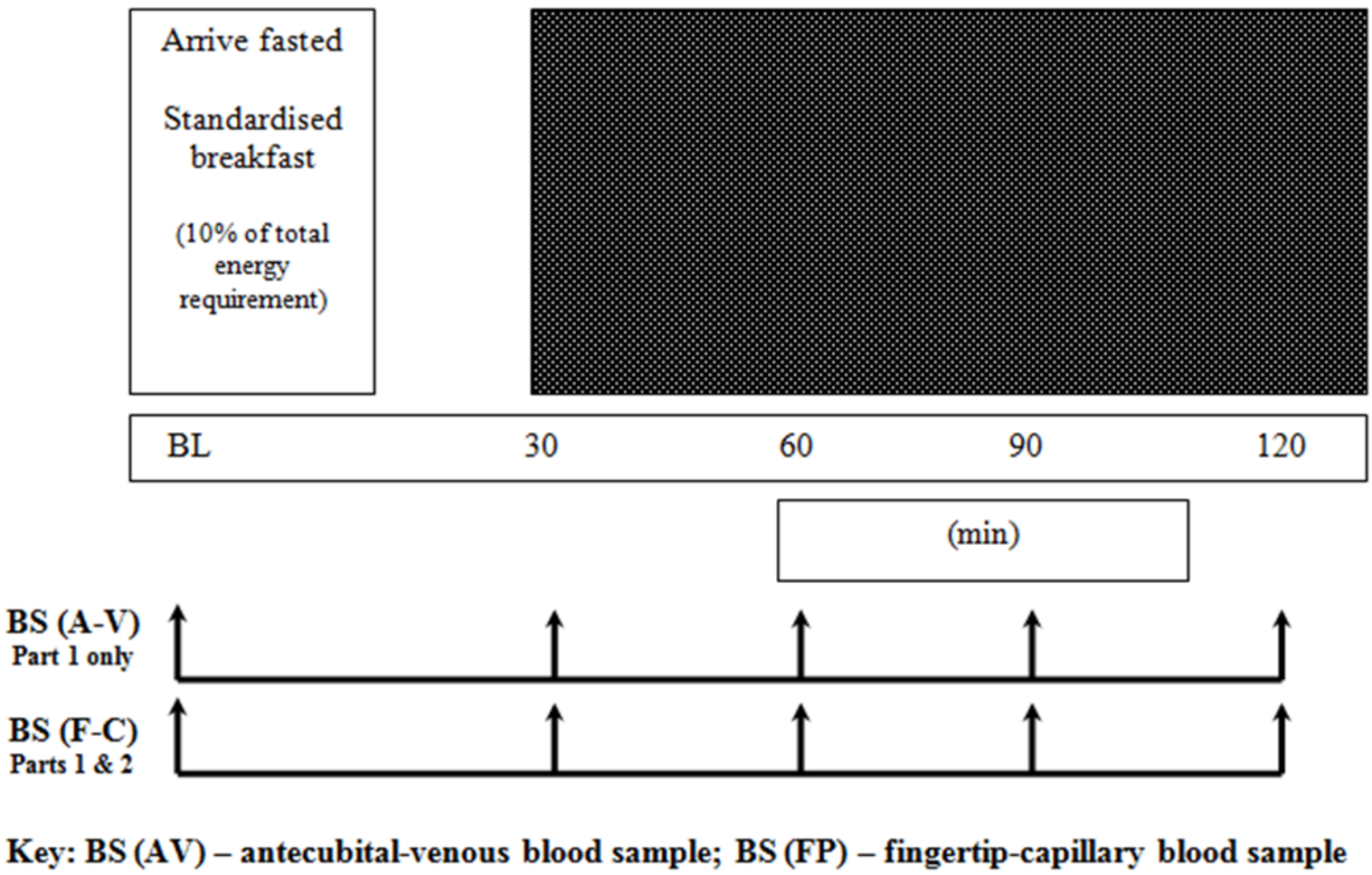

Figure 2.0 Schematic representation of the experimental study design. Note: Antecubital-venous blood samples were collected one only in part 1.

2.1.3 Blood sampling

At five separate intervals, simultaneous fingertip-capillary (0.3 mL) and antecubital-venous blood samples (8.0 mL) were drawn into pre-cooled EDTA-treated microvettes and monovettes, respectively. Samples were collected at baseline (t = 0 min) and at 30, 60, 90 and 120 min following breakfast consumption for the determination of plasma GLP-$1_{7\text{-}36}$, glucagon, insulin and leptin. Venous blood was drawn from an indwelling cannula (Venflon 20G, Becton Dickinson & Company), inserted into an antecubital forearm vein. Patency of the cannula was preserved by flushing a small volume of nonheparinised saline (0.9% NaCl; Becton, Dickinson and Company, USA) through the connector tube on completion of each antecubital-venous sample. Residual saline waste was discarded immediately prior to succeeding sample points, avoiding contamination and dilution of antecubital-venous blood. Fingertip-capillary blood was simultaneously obtained from a pre-warmed fingertip pierced with a sterile automated lancet (Accu-Check, Mannhein, Germany). Fingertip-capillary blood samples were also collected for the assessment of reproducibility for part 2 of the study. Approximately 3-5 min preceding each fingertip-capillary sample, the entire sample-hand was pre-warmed in warm-water, to promote an adequate flow of fingertip-capillary blood. On removal, the hand was dried thoroughly and the identified site for puncture was further cleansed with an aseptic alcohol wipe. On puncturing the fingertip, the first drop of blood was removed before subsequent collection, where care was taken not to apply excessive pressure. All blood samples were obtained while participants lay in a semi-supine position.

Blood collection tubes contained aprotinin (33 μL·mL blood) and a DPP-IV inhibitor (30 μL·mL blood) for the preservation of GLP-$1_{7\text{-}36}$ and glucagon by proteases. Sample pre-treatment and the addition of protease inhibitors were applied to both microvettes and monovettes and performed preceding sample collection. Of note, the addition of protease inhibitors to plasma samples for the preservation GLP-$1_{7\text{-}36}$ and glucagon does not influence measured concentrations of plasma leptin and insulin (Bielohuby, Popp, & Bidlingmaier, 2012). Following collection, samples

were placed on ice and immediately centrifuged. Monovettes were spun at 3000 rpm for 10 min at 4°C in a refrigerated multispeed centrifuge and microvettes spun at 3000 rpm for 10 min in a multispeed micro-centrifuge. Aliquots of plasma supernatant were housed in appropriately labelled eppendorfs and stored at −80ºC for the determination of plasma GLP-$1_{7\text{-}36}$, glucagon, insulin and leptin concentrations.

2.1.4 Breakfast meal

Following baseline measures, participants consumed a standardised breakfast meal consisting of semi-skimmed milk (Sainsbury, UK) and Kellogg's Rice Krispies (Kelloggs, Manchester, UK), distributed at a cereal to milk ratio of 30 g: 125 mL. The quantity issued was designed to provide 10% of the participants estimated daily energy requirement for protein, fat and carbohydrate (14%, 14% and 72%, respectively) as used previously (Astbury, Stevenson, Morris, Taylor, & Macdonald, 2010). Individual daily energy requirements were computed according to age and sex specific calculations (Schofield, 1985), providing an estimate of basal metabolic rate. Estimated values of basal metabolic rate were further multiplied against a self-perceived physical activity factor. Participants were given 15 min to consume the entire contents of the breakfast meal.

2.1.5 Electrochemiluminescence

Quantitative assessments of GLP-$1_{7\text{-}36}$ (pg·mL), glucagon (pg·mL), leptin (pg·mL) and insulin (pmol·L) were simultaneously determined in duplicate in 40 μL of plasma by electrochemiluminescence using a human hormone multiplex assay kit (Sector Imager 2400, MesoScale Discovery, Maryland, USA). To facilitate quantification, a standard curve was produced from a stock calibrator of known hormone concentration. The stock calibrator was provided by the manufacturer and diluted (4-fold serial dilutions) accordingly to generate an 8-point standard curve

with a supplied 'Metabolic Assay Working Solution'. As per manufacturer's instructions, calibrators were analysed in duplicate and included for each set of unknown samples and on each assay plate. Using linear regression analysis, linearity and the quality of the curve-fit corresponded to $r^2 \geq 0.95$ for all peptides and across all assays. The lower limit of detection (sensitivity) for GLP-$1_{7\text{-}36}$, glucagon, leptin and insulin was 0.7 ± 0.2 pg·mL, 58.4 ± 9.2 pg·mL, 101.6 ± 6.8 pg·mL and 2.0 ± 1.2 pmol·L, respectively, as determined from in house analysis. Concentrations below the detection limit were left blank, yet accounted for within the time-averaged AUC calculation. To eliminate inter-assay variation, samples from each participant were analysed within the same run. In part 1, average intra-assay coefficients of variation were 12%, 7%, 15% and 12% for GLP-$1_{7\text{-}36}$, glucagon, leptin and insulin, respectively. For part 2, average intra-assay coefficients of variation were 10%, 8%, 6% and 12% for GLP-$1_{7\text{-}36}$, glucagon, leptin and insulin, respectively. Intra-assay coefficients of variation were determined for part 1 by the repeated measurement of a single baseline antecubital-venous plasma sample five times. For part 2, intra-assay coefficients of variation were determined by the repeated measurement of a single baseline fingertip-capillary plasma sample three times (primarily due to the reduced volume of plasma available for analysis). Please refer to Appendix A for a detailed description of the electrochemiluminescence procedure (**see page 223**).

2.1.6 Statistical analysis

Descriptive data are presented as means ± standard deviation ($_{SD}$), whereas graphical depictions are presented as means ± $_{SEM}$. The time-averaged AUC score was computed for each peptide, in antecubital-venous and fingertip-capillary blood, using the trapezoidal rule. Agreement between fingertip-capillary and antecubital-venous-derived measurements were subsequently assessed using Deming regression (to test for systematic and proportional bias) (Deming, 1943), Bland-Altman (Bland & Altman, 1986) limits of agreement (LOA; to quantify random error) and typical error of

the estimate as a coefficient of variation (CV_a, %) to compare random error between measures (WG. Hopkins, 2009). Assumptions of normal distribution and non-dependence of measurements were assessed using boxplots and scatterplots, respectively (Newell, Aitchison, & Grant, 2010). For Deming regression, systematic and proportional bias was evaluated by means of the intercept and slope, respectively (Martin, 2000). For LOA, heteroscedasticity was assessed by inspecting scatterplots and associated Pearson's correlation coefficients of the absolute differences (errors) and measurement means (Bland et al., 1986). Where significant heteroscedasticity existed (defined as an r value > 0.4), data were log transformed (natural) and reported as a geometric mean and ratio (×/÷) LOA. Statistical significance was accepted at $p < 0.05$ for all analyses. Between-day test-retest reproducibility of fingertip-capillary measures was assessed using paired-samples t-tests and typical error as a coefficient of variation (CV_r, %).

For the purpose of this study, pre-determined clinically significant differences were computed for each peptide prior to data collection. Differences employed were facilitated through literature informed choices and their associated effects on subjective appetite, feeding behaviour, and within-subject day-to-day biological variation. For plasma concentrations of GLP-$1_{7\text{-}36}$ and glucagon, pre-determined time-averaged AUC differences of 1.4 pg·mL (Verdich et al., 2001) and 4.9 pg·mL (Celga et al., 2013) respectively, between methodological approaches and visits were deemed clinically important discrepancies. Further, differences of 62.0 pmol·L (Nair et al., 2009) and 148.0 pg·mL (Liu, Askari, & Dagogo-jack, 1999) were deemed clinically important for plasma concentrations of insulin and leptin, respectively, between methodological approaches and visits. Furthermore, based on the within-subject variation of antecubital-venous plasma glucagon (19% (Widjaja et al., 1999)), leptin (20% (Liu et al., 1999)) and insulin (26% (Widjaja et al., 1999)), typical error of the estimate when reported as a CV% were deemed strong when below 10%, modest when equal or similar to previously reported variation, and poor when substantially greater than this.

2.2 Results

In total, 19 participants completed the agreement study (part 1). However, due to difficulties associated with cannulation and consequently blood collection, results for GLP-$1_{7\text{-}36}$, glucagon, and insulin are presented for 17 participants, and 16 participants for leptin. All 18 participants successfully completed the between-day reproducibility study (part 2) and results for GLP-$1_{7\text{-}36}$, glucagon, and insulin are provided. For leptin, however, results are available from 17 participants. One participant's data was excluded on the bases of yielding a time-averaged AUC value 3_{SDs} above the group mean.

2.2.1 Agreement between antecubital-venous & fingertip-capillary-derived peptides

Deming regression analysis revealed no evidence of systematic [intercept (95% CI) = -5.8 (-31.0 to 19.5)] or proportional bias [slope (95% CI) = 1.1 (0.8 to 1.3)] between antecubital-venous (mean ± $_{SD}$, 90.1 ± 23.8 pg·mL) and fingertip-capillary (90.0 ± 37.2 pg·mL) time-averaged AUC estimates of plasma glucagon (**Figure 2.1, panel A**). Similarly, Deming regression analysis of fingertip-capillary and antecubital-venous measures of plasma GLP-$1_{7\text{-}36}$ (mean ± $_{SD}$, 8.6 ± 3.4 pg·mL vs. 9.1 ± 3.0 pg·mL, respectively, **Figure 2.1, panel B**) and leptin (mean ± $_{SD}$, 664.5 ± 350.3 pg·mL vs. 741.0 ± 375.2 pg·mL, respectively, **Figure 2.2, panel A**) demonstrated no evidence of systematic or proportional bias between methodological approaches. For insulin, Deming regression revealed no evidence of systematic bias [intercept (95% CI) = -28.4 (-106.8 to 50.1)] between antecubital-venous (mean ± $_{SD}$, 302.4 ± 154.7 pmol·L) and fingertip-capillary (mean ± $_{SD}$, 236.2 ± 113.0 pmol·L) blood sampling, however, there was a proportional difference between measurements at higher concentrations [slope (95% CI) = 1.4 (1.1 to 1.7, intercept (95% CI) = -28.4 (-106.8 to 50.1), **Figure 2.2, panel B**].

Agreement between fingertip-capillary and antecubital-venous-derived measures of glucagon (CV_a = 21.0%, LOA ± 31.5 pg·mL, **Figure 2.1, panel B**) was good and modest for GLP-

$1_{7\text{-}36}$ (CV_a = 24.0%, LOA ± 2.5 pg·mL, **Figure 2.1, panel A**) and leptin (CV_a = 9.0%, LOA ×/÷ 1.19, **Figure 2.2, panel A**). For insulin, although the pattern of response to the standardised meal was similar, agreement between fingertip-capillary and antecubital-venous estimates was poor (CV_a = 36.0%, LOA ×/÷ 1.79, **Figure 2.2, panel B**).

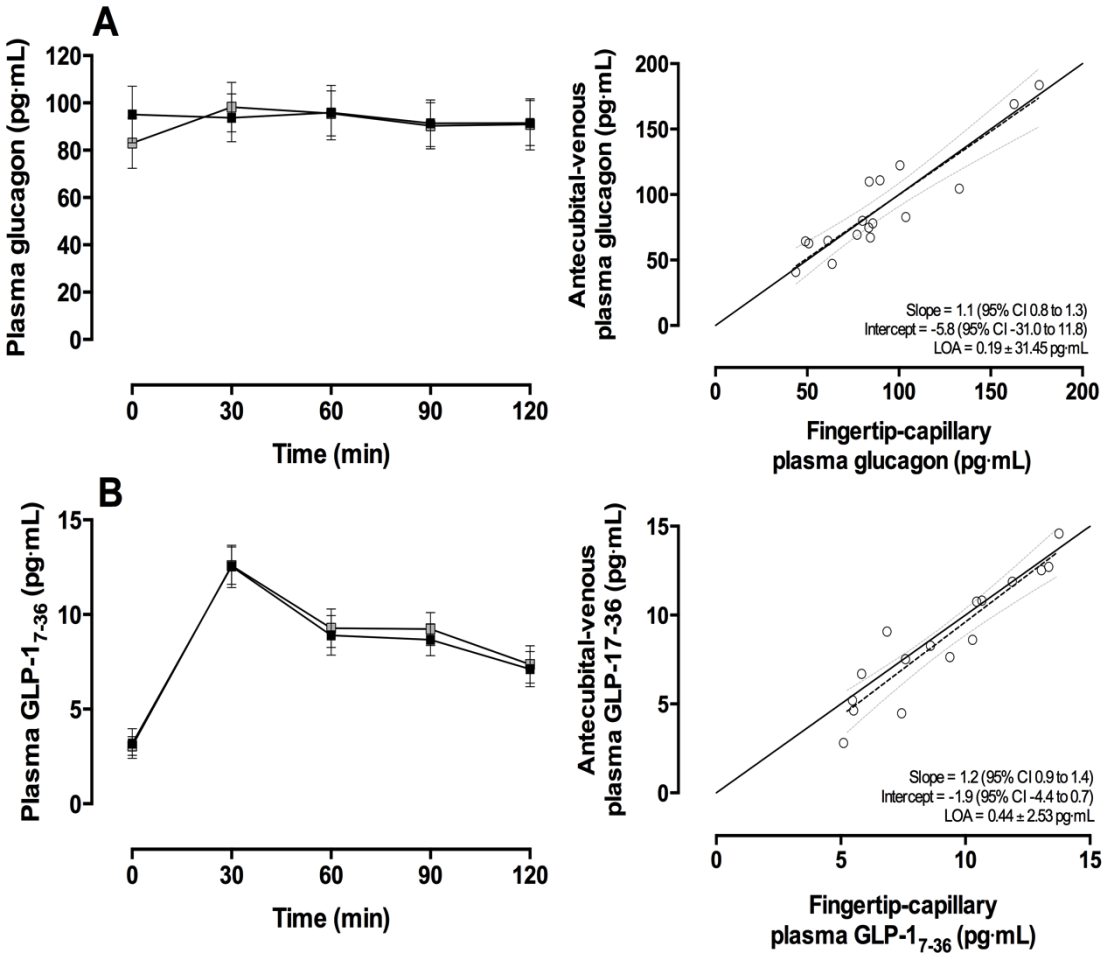

Figure 2.1 Mean ± $_{SEM}$ comparison of plasma glucagon (pg·mL; Panel A, n = 17) concentrations obtained simultaneously from fingertip-capillary and antecubital-venous blood samples (left). Deming regression scatterplot (right). The solid line represents the line of equality. The dashed line denotes the regression line with corresponding 95% confidence intervals represented in the grey hashed area. Mean ± $_{SEM}$ comparison of plasma GLP-$1_{7\text{-}36}$ (pg·mL; Panel B, n - 17) obtained from simultaneous fingertip-capillary and antecubital-venous blood samples (left). Deming regression scatterplot (right). The solid line represents the line of equality. The dashed line denotes the regression line with corresponding 95% confidence intervals represented in the grey hashed area. All values are expressed as mean ± $_{SEM}$. Grey shaded boxes (-□-) represent values obtained from fingertip-capillary blood sampling, whereas black shaded boxes (-■-) represent concentrations in antecubital-venous blood. To convert GLP-$1_{7\text{-}36}$ (pg·mL) and plasma glucagon (pg·mL) to their corresponding SI units multiply values by 0.298 and 0.287, respectively.

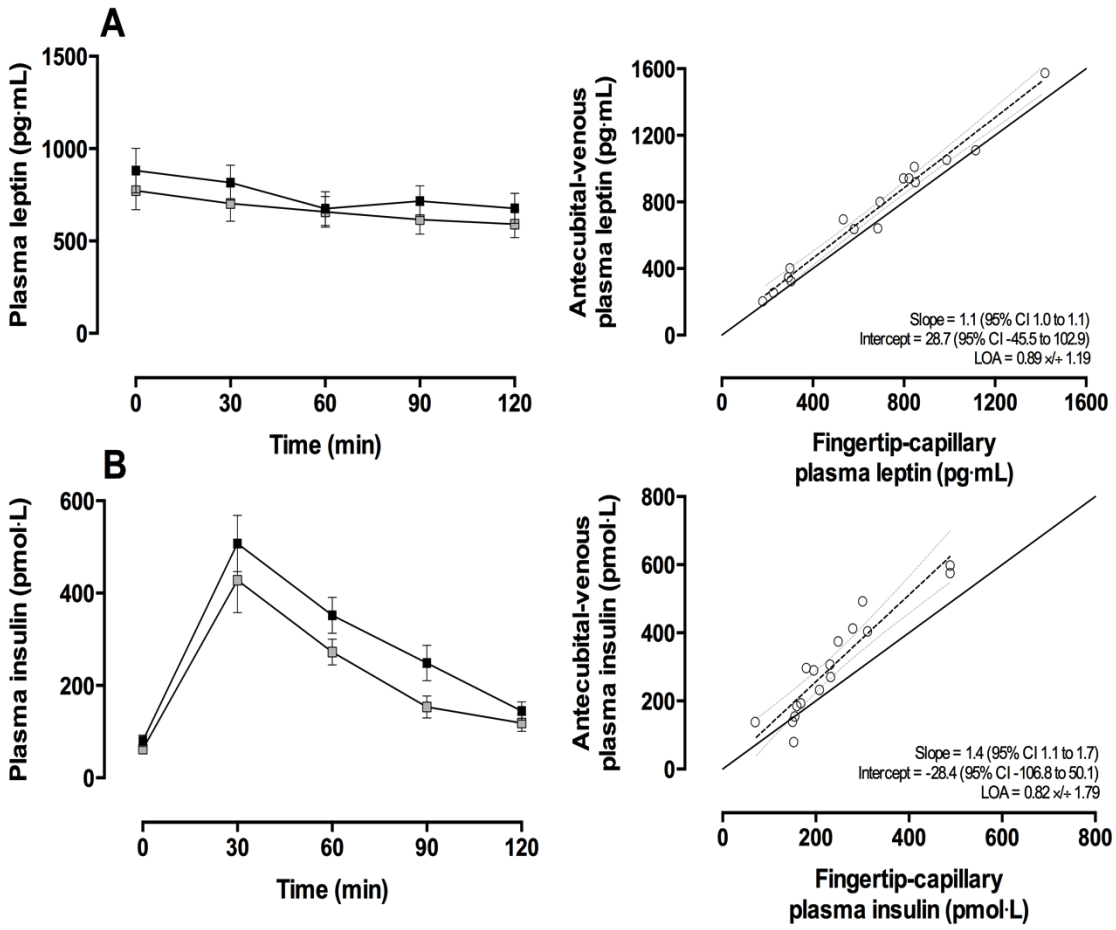

Figure 2.2 Mean ± $_{SEM}$ comparison of plasma leptin (pg·mL; Panel A, n = 16) concentrations obtained simultaneously from fingertip-capillary and antecubital-venous blood samples (left). Deming regression scatterplot (right). The solid line represents the line of equality. The dashed line denotes the regression line with corresponding 95% confidence intervals represented in the grey hashed area. Mean ± $_{SEM}$ comparison of plasma insulin (pmol·L; Panel B, n = 17) obtained from simultaneous fingertip-capillary and antecubital-venous blood samples (left). Deming regression scatterplot (right). The solid line represents the line of equality. The dashed line denotes the regression line with corresponding 95% confidence intervals represented in the grey hashed area. All values are expressed as mean ± $_{SEM}$. Grey shaded boxes (-□-) represent values obtained from fingertip-capillary blood sampling, whereas shaded boxes (-■-) represent concentrations in antecubital-venous blood.

2.2.2 Between-day reproducibility of fingertip-capillary-derived peptides

No systematic bias existed for time-averaged AUC measurement of any fingertip-capillary-derived peptides between visits ($p \geq 0.05$ for all). Reproducibility of plasma glucagon was strong between visits (mean ± $_{SD}$, 103.3 ± 36.9 pg·mL vs. 103.6 ± 32.9 pg·mL, CV_r = 8.2%, **Figure 2.3, panel B**). Plasma GLP-$1_{7\text{-}36}$ (8.6 ± 3.2 pg·mL vs. 8.8 ± 3.0 pg·mL, **Figure 2.3, panel A**) and leptin (3870.8 ± 3482.7 pg·mL vs. 3774.9 ± 3813.3 pg·mL, **Figure 2.3, panel C**) demonstrated modest reproducibility (CV_r = 22.7 and 25.0%, respectively). Plasma insulin exhibited the greatest variability (291.7 ± 142.1 pmol·L vs. 253.9 ± 94.7 pmol·L, CV_r = 36.0%, **Figure 2.3, panel D**), indicating a large degree of random error between visits.

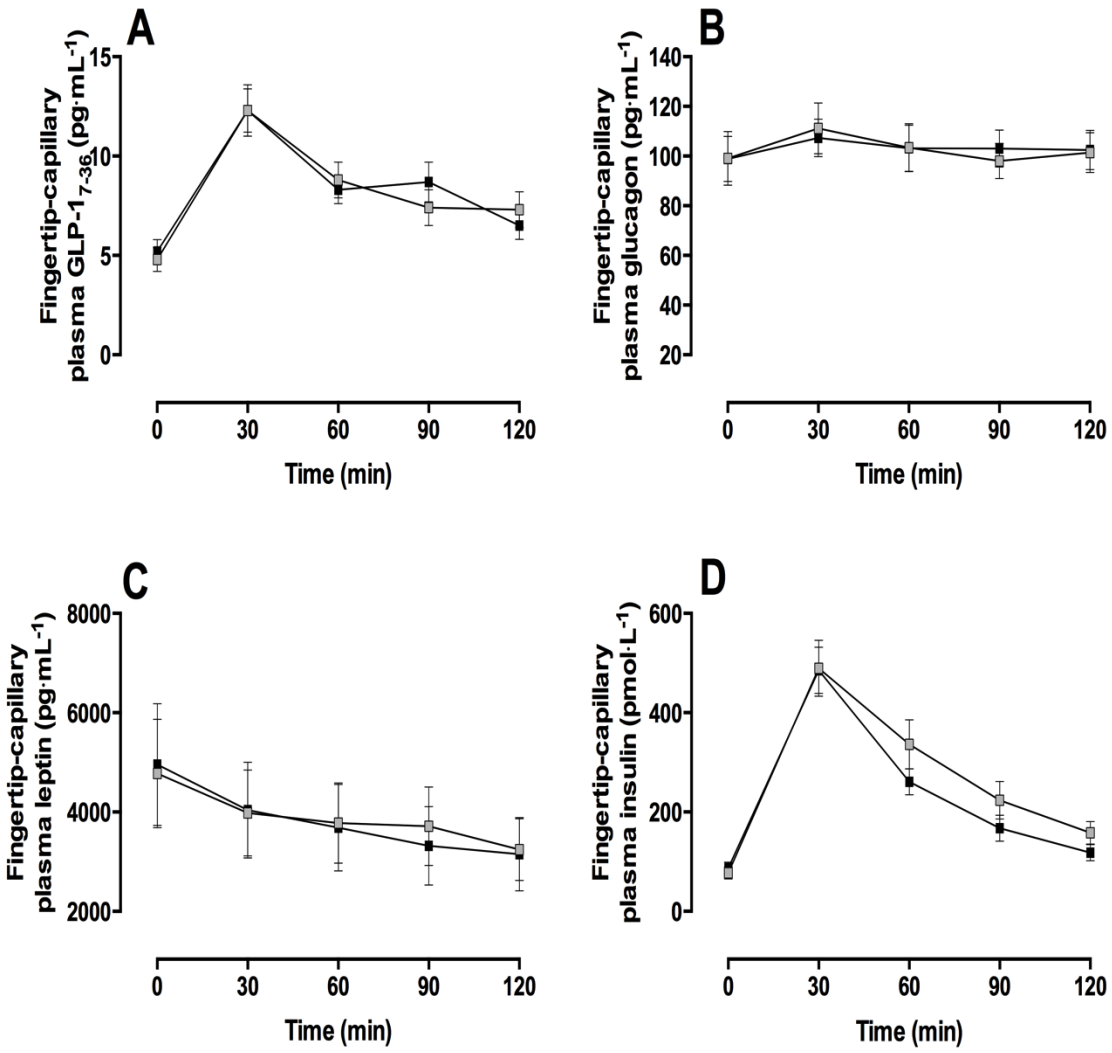

Figure 2.3 Between day comparison of fingertip-capillary-derived measures of plasma GLP-$1_{7\text{-}36}$ (pg·mL; Panel A, n = 18), plasma glucagon (pg·mL; Panel B, n = 18), plasma leptin (pg·mL; Panel C, n = 17) and plasma insulin (pmol·L; Panel D, n =18). All values are expressed as mean ± $_{SEM}$. Grey shaded boxes (-□-) represent values obtained from fingertip-capillary blood during visit 1, whereas shaded boxes (-■-) represent concentrations in fingertip-capillary blood during visit 2. Laboratory visits were separated by 7 days and were conducted at consistent times between successive trials. To convert GLP-$1_{7\text{-}36}$ (pg·mL), plasma glucagon (pg·mL) to their corresponding SI units multiply values by 0.298 and 0.287, respectively.

2.2 Discussion

This study is the first to examine the agreement between fingertip-capillary and antecubital-venous blood sampling for the determination of plasma GLP-1_{7-36}, glucagon, insulin and leptin, and the between-day reproducibility of these hormonal peptides measured utilising fingertip-capillary blood. The results presented here support the use of fingertip-capillary-derived estimates of glucagon, and to a lesser extent for GLP-1_{7-36} and leptin. Caution should be exercised when utilising fingertip-capillary blood sampling for insulin quantification. The presence of proportional bias between fingertip-capillary and antecubital-venous estimates of insulin suggests these methods should not be employed interchangeably. The results of this chapter hold important implications for researchers and practitioners who wish to quantify and compare the expression of these appetite- and metabolism-related peptides in various populations or field-based scenarios where antecubital-venous blood sampling might be contraindicated. This is especially important with regards to children and adolescents, and also when exploring appetite- and metabolism-related peptide expression following milk-based dairy food consumption.

In the present study, plasma glucagon and GLP-1_{7-36} illustrated no evidence of systematic or proportional bias between methodological approaches, and no systematic bias was detected for between-day fingertip-capillary sampling, suggesting fasting and postprandial concentrations of these peptides are comparable between methodological approaches (**Figure 2.1**) and reproducible between visits (**Figure 2.3**). This observation coupled with the overall bias generated between fingertip-capillary and antecubital-venous time-averaged AUC for glucagon (−0.2 pg·mL) and GLP-1_{7-36} (0.4 pg·mL) and between-day fingertip-capillary sampling (0.3 pg·mL and 0.2 pg·mL, respectively) were not deemed clinically relevant discrepancies as based on our pre-determined values [4.9 pg·mL (Celga et al., 2013) and 1.4 pg·mL (Verdich et al., 2001), respectively]. Between methodological approaches, the typical percentage error (CV_a, %) of plasma glucagon and GLP-1_{7-36} was similar (CV_a = 21.0 and 24.0%, respectively), demonstrating modest agreement. Under test-rest conditions, fingertip-capillary-derived estimates of glucagon displayed strong reproducibility

with an acceptable level of random error (CV_r = 8.2%), yet remained similar for plasma GLP-1_{7-36} (CV_r = 22.7%). The results concerning plasma glucagon are considerably lower than the biological variation presented by Widjaja et al. (1999). Widjaja and colleagues (1999) assessed the variation (within- and between-subject variation over 12 consecutive days) of several biochemical variables in healthy adults, and illustrated antecubital-venous plasma glucagon exerted a daily variation of 19.0 and 28.0% within- and between-subjects, respectively (Widjaja et al., 1999). Results of the current study may therefore suggest estimates of fingertip-capillary plasma glucagon are comparable to their antecubital-venous equivalents, and more reliable when obtained within fingertip-capillary blood. To the authors' knowledge, this is the first study to report the test-retest reproducibility of plasma GLP-1_{7-36}. Glucagon-like peptide-1 is produced and secreted into the circulation from the L cells of the intestinal mucosa in response to meal ingestion, and is recognised as a key effector in glucose regulation, gastrointestinal motility, and appetite (Chelikani, Haver, & Reidelberger, 2005; Edholm et al., 2010). In the present study, plasma GLP-1_{7-36} demonstrated moderate CV's with negligible bias between methodological approaches and days. Similar to plasma glucagon, results of the current study may suggest estimates of GLP-1_{7-36} are more reliable when obtained within fingertip-capillary blood. Nonetheless, further research quantifying the biological variation of GLP-1_{7-36} is warranted to elucidate the within- and between-subject variation of this peptide. Taken together, fingertip-capillary blood sampling provides reliable and comparable measures of glucagon and GLP-1_{7-36} to antecubital-venous samples, indicating the two methodological approaches may be interchanged. These results are of importance as they permit comparison of results obtained in studies utilising fingertip-capillary blood sampling to studies using antecubital venous blood sampling.

Although Deming regression revealed no evidence of systematic or proportional bias between methodological approaches (**Figure 2.2, panel A**), and no systematic bias for between-day fingertip-capillary sampling, estimates of fingertip-capillary plasma leptin were consistently

underestimated in comparison to their antecubital-venous equivalents. It might be unsurprising that concentrations of leptin differed between methodological approaches given its production site, manner of collection and differing sampling location. Leptin is predominantly secreted from the adipose tissue (Klein, Coppack, Mohamed-Ali, & Landt, 1996) whereas all other hormones analysed within this study are secreted from either the pancreas or the gastrointestinal tract. The antecubital vein drains a mixture of forearm muscle, adipose and skin tissue (Macdonald, 1999), compared to the fingertip which drains an assortment of blood from venules, arterioles, intracellular and interstitial fluid, and of course the capillaries. Concentrations of plasma leptin may therefore be more pronounced in venous outflow, as illustrated in the present study. Despite this tendency, the bias generated between methodological approaches (−77.5 pg·mL, CV_a = 9.0%) and between-days (95.9 pg·mL) were not deemed clinically relevant discrepancies and likely to alter research interpretation as based on our pre-determined values (148.0 pg·mL), which takes into account the within-subject daily variation of plasma leptin (Liu et al., 1999). Under test-reset conditions fingertip-capillary-derived estimates of leptin demonstrated modest reproducibility, with a satisfactory typical percentage error (CV_r = 25.0%). Leptin, a tonic adipocyte hormone, indicates long-term energy balance (Duca et al., 2012; Woods, 2005) and signals chronic nutritional state. It may be plausible that the increased bias and level of typical error between-days may be attributable to dietary standardisation. In the present study dietary standardisation was only implemented from the evening meal preceding data collection periods. Standardisation to promote identical nutritional states may therefore require longer dietary replication. Nonetheless, the typical error reported between-days was similar to that reported in earlier investigations (Chia et al., 2008; Liu et al., 1999). These researchers established the reproducibility of fasting plasma leptin among lean and obese individuals in antecubital-venous blood, and provided evidence that plasma leptin exerts a daily variation of 20% (Liu et al., 1999). Based on the typical percentage error, we report that fingertip-capillary blood sampling is an acceptable approach for leptin quantification, but should not be interchanged with antecubital-venous blood sampling.

For insulin quantification, Deming regression revealed evidence of proportional difference between fingertip-capillary and antecubital-venous blood (slope = 1.4, 95% CI for slope = 1.1 to 1.7, 95% CI for intercept = -106.8 to 50.1), and visual inspection of scatterplots indicated worse agreement between measurements at higher concentrations (**Figure 2.2, panel B**). The identification of proportional bias between fingertip-capillary and antecubital-venous blood sampling illustrate these techniques cannot be used interchangeably. The typical percentage error of plasma insulin was similar between methodological approaches and between visits (CV_a and CV_r = 36.0%, respectively), representing a large level of random error. The CV's demonstrated here are higher than the within-subject variation of plasma insulin (26%) as illustrated by Widjaja et al. (1999). Despite this, the bias generated between days (−37.8 pmol·L) was not deemed a clinically relevant discrepancy according to our pre-determined values (62.0 pmol·L), which took into account the within-subject daily variation. Nonetheless, compared to antecubital-venous blood, Deming regression, typical percentage error and LOA suggest fingertip-capillary represents an inappropriate alternative for insulin quantification. Perhaps fingertip-capillary blood, being arterialised, is comparable and more reflective of arterial-derived estimates. Further work to elucidate this is necessary. Researchers and practitioners wishing to implement this technique should take the random error between techniques in to consideration when interpreting study findings.

No data concerning differences in any of the peptides reported here based on antecubital-venous and fingertip-capillary blood sampling exists. The error observed (differences observed for each analyte between methodological approaches and between visits) may be attributable to pre-analytical (e.g. specimen collection, timing of collection) and analytical error (e.g. collection techniques, sample handling). As mentioned, blood attained following fingertip-capillary blood sampling is characteristically dissimilar to that of antecubital-venous blood, and may be more reflective of arterial blood (Merton et al., 2000). Further, discrepancies between fingertip-capillary

and antecubital-venous-derived peptides might have been influenced by the introduction of cytoplasmic matter (intracellular fluid) to the fingertip-capillary sample. Excessive squeezing during fingertip-capillary blood sampling encourages haemolysis of the erythrocytes, thus influencing the introduction of intracellular contents and interstitial fluid to the surrounding blood plasma consequently diluting samples and affecting assay effectiveness (Godfrey, Whyte, McCarthy, Nevill, & Head, 2004). Granted the methodological approach of fingertip capillary blood sampling presents reduced complexity, substantial proficiency is essential to ensure an appropriate amount of blood is collected in a reduced amount of time (30-60 sec). In the present study, highly trained research staff collected all fingertip-capillary blood samples and care was taken to avoid excessive pressure whilst sampling in an attempt to minimise the risk of such error. When performing fingertip capillary sampling we highly recommend, where possible, pre-warming the entire sample-hand (in either a warm towel or warm-water) for approximately 3-5 min preceding sample collection, in an attempt to promote adequate blood flow. Further, we encourage the collection of at least 300 μL of whole blood to ensure a sufficient volume of plasma is available for biochemical analysis. For samples taken as EDTA plasma, the respective additives [aprotinin (33 μL·mL blood) and a DPP-IV inhibitor (30 μL·mL blood)] should be added prior to sample collection in order to maintain the integrity of GLP-$1_{7\text{-}36}$ and glucagon by proteases. The authors' acknowledge that values for CV% in the current study appear relatively high, particularly for GLP-$1_{7\text{-}36}$ and leptin, yet are consistent with the variability expected between plasma samples from the same sampling location. For this reason, the findings of the present study suggest fingertip-capillary blood sampling represents a suitable and comparable alternative to antecubital-venous methods for the estimation of certain appetite-related peptides in various population groups.

In conclusion, the key findings arising from this chapter demonstrate that at rest fingertip-capillary blood sampling offers and appropriate methodological and reproducible approach for the quantification of plasma glucagon and to a lesser extent for GLP-$1_{7\text{-}36}$, leptin and insulin in response

to a standardised breakfast meal. For plasma insulin quantification, in repeated measures designs, fingertip-capillary and antecubital-venous blood sampling should not be interchanged. These findings will allow for appropriate comparison of capillary and venous sampling techniques, especially in investigative settings where antecubital-venous blood sampling of appetite- and metabolism-related peptides may be contraindicated. With specific regard to the broader aim of this thesis, in addition to the link between milk-based dairy consumption and adiposity, fingertip-capillary blood sampling represents an appropriate technique to further establish the influence of milk-based dairy consumption on appetite and feeding behaviour in children and adolescents. Consequently, it would be advantageous to assess patterns of milk-based dairy consumption in populations relevant to the thesis. Specifically, to establish dairy food popularity (types, frequencies and amounts) and identify potential populations (sex & age) to inform the intervention-based sections of this thesis as to the most appropriate dairy food to intervene with and population to focus on.

CHAPTER THREE

PATTERNS OF MILK-BASED DAIRY CONSUMPTION IN FREE-LIVING CHILDREN AND ADOLESCENTS

3.0 Introduction

In chapter two of this thesis, an alternative approach to antecubital-venous blood sampling was evaluated. The results presented within chapter two provide valuable information for researchers and practitioners regarding the utility of fingertip-capillary blood sampling for the estimation of appetite- and metabolism-related peptides for use in future appetite intervention studies in paediatric populations. In this sense, the key findings arising from this chapter demonstrated that at rest fingertip-capillary blood sampling offers an appropriate methodological and reproducible approach for the quantification of plasma glucagon and to a lesser extent for GLP-$1_{7\text{-}36}$, leptin and insulin in response to a standardised breakfast meal. With specific regard to the broader aim of this thesis, fingertip-capillary blood sampling represents an appropriate technique to further establish the link between milk-based dairy consumption, appetite and feeding behaviour in children and adolescents. Consequently, it would be advantageous to assess patterns of milk-based dairy consumption in populations relevant to the thesis. Specifically, to establish dairy food popularity (types, frequencies and amounts) and identify potential populations (sex & age) to inform the intervention-based sections of this thesis as to the most appropriate dairy food to intervene with and population to focus on.

As mentioned earlier in section 1.1 of the general introduction and literature, rates of childhood and adolescent overweight and obesity in England have increased dramatically over the past few decades (Ryley, 2013). Evidence sought from the most recent HSE illustrate that 29.5% of children and adolescents (2-15 y) are presently classified as being either overweight (≥ 85th centile) or obese (≥ 95th centile) (Cole et al., 1995; Ryley, 2013). In general, the estimated rates of obesity have increased from 11.1% and 12.2% in 1995 to 15.7% and 14.7% in 2013 among boys and girls (2-15 y), respectively. Interestingly, trends concerning the incidence of overweight and obesity are concurrent with declining levels of dairy consumption, which may suggest that milk-based dairy foods offer a protective effect against adiposity. Indeed, an emerging body of evidence exists regarding the association of dairy intake and weight regulation in children and adolescents (Abreu

et al., 2013; Barba et al., 2005; Moore et al., 2008). The underlying relationship between the consumption of dairy and adiposity has not been clearly elucidated, however, several plausible mechanisms have been identified. Greater intakes of dairy calcium may inhibit lipid accretion and influence adipocyte lipid metabolism (Dougkas et al., 2012), increase postprandial fat oxidation (Mealanson et al. 2005; Van Loan, 2009), and faecal fat excretion (Bendsen et al., 2008). Furthermore, evidence suggests that dairy proteins may facilitate body mass regulation through dietary and appetite regulation and are associated with increased plasma concentrations of appetite-regulating peptides (Anderson et al., 2004; Bowen et al., 2006; Luhovyy et al., 2007; Schneeman et al., 2003), subsequently influencing energy intake (Dougkas et al., 2012; Lluch et al., 2010) and reducing gastric emptying. This may also be true for child and adolescent populations (Vien et al., 2014), however, only one investigation to date (comprising two experiments) has sought to establish the effect of milk-based dairy food consumption on appetite and feeding behaviour in children and adolescents (9-14 y), where subjective appetite and appetite-related peptides were measured (Vien et al., 2014). Consequently, knowledge concerning the milk-based dairy food consumption and the mechanisms impacting on appetite and feeding behaviour in children and adolescents is not fully understood. Consequently, it would also be advantageous to assess patterns of milk-based dairy consumption in populations relevant to the thesis. Specifically, to establish dairy food popularity (types, frequencies and amounts) and identify potential populations (sex and age) to inform the intervention-based sections of this thesis as to the most appropriate dairy food to intervene with and population to focus on.

To date, the NDNS (2011-12) remains the only surveillance programme in the UK providing a nationally representative assessment of dietary habits of the general population (1.5-3 y, 4-10 y, 11-18 y, 16-64 y and 65 y and older). Current recommendations generally encourage two to three servings daily (Gidding et al., 2006), however, national survey data continues to fuel concern over consumption rates, especially throughout adolescence. Statistics obtained from the most recent NDNS indicate that in 2011-12, the consumption of milk and dairy foods was approximately 30%

greater in children (4-10 y: 275 $g \cdot d^{-1}$) compared with their adolescent counterparts (11-18 y: 197 $g \cdot d^{-1}$) (Bates et al., 2014). Furthermore, among male and female adolescents, per capita milk-based dairy food consumption has fallen by 14.1% in comparison to reported intakes in 1997. Originally established in 1992, methodological approaches used to collect dietary information in the NDNS have varied throughout the series of cross-sectional assessments. Consequently, this limits comparability between preceding surveys. In addition, dietary surveys typically rely on retrospective dietary assessment methods, which pose complications of misreporting error and are therefore not entirely robust. In general, it appears from the aforementioned surveys that milk-based dairy consumption declines with increasing age, particularly through the adolescent period. It should be noted, however, the NDNS exercise wide-ranging age groupings (e.g. 4-10 y; 11-18 y) which make it difficult to differentiate between consumption patterns in middle-childhood and adolescence. While evaluations are confounded by methodological disparities, trends concerning milk-based dairy consumption may represent methodological inadequacies rather than changes in habitual dietary practices between middle-childhood and adolescence.

Accurately quantifying dietary intakes in children and adolescents is fundamentally important, especially in free-living environments (Rumbold, St Clair Gibson, Stevenson, et al., 2011), yet it is often problematic due to the unique methodological considerations associated with respondents (Livingstone et al., 2000). Adolescence is the transitional phase between childhood and the onset of adulthood (13-19 y), and is a critical time that, alongside growing independence and marked physical development, typically behavioural changes toward dietary habits are evident (Alberga et al., 2012). Dietary practices shaped throughout childhood and adolescence may track into adulthood (Hallal, Victora, Azevedo, & Wells, 2006). These modifications warrant attention as persistence of dietary practices deemed unhealthy may incur nutritional and health-related implications including: cardiovascular disease, hypertension, dyslipidemia, and insulin resistance (Ogden et al., 2007; Wang et al., 2011). Indeed, continual dairy food avoidance as highlighted throughout the NDNS may incur detrimental nutritional and health-related implications, particularly

among adolescents, leaving populations vulnerable to micronutrient deficiencies and thus lasting health implications (Nicklas, 2003). As dietary behaviours can vary widely from childhood to adolescence, the aforementioned evaluations may not hold true between middle-childhood and adolescence. Accordingly, the present study set out to examine and compare patterns of dairy food consumption among a sample of children (9-11 y) and adolescents (15-18 y), exercising more finite age boundaries and more robust dietary assessment tools. Specifically, this study aimed to establish dairy food popularity (types, frequencies and amounts) and identify potential populations (sex and age) to inform the intervention-based sections of this thesis as to the most appropriate dairy food to intervene with and population to focus on.

3.1 Materials and methods

3.1.1 Study design

Using a between-groups cross-sectional design, the present study assessed free-living dairy consumption among a sample of children and adolescents over a period of 4 days. All testing took place during school term-time.

3.1.2 Study population

This study comprised a convenience sample of participants aged 9-11 y (n = 40; 15 boys and 25 girls) and 15-18 y (n = 40; 20 males and 20 females), recruited from a local primary and secondary school respectively, in the North-East of England. After an initial seminar detailing study procedures, parental information letters and consent forms were issued to all eligible participants. The study was conducted according to the guidelines laid down in the 2013 Declaration of Helsinki (WMA, 2013), and all procedures involving human participants were approved by the Faculty of Health and Life Sciences Ethics Committee at the University of Northumbria at Newcastle. All

participants provided parental written informed consent prior to data collection. Please refer to Appendix C for an example information sheet and consent form (**see page 234**).

3.1.3 Preliminary testing and familiarisation

One week preceding data collection, all adolescent participants attended a practical workshop. For child participants, parents and/or guardians of the participating child were also invited to attend. The workshop took place on school campus and acted as a preliminary testing and familiarization session. For logistical purposes, workshops ran on several occasions and generally comprised of 10 participants. The aims of the workshops were threefold. Firstly, participants were trained and educated on the correct food weighing and recording procedures, as recommended by Livingstone et al. (1992). This included weighing all food and drink items, prior to and following consumption (if leftovers were present). Secondly, adolescent participants were habituated with the two-pass 24-hour dietary recall procedure (Ashley et al., 2003). Lastly, workshops served as an initial assessment session to enable anthropometric measures to be taken.

3.1.4 Anthropometry

Measures of body mass were determined to the nearest 0.1 kg, using portable scales (Seca, Birmingham, UK), with children and adolescents shoeless and wearing light weight clothing. Stature was measured to the nearest 0.01 m using a portable stadiometer (Holtain Ltd, Pembs, UK). From these parameters a measure of BMI ($kg \cdot m^2$) was computed.

3.1.5 Adolescent dietary assessment

Adolescent (15-18 y) free-living dietary intake was evaluated utilising a combined weighed self-reported food record and 24 h dietary recall technique, used previously with adolescent populations (Rumbold, St Clair Gibson, Stevenson, et al., 2011). The reporting accuracy of this combined

approach has demonstrated it be an effective method to collect dietary information in adolescents (confidence intervals for bias ranging from 0.00 – 0.95 MJ) (Rumbold, St Clair Gibson, Stevenson, et al., 2011). Reported dietary intakes were subsequently explored to determine types, amounts and frequency of dairy food consumption. Food records were collected during school term-time over four consecutive days, including two weekdays and two weekend days. To collect sufficient detail concerning habitual milk-based dairy food consumption the present study utilised an observation period encompassing both weekday and weekend days. Reasons for this were facilitated through literature informed recommendations considering dietary intake behaviours in children and adolescents have been reported to differ between weekdays and weekend days (Bjelland et al., 2011; Cullen et al., 2002; Rothausen et al., 2012; Svensson et al., 2014). During this period, adolescent participants, with parental support where necessary, were requested to give full comprehensive recordings of all food and drink items consumed, weighing all items prior to and following consumption (if leftovers were present). Additional information deemed necessary included methods of preparation and cooking, names of branded products and condiment use. For homemade dishes, participants were asked to record individual ingredients and quantities for the whole dish, along with a brief description of cooking method and how much of the dish they consumed. Please refer to Appendix B for an example food record template (**see page 228**). For those individuals who consumed meals at school, participants were requested not to estimate food portion sizes as this may have influenced usual feeding behaviour (Livingstone et al., 1992), subsequently impacting on habitual dietary practices. Instead, as implemented previously in young people (Rumbold, St Clair Gibson, Allsop, et al., 2011), to obtain information concerning school meals researchers communicated with kitchen staff to obtain the required nutritional information.

Following each day of dietary data collection, research staff visited the adolescent participants separately on school premises (apart from weekends) and completed 24 h recall interviews. Interviews exercised a two-pass approach (Ashley et al., 2003), and lasted

approximately 15 min per participant. Initially, participants were requested to recollect all eating episodes of the preceding day, highlighting the main food and drink items consumed. Researchers subsequently encouraged participants to further elaborate on information relating to: brand names, condiment use, forgotten food or drink items, portion size and food handling. Absent portion sizes were substituted, if not rectified during recall interviews, using previously recorded portion sizes of the same food or drink item. If a substitute was not available, standard portion estimates from the nutrition software package (Nutritics Professional v3.09, Nutritics, Ireland) were used. All interviews took place at the same time each day and were conducted by the same researcher.

3.1.6 Children's dietary assessment

For children (9-11 y), free-living dietary intake was evaluated through parental weighed food records. As with their adolescent counterparts, food records were collected during school term-time over four consecutive days, including two weekdays and two weekend days. Reported dietary intakes were subsequently explored to determine types, amounts and frequency of dairy food consumption. During periods of dietary data collection, a parent and/or guardian was requested to complete the 4-day diary, with assistance from the participating child where necessary. Parental food records have successfully been employed when reporting dietary habits of children (4-10 y), and have shown to be comparable to measures of energy expenditure using doubly labelled water (Bates et al., 2014; Bates, Lennox, Prentice, Bates, & Swan, 2011, 2012). Parents were given instructions to record all food and drink items consumed by the participating child in and out of the home environment. Parents were requested to give full comprehensive recordings of all food and drink items consumed, weighing all items prior to and following consumption (if leftovers were present). Additional information deemed necessary for inclusion included: methods of preparation and cooking, names of branded products and condiment use. For homemade dishes, parents were asked to record individual ingredients and quantities for the whole dish, along with a brief

description of cooking method and how much of the dish the participating child consumed. Again, for those individuals who consumed meals at school, participants were requested not to estimate food portion sizes as this may have influenced usual feeding behaviour (Livingstone et al., 1992), subsequently impacting on habitual dietary practices. Instead, as implemented previously in young people (Rumbold, St Clair Gibson, Allsop, et al., 2011), to obtain information concerning school meals researchers communicated with kitchen staff to obtain the required nutritional information.

3.1.7 Free-living milk-based dairy consumption

All food records were analysed utilising the nutritional software package Nutritics (Nutritics Professional v3.09, Nutritics, Ireland). Food records were explored to determine types, amounts and frequency of dairy food consumption. Dairy food categories in the present study included: milk (whole, reduced-fat, fat-free, and flavoured milk), yogurt (all yogurt types), cheese (all cheese types), butter, ice cream, cream and custard. For the present study, a serving of dairy equated to 150 mL milk, 25 g cheese, 120 g yogurt, 5 g butter, 75 g ice cream, 15 mL cream and 75 g of custard. As recommended serving sizes are not available for the UK, servings were adapted based on portion sizes of milk-based dairy foods to meet calcium needs for children and adolescents (The Dairy Council, 2015). Patterns of dairy consumption were dichotomised according to overall consumption and average daily servings.

3.1.8 Statistical analysis

The statistical software package SPSS v 21.0 (SPSS Inc., Chicago, IL) was used for all data analysis. Descriptive data are presented as means and standard deviation (SD). Results are presented by groups according to sex and age (boys 9-11 y; boys 15-18 y; girls 9-11 y; girls 15-18 y). Differences in overall consumption and average daily servings of milk, cheese, yogurt, butter,

ice cream, cream and custard were analyzed using a between group 2 x 2 (age x sex) analysis of variance. Following a significant interaction, LSD *post hoc* test was used to determine the location of variance, and all *p* values reported have been adjusted for multiple comparisons. Statistical significance was accepted at $p < 0.05$ for all analysis.

3.2 Results

In agreement with previous adolescent studies, dietary intake data were excluded from statistical analysis if food records were deemed incomplete or when cases of extreme reporting were present (e.g. <500 kcal or >5000 kcal), as this was believed to be considered implausible (Ambrosini, de Klerk, O'Sullivan, Beilin, & Oddy, 2009; Greatwood, Daly-Smith, McGregor, & McKenna, 2013). In total, data are presented for 75 participants [9-11 y (n = 40; 15 m, 25 f) and 15-18 y (n = 35; 18 m, 17 f)]. Three participants were excluded due to unsatisfactory food record completion, and two withdrew due to their lack of interest to participate during the data collection. Characteristics for participating children and adolescents are shown in **Table 3.0**. Data concerning types, amounts and frequencies of consumed dairy foods are presented in **Table 3.1** by age and sex, dichotomised according to overall consumption and average daily servings.

Table 3.0 Participant characteristics dichotomised according to age

	9-11 y N = 40		15-18 y N = 35	
Participant Characteristics	Mean	(SD)	Mean	(SD)
Age (y)	9.4	0.5	16.1	1.0
Stature (m)	1.41	0.07	1.71	0.1
Body Mass (kg)	33.49	7.46	66.2	15.1
BMI (kg/m^2)	16.8	3.1	22.6	3.5

Statistical analysis revealed a main effect for sex on overall milk consumption ($F_{1,71} = 7.07$, $p = 0.010$) and daily milk portions ($F_{1,71} = 6.79$, $p = 0.011$), indicating that independent of age, boys consumed greater amounts of milk compared to girls (**Table 3.2**). No interaction or main effect for any other variable (cheese, yogurt, butter, ice cream, cream and custard) was evident. Total daily dairy servings did not differ significantly between ages ($F_{1,71} = 2.12$, $p = 0.150$) and sexes ($F_{1,71} = 2.48$, $p = 0.120$). Patterns of milk, cheese, yogurt and total daily dairy servings remained widely stable among girls and female adolescents (**Table 3.1**). For boys and adolescent males, a downward trend of milk and dairy food consumption with increasing age was noted, although differences were not significant (**Table 3.1**). The major contributing source of dairy was milk, consumed by 91% of participants (across all ages and sexes). This was followed by cheese and yogurt consumption, 60% and 56% respectively.

Table 3.1. Milk-based dairy food consumption of children (8-11 y) and adolescents (15-18 y) dichotomised according to overall consumption and average daily servings. Differences in overall consumption and average daily servings were analysed using a between group 2 x 2 (age x sex) ANOVA.

Dairy Product	Males 9-11 y (n = 15)	Males 15-18 y (n =18)	Females 9-11 y (n = 25)	Females 15-18 y (n = 17)	Ages Combined Males 9-18 y (n = 33)	Females 9-18 y (n = 42)	*p* Value
Milk (mL)	716.7 ± 516.8	573.4 ± 451.6	392.6 ± 300	389.3 ± 384.5	638.5 ± 480.0	391.3 ± 332.2*	$p = 0.010$
Daily Milk Servings	1.2 ± 0.9	1.0 ± 0.8	0.7 ± 0.5	0.6 ± 0.6	1.1 ± 0.8	0.7 ± 0.6*	$p = 0.011$
Cheese (g)	49.3 ± 34.6	35.4 ± 32.2	30.3 ± 35.0	26.4 ± 39.8	41.7 ± 33.5	28.7 ± 36.6	NS
Daily Cheese Servings	0.5 ± 0.4	0.4 ± 0.3	0.3 ± 0.4	0.3 ± 0.4	0.4 ± 0.3	0.3 ± 0.4	NS
Yogurt (g)	153.8 ± 154.9	69.6 ± 136.7	105 ± 134	123.7 ± 225.1	107.8 ± 149.1	112.6 ± 174.3	NS
Daily Yogurt Servings	0.3 ±0.3	0.1 ± 0.3	0.2 ± 0.3	0.3 ± 0.5	0.2 ± 0.3	0.2 ± 0.4	NS
Butter (g)	14.3 ± 13.1	10.5 ± 10.2	13.2 ± 15.1	14.8 ± 14.7	12.2 ± 11.6	13.8 ± 14.8	NS
Daily Butter Servings	0.7 ± 0.7	0.5 ± 0.5	0.7 ± 0.8	0.7 ± 0.7	0.6 ± 0.6	0.7 ± 0.7	NS
Ice-Cream (g)	21.2 ± 39.7	22.5 ± 42.4	21.2 ± 36.0	37.6 ± 60.5	21.9 ± 40.5	27.9 ± 47.4	NS
Daily Ice-Cream Servings	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.2	NS
Cream (g)	4.3 ± 9.0	0.0 ± 0.0	2.8 ± 11.3	2.1 ± 6.4	2.0 ± 6.4	2.5 ± 9.5	NS
Daily Cream Servings	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1	NS
Custard (g)	13.3 ± 39.9	0.0 ± 0.0	8.7 ± 21.8	0.0 ± 0.0	6.1 ± 27.3	5.2 ± 17.2	NS
Daily Custard Servings	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	NS
Total Daily Servings	2.9 ±1.4	2.1 ± 1.2	2.0 ± 1.2	2.0 ± 1.0	2.4 ± 1.3	2.0 ± 1.1	NS

Values are presented as mean ± SD. * denotes significant difference between sexes ($p < 0.05$). Statistical analysis revealed a main effect for sex on total milk (mL) ($F_{1,71} = 7.07$, $p = 0.010$) and number of daily milk portions ($F_{1,71} = 6.79$, $p = 0.011$), indicating that independent of age, boys consumed greater amounts of milk compared to girls A serving of dairy equated to 150 mL milk, 25 g cheese, 120 g yogurt, 5 g butter, 75 g ice cream, 15 mL cream and 75 g of custard. Total dairy portions group comprised all dairy subtypes.

3.3 Discussion

The present study investigated patterns of dairy food consumption in a sample of free-living children and adolescents, from the North-East of England, in order to establish dairy food popularity (types, frequencies and amounts) and identify potential populations (sex and age) to inform the intervention-based sections of this thesis as to the most appropriate dairy snack to intervene with. Analysis revealed a main effect for sex on overall milk consumption ($F_{1,71} = 7.07$, $p = 0.010$) and daily milk portions ($F_{1,71} = 6.79$, $p = 0.011$), indicating that independent of age, boys consumed greater amounts of milk compared to girls. This was apparent for both overall milk consumption and daily milk servings and is consistent with other cross-sectional data indicating boys consume greater quantities of milk than girls (Forshee & Storey, 2003; Mensink, Kleiser, & Richter, 2007; Novotny et al., 2003). In addition, findings reported throughout this chapter indicate that milk was the most favorable dairy food consumed among children and adolescents, consumed by 91% of participants (across all ages and sexes). Although we sought no statistical evidence that milk-based dairy food consumption differed significantly between middle-childhood and adolescence, for boys and adolescent males, a downward trend of total daily dairy food consumption with increasing age was noted whereas patterns of milk and dairy food consumption remained widely stable among girls and female adolescents (**Table 3.1**). In this sense, for boys and adolescent males total daily dairy food servings declined from 2.9 at age 9-11 y to 2.1 at age 15-18 y, respectively. This finding is not consistent with the current literature as intakes of milk and dairy food consumption have reportedly declined among both boys, girls and male and female adolescents (Baird et al., 2012; Cavadini et al., 2000). In Addition, Parker et al (2012) reported milk and dairy product servings have remained stable among boys and adolescents males, whereas patterns of milk and dairy food consumption have declined among girls and female adolescents. As dietary habits shaped throughout this phase may ultimately track into adulthood (Lake et al., 2006), continual milk and dairy food avoidance could be disadvantageous, particularly among adolescents, leading to lasting nutritional and health-related implications. Additionally, continual milk and dairy

food avoidance may present an increased risk of developing overweight and obesity as demonstrated throughout chapter one of this thesis. For this reason, it may be appropriate to target the intervention-based studies on adolescent males (15-18 y).

Despite the acknowledged nutritional and health-related benefits of milk-based dairy consumption as part of a healthy balanced diet health (Fiorito, Mitchell, et al., 2006), consumption of dairy (as based on national survey data) has declined in recent years especially from childhood through to adolescence, and declines further with increasing age (Bates et al., 2014). Contrary to existing literature (Baird et al., 2012; Bates et al., 2014; Cavadini et al., 2000; Morton et al., 1998; C. E. Parker et al., 2012), the results reported here suggest there is no difference in dairy consumption (types, amounts and frequency) between middle-childhood and adolescence. In the present study, daily dairy servings complied with current dietary recommendations (two to three servings daily) (Gidding et al., 2006) for all age groupings and between sexes, although are on the lower end of the recommendation. These findings are consistent with previous studies that have shown poor compliance of dairy consumption among U.S children and adolescents (Cavadini et al., 2000; Fiorito, Mitchell, et al., 2006). This is of particular concern as dairy food avoidance and low dairy consumption could be disadvantageous, particularly among adolescents, leading to nutritional and health-related implications. Milk-based dairy foods represent a nutrient-dense foodstuff, and encompass a host of bioactive constituents pertinent to human health (Fiorito, Mitchell, et al., 2006). Daily consumption of dairy contributes significantly to intakes of high-quality proteins and various essential micronutrients [such as calcium, riboflavin, and vitamin D (if fortified)], helping children and adolescents achieve nutrient intake recommendations and augment dietary quality (C. Weaver et al., 2013).

Although the findings reported here cannot be used to elucidate motivations for differences in dairy food consumption between boys and girls, numerous observations indicate that participants associate the consumption of dairy with increased body mass (Nolan-Clark et al., 2011; Wham et al., 2001). Research suggests female adolescents decrease dairy food consumption due to concerns

regarding weight gain and the notion that dairy foods are fattening (Gulliver et al., 2001; Neumark-Sztainer et al., 1997). Indeed, dairy foods contain saturated fats, and historical views concerning elevated plasma cholesterol following consumption have been linked to increased cardiovascular risk (Soerensen et al., 2014; Tunick et al., 2014). Taken together, the belief that dairy foods are fattening may have contributed to the assumption that dairy is a factor in obesity (Elwood et al., 2010), and consumption should consequently be limited. However, trends concerning the incidence of overweight and obesity are concurrent with declining levels of dairy consumption, suggesting that dairy may confer a direct or indirect protective effect against adiposity. An expanding body of research, suggesting a potential role of milk and dairy consumption in the prevention of weight gain is emerging (Abreu et al., 2013; Barba et al., 2005; Moore et al., 2008). Research concerning the impact of dairy foods on mechanisms facilitating the relationship between dairy and adiposity, however is sparse, particularly within the free-living environment. Further research is therefore warranted to verify the magnitude of the effect, if any, of dairy food consumption among adolescents on the aforementioned mechanisms highlighted previously.

In agreement with figures from Australian children and adolescents (2-16 y) (Baird et al., 2012), National Health and Nutrition Examination Survey (Moore et al., 2008), and the NDNS (Bates et al., 2014), milk was the most favoured dairy food among both children and adolescents, consumed by 91% of participants. This was followed by cheese and yogurt, consumed by 60% and 56% of participants, respectively. In general, overall consumption and daily servings of milk and cheese were greater among boys and adolescent males compared with their female counterparts; however, the opposite was apparent for yogurt and ice cream. Indeed, this appears to be a common finding in the literature (Baird et al., 2012; Cavadini et al., 2000). Nonetheless, daily servings remained similar between females and for milk appeared to decline for boys and adolescent males.

It is relevant to acknowledge that the results of this study are limited to a relatively small population of children and adolescents from the North-East of England. Further, limitations of this

study warrant consideration. Data regarding free-living dairy consumption were obtained through self-reported records. A major drawback of participant centered data collection is that such approaches present opportunities for bias and misreporting (Illner, Nöthlings, Wagner, Ward, & Boeing, 2010). However, in the present study two methodological approaches were exercised to explore dairy consumption, which the authors' believe to be a novel aspect of the study. Adolescent free-living dairy intake was evaluated utilising a combined weighed self-reported food record and 24 h dietary recall technique, and through parental centred food records for children in an attempt to combat opportunities for bias and misreporting. In summary, these findings contribute valuable information for researchers and practitioners regarding milk-based dairy consumption between middle-childhood and adolescence in the UK. Findings reported here indicate that milk is the most favourable dairy food consumed among children and adolescents. Furthermore, this study suggests that independent of age, boys consume greater amounts of milk compared to girls. Although we sought no statistical evidence that daily milk and dairy food dairy consumption differed significantly between middle-childhood and adolescence, for boys and adolescent males, a downward trend of total milk and dairy food consumption with increasing age was noted whereas patterns of milk and dairy food consumption remained widely stable among girls and female adolescents. Considering the nutritional and health-related implications associated with continual dairy avoidance, it seems necessary to target intervention based studies on adolescent males (15-18 y).

CHAPTER FOUR

ACUTE APPETITE AND METABOLIC RESPONSES TO A MID-MORNING DAIRY SNACK IN ADOLESCENT MALES

4.0 Introduction

In chapter three of this thesis, patterns of milk-based dairy consumption were assessed in a sample of free-living children and adolescents. The results presented within chapter three indicate that milk was the most favorable dairy food consumed among children and adolescents, consumed by 91% of participants (across all ages and sexes). In addition, although we sought no statistical evidence that milk-based dairy food consumption differed significantly between middle-childhood and adolescence, for boys and adolescent males, a downward trend of total milk and dairy food consumption with increasing age was noted whereas patterns of milk and dairy food consumption remained widely stable among girls and female adolescents. For this reason, it may be appropriate to intervene with adolescent populations utilising milk for the intervention-based studies. In addition to this, in chapter two of this thesis an appropriate methodological and reproducible approach to systematically quantify appetite- and metabolism-related peptide expression (plasma GLP-$1_{7\text{-}36}$, glucagon, insulin and leptin) was established. Taken together, the establishment of a less-invasive approach to quantify measure of appetite- and metabolism-related peptides and identification that milk consumption declines from middle-childhood to adolescence provides a strong basis to target future intervention-based studies on adolescent males (15-18 y). Indeed, this would provide further insight into the influence of milk-based dairy consumption on appetite and feeding behaviour in adolescents.

In recent years snacking has become commonplace and characterises an element of the modern diet, yet is often regarded as a contributing factor to the development of overweight and obesity in adolescents (Mattes et al., 2011). Snacking represents an eating behaviour or episode that occurs between typical main meals (Chapelot, 2011), and includes all food and beverage items consumed outside the context of the aforementioned main meals. For adolescents, sugar-sweetened beverages, fruit-juice drinks and milks (plain and flavoured) represent several common snack items consumed between main meals (Duffey et al., 2012). Sugar-sweetened beverages hold a negligible nutritive value and a recent systematic review has concluded that high rates of consumption are

linked with increased obesity in children and adolescent (Woodward-Lopez et al., 2011), while the opposite is apparent for milk-based dairy food consumption (Dror, 2014). Indeed, an emergent body of literature exists supporting the hypothesis that milk-based dairy food consumption provides a protective effect against adiposity in adolescents (Dror, 2014), conveying beneficial effects on abdominal adiposity, body mass and body fat (Abreu et al., 2013; Barba et al., 2005; L. L. Moore et al., 2008).

The inclusion of milk-based foodstuff as a component of a healthy balanced diet is recognised extensively, providing a significant contribution of several essential nutrients. Consequently, milk-based dairy foods provide the potential to impact favourably on human health (Fiorito, Mitchell, et al., 2006). Despite this, per capita consumption has reduced over time and declines further with age, particularly throughout adolescence. According to statistics obtained from the NDNS, dairy food consumption has declined by 14% between 1997 and 2011-12 among adolescents (11-18 y). Additionally, in 2011-12 milk-based dairy food was approximately 30% greater in children (4-10 y: 275 $g \cdot d^{-1}$) compared with their adolescent counterparts (197 $g \cdot d^{-1}$) (Bates et al., 2014). Indeed, similar patterns have been shown throughout chapter three of this thesis, especially concerning male adolescents (15-18 y). Interestingly, the temporal trends concerning milk-based dairy food consumption are concurrent with the increasing prevalence of childhood and adolescent overweight and obesity, which may suggest that the two are aetiologically related. Indeed, an emergent body of evidence exists regarding the association of dairy and weight regulation in children and adolescents (Abreu et al., 2013; Barba et al., 2005; L. L. Moore et al., 2008).

The underlying mechanisms responsible for the relationship between dairy consumption and adiposity have not been clearly defined, however, it has been suggested that constituents of milk-based dairy foods (such as proteins, calcium medium-chain triglycerides and conjugated linioleic acid) contribute to adiposity regulation through actions on appetite and feeding behaviour

(Aziz et al., 2007). In adults, consumption of dairy proteins is associated with increased concentrations of plasma appetite-regulating peptides (Anderson et al., 2004; Bowen et al., 2006; Luhovyy et al., 2007; Schneeman et al., 2003), subsequently reducing energy intake (Dougkas et al., 2012; Lluch et al., 2010) and gastric emptying. Furthermore, milk-based dairy food consumption may result in an increased rate of energy expenditure in comparison to isocaloric sugar-sweetened beverages (St-Onge et al., 2004). Taken together, it appears that milk-based dairy foods and their properties work synergistically impacting favourably on appetite, feeding behaviour and metabolism. The majority of this appetite and metabolic research, however, has been conducted in adult populations. At present, there are only four studies in children and adolescents exploring acute appetite and energy intake responses (Birch et al., 1993; Mehrabani et al., 2014; Vien et al., 2014; Zandstra et al., 2000) and only one exploring postprandial metabolism following milk-based dairy consumption in children and adolescents published only in abstract form (Apolzan et al., 2006).

In these studies, the effect of ice-cream (Birch et al., 1993), yogurt (Zandstra et al., 2000) and low-fat milk (Mehrabani et al., 2014) on appetite or energy intake have been examined in children (3-12 y). The energetic content provided by the test preloads ranged between 334 kJ and 1.2 MJ, and were offered 75 to 300 min preceding *ad libitum* energy intake assessment. All of these studies reported significant reductions in energy intake at *ad libitum* assessments, suggesting that milk-based dairy consumption influences feeding behaviour in an acute setting among children. It remains difficult to discuss the findings of the aforementioned studies, considering the preloads differed according to volume and energetic content. Furthermore, no quantitative measures of subjective appetite and/or appetite- and metabolism-related peptides were included which may have provided valuable insights concerning the mechanisms impacting on appetite and feeding behaviour. It is relevant to acknowledge that the common methodological approach to assess appetite-related peptide expression in clinical and research practice (venepuncture or antecubital-

venous catheterisation) presents increased ethical concern in vulnerable populations, certain exercise and field settings. It is probable this may explain the dearth of literature concerning appetite- and metabolism-related peptide expression in children and adolescents. In chapter two of this thesis, however, it was demonstrated that fingertip-capillary blood sampling provides an appropriate methodological and reproducible alternative approach to venous blood sampling for the quantification of plasma GLP-$1_{7\text{-}36}$, glucagon, insulin and leptin (Green, Gonzalex, Thomas, Stevenson & Rumbold, 2014). Indeed, this provides a method for paediatric researchers and practitioners where quantification of these peptides in studies concerning appetite, feeding behaviour and metabolism is important.

To date, only one investigation (comprising two experiments) has sought to establish the effect of dairy food consumption on appetite and feeding behaviour in children and adolescents (9-14 y), where subjective appetite and appetite-related peptides were measured (Vien et al., 2014). In both experiments, preloads (*experiment 1*: 1% fat chocolate milk, 2% fat milk, 1.5% fat yogurt drink, fruit punch or a water drink; *experiment 2*: 2% fat milk or a fruit punch) were provided 60 min preceding and during an *ad libitum* pizza meal. All preloads were matched for volume (250 mL) and energy content (130 kcal, 543.9 kJ). The first experiment comprised measures of subjective appetite, whereas the second experiment included measures subjective appetite together with appetite-related peptides (serum glucose, insulin and plasma GLP-1 and peptide YY). In the first experiment, Vein et al. (2014) illustrated reduced energy intake ($p < 0.01$) at a pizza meal offered 60 min following chocolate milk and yogurt consumption compared to a water drink. Consistent with a reduction in energy intake, subjective appetite (combined appetite score) was lower following 2% fat milk consumption compared with the yogurt drink only ($p < 0.01$). No additional effects were observed concerning energy intake following the consumption of 2% fat milk and fruit punch or on subjective measures of appetite after 1% fat chocolate milk, 1.5% fat yogurt drink, fruit punch or water. In the second experiment, identical procedures were followed, however antecubital-venous concentrations of serum glucose and insulin and plasma GLP-1 and

peptide YY were collected. Compared with the fruit punch preload, milk consumption resulted in a greater GLP-1 AUC (p = 0.03). Nonetheless, *ad libitum* energy intake, insulin and glucose AUC were comparable between trials (Vien et al., 2014).

Accurately quantifying human appetite and feeding behaviour is of great importance when conducting investigations exploring energy regulation. To date, there remains a paucity of literature with regards to the short-term effect of milk-based dairy consumption on appetite, energy intake and metabolism, especially in adolescent populations. Studies are primarily limited to child populations, in which the findings are equivocal. Without a better understanding concerning the mechanisms impacting on appetite and feeding behaviour following dairy consumption, it remains challenging to reconcile the potential effects of different dairy foods on energy regulation in children and adolescents. Accordingly, the present study aimed to examine the effects of mid-morning dairy consumption on subsequent appetite, energy intake and metabolic responses relative to an isoenergetic and isovolumetric serving of fruit-juice in healthy adolescent males.

4.1 Materials and methods

4.1.1 Study design

Using a randomised counterbalanced crossover design, participants completed three main trials. Trials consisted of mid-morning milk (< 2% fat), plain yogurt and fruit-juice (control) consumption, each separated by 7 days. All testing took place during school term-time.

4.1.2 Study population

In total, eleven male adolescents (mean ± SD; age 16.5 ± 0.8 y, stature 1.8 ± 0.1 m, body mass 73.4 ± 11.5 kg, and BMI 23.3 ± 3.3 kg·m^2) participated in this study. Participants were recruited from a local secondary school in the North-East of England, after attendance at an initial advertisement seminar. For the purpose of this study, sample size assessment was computed based on a pre-

determined clinically significant time-averaged AUC difference and the reported typical percentage error (8.2%) of between-day fingertip-capillary blood sampling for plasma glucagon (B. P. Green et al., 2014). Consequently, it was estimated that 10 participants would provide > 80% chance of statistically detecting a difference with $p < 0.05$. The study was conducted according to the guidelines laid down in the 2013 Declaration of Helsinki (WMA, 2013), and all procedures involving human participants were approved by the Faculty of Health and Life Sciences Ethics Committee of the University of Northumbria. Parental and adolescent written informed consent was obtained from all prior to data collection.

4.1.3 Preliminary testing and familiarisation

Approximately 1 week preceding the volunteers' first experimental visit, all participants visited the clinical testing laboratories and attended a practical workshop. The workshop acted as a preliminary testing and familiarisation session. The aims of the workshop were threefold. Firstly, participants were habituated to the equipment and methodological procedures that were employed in the study. In this sense, participants were familiarised with the blood sampling equipment, gas collection techniques, subjective appetite scales and test foods that were employed throughout. Secondly, participants were trained and educated about the food weighing and recording procedures, as recommended by Livingstone et al. (1992), to enable free-living feeding behaviour to be documented by the participants upon leaving the laboratory environment. Lastly, the workshops served as an initial assessment session where measures of anthropometry were determined.

4.1.4 Anthropometry

Measures of anthropometry (body mass and stature) were determined during practical workshops. Measures of body mass were determined to the nearest 0.1 kg, using portable scales (Seca, Birmingham, UK) shoeless and wearing light weight clothing. Stature was measured to the nearest

0.01 m using a portable stadiometer (Holtain Ltd, UK). From these parameters a measure of body mass index (kg/m^2) was computed.

4.1.5 Study protocol

Please refer to **Figure 4.0** for a schematic representation of the study design. Food and fluid consumption were recorded for the 24 h preceding each participant's initial visit using self-reported, weighed food diaries. Participants were requested to replicate these dietary behaviours before subsequent visits. Photocopies of the self-reported weighed food diaries were distributed to participants to facilitate dietary replication. Participants refrained from caffeine consumption (≥ 12 h) and avoided any form of strenuous activity (≥ 24 h) before data collection. Upon waking and until arrival at the clinical testing laboratory the consumption of water only was permitted. Participants were requested to record, document and replicate morning water consumption (if any) for subsequent trials.

On study days, participants reported to the clinical testing laboratory at 0830 h, after a 12 h overnight fast. Within 30 min of arrival, participants provided a baseline fingertip-capillary blood sample, expired gas sample (300 sec resting sample) and completed a series of VAS. Following the completion of baseline measurements (t = 0), participants were provided with a standardised cereal and milk breakfast. Participants remained at rest in the laboratory for 180 min. This period started upon the first mouthful of the breakfast meal. Further samples of fingertip-capillary blood and VAS were collected at 30, 60, and 90 min foregoing mid-morning snack consumption, and again at 30, 60 and 90 min (120, 150 and 180 min) after mid-morning snack consumption. The intervals between test meals were selected to be representative of a typical school day. Expired gas samples (300 sec) were collected at 25-30, 55-60, 85-90, 115-120, 145-150, and 175-180 min. Additional food and fluid consumption was prohibited until trial termination, apart from water that was offered *ad libitum*. In trial *ad libitum* fluid consumption (if any) was documented and matched for

subsequent trials. At 180 min, a homogenous *ad libitum* pasta meal was provided. Throughout test periods, participants remained sedentary in an environment free from food cues. On completion of the *ad-libitum* pasta meal, participants were returned to school. For the remainder of the test day, participants recorded all food and drink items consumed utilising a combined weighed self-reported food record and 24 h dietary recall technique (Rumbold, St Clair Gibson, Stevenson, et al., 2011), used previously with adolescent populations (chapter three). Following each study day, research staff visited the adolescent participants separately on school premises and completed 24 h recall interviews.

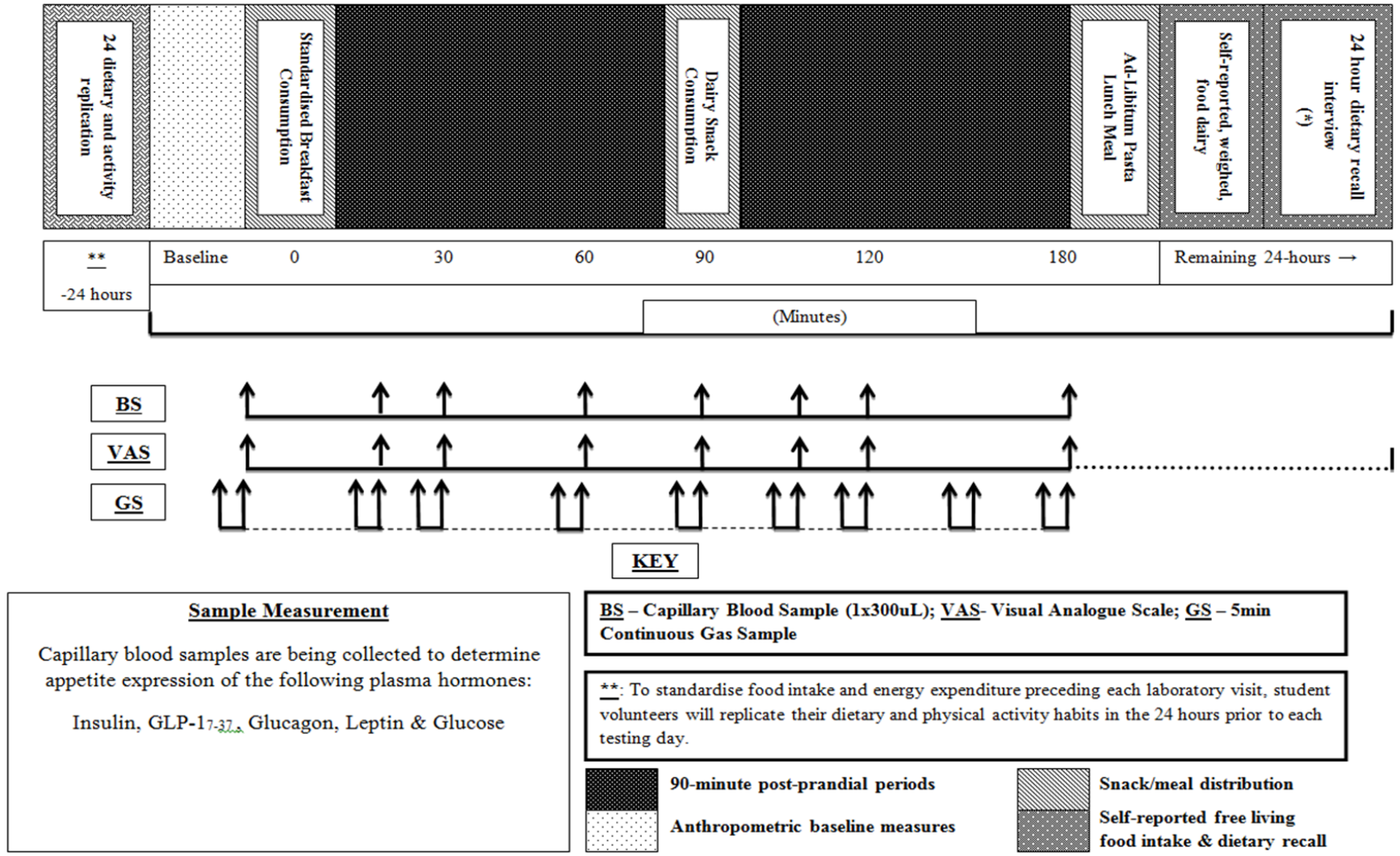

Figure 4.0. Schematic representation of the study design exercised in the present study.

4.1.6 Subjective appetite profile

Subjective measures of appetite (hunger, gut fullness, satisfaction and prospective food consumption) were assessed using validated (A. Flint et al., 2000) 100 mm, paper based, VAS. Scales were anchored with diametrically opposed feelings of extremity. Questions asked addressed hunger [‘how hungry do you feel?’ anchored with ‘*not hungry at all*’ (0 mm) and ‘*very very hungry*’ (100 mm)], gut fullness [‘how full do you feel?’ anchored with ‘*not full at all*’ (0 mm) and ‘*very very full*’ (100 mm)], prospective food consumption [‘how much do you think you can eat?’ anchored with ‘*nothing at all*’ (0 mm) and ‘*a lot*’ (100 mm), and satisfaction [‘how satisfied do you feel?’ anchored with ‘*I am completely empty’* (0 mm) and *‘I cannot eat another bite’* (100 mm)]. In response to each question, participants were requested to place a vertical mark between the diametric phrases corresponding to their feelings at the time of administration. Participants were required to report their self-perceived appetite immediately following each fingertip-capillary blood sample (t = 0, 30, 60, 90, 120, 150 and 180 min). Scales were issued in the same order at each sample point, and ratings measured by the same researcher to minimise discrepancies. An example visual analogue scale is given in Appendix D (**see page 240**).

4.1.7 Fingertip-capillary blood sampling

At seven separate intervals, fingertip-capillary (0.3 mL) blood samples were drawn into pre-cooled EDTA-treated microvettes. Samples were collected at baseline (t = 0 min) and at 30, 60, 90, 120, 150 and 180 min following breakfast consumption. At each time-point, fingertip-capillary blood samples were obtained from a pre-warmed fingertip pierced with a sterile automated lancet (Accu-Check, Mannhein, Germany) for the determination of plasma GLP-$1_{7\text{-}36}$, glucagon, insulin, leptin. In chapter two of this thesis we provided affirmation that in a resting state fingertip-capillary blood sampling offers an appropriate methodological and reproducible approach for the quantification of appetite- and metabolism-related peptides. Furthermore, as utilised by Green and colleagues (2014),

approximately 3-5 min preceding each sample, the entire sample-hand was pre-warmed in warm-water, to promote an adequate flow of fingertip-capillary blood. On removal, the hand was dried thoroughly and the identified site for puncture was further cleansed with an aseptic alcohol wipe. On puncturing the fingertip, the first drop of blood was removed before subsequent collection, where care was taken not to apply excessive pressure. All blood samples were obtained while participants lay in a semi-supine position. Additional fingertip-capillary (0.02 mL) blood samples for concentrations of blood glucose were drawn into sodium heparinised capillary tubes and transferred into eppendorfs containing 1 mL haemolysis solution (EKF Diagnostics). Samples were subsequently shaken to encourage haemolysis, placed on ice and processed immediately. Concentrations of blood glucose were quantified instantaneously by glucose oxidase method (BiosenC_line, EKF Diagnostics).

Blood collection tubes contained aprotinin (33 μL·mL blood) and a DPP-IV inhibitor (30 μL·mL blood) for the preservation of GLP-1_{7-36} and glucagon by proteases. Of note, the addition of protease inhibitors to plasma samples for the preservation GLP-1_{7-36} and glucagon does not influence measured concentrations of plasma leptin and insulin (Bielohuby et al., 2012). Following collection, samples were placed on ice and immediately centrifuged. Microvettes were spun at 3000 rpm for 10 min in a multispeed micro-centrifuge. Aliquots of plasma supernatant were housed in appropriately labelled eppendorfs and stored at −80ºC for the determination of plasma GLP-1_{7-36}, glucagon, insulin and leptin concentrations.

4.1.8 Gas analysis

To collect gas samples, a mouthpiece attached to a two-way, non-rebreathing valve (model 2730, Hans Rudolph, Kansas City, Missouri), was used. Gas samples, collected in Douglas Bags, were analysed for concentrations of oxygen and carbon dioxide using a paramagnetic and infrared transducers, respectively (Servomex 5200S, Crowborough, East Sussex, UK). In addition, bag

volume and temperature of expired gas samples were determined using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, UK) and thermistor (model 810-080, ETI, Worthing, UK), respectively. Sensors were turned on for 30 – 45 min before calibration. A two-point calibration (zero: 100% nitrogen; span: 16.93% oxygen and 5.04% carbon dioxide) was completed using certified gases (BOC Industrial Gases, Linde AG, Munich, Germany). Expired gas samples (300 sec) were collected at 25-30, 55-60, 85-90, 115-120, 145-150, and 175-180 min. Rates of energy expenditure (kJ), and substrate oxidation were estimated based on caloric equivalents of carbohydrate utilisation and lipid oxidation, using stoichiometric equations as described by (Frayn, 1983) with the assumption protein oxidation was negligible:

Carbohydrate oxidation (CO; g·min) = 4.55 $\dot{V}CO_2$ – 3.21 $\dot{V}O_2$

Fat oxidation (FO; g·min) = 1.67 $\dot{V}CO_2$ – 1.67 $\dot{V}O_2$

4.1.9 Test food

4.1.9.1 Breakfast meal

Following baseline measures, participants consumed a standardised breakfast meal consisting of semi-skimmed milk (Sainsbury, UK) and Kellogg's Rice Krispies (Kelloggs, Manchester, UK), distributed at a cereal to milk ratio of 30 g: 125 mL. The quantity issued was designed to provide 10% of the participants estimated daily energy requirement for protein, fat and carbohydrate (14%, 14% and 72%, respectively) as used previously (Astbury et al., 2010). Individual daily energy requirements were computed according to age and sex specific calculations (Schofield, 1985), providing an estimate of basal metabolic rate. Estimated values of basal metabolic rate were further multiplied against a self-perceived physical activity factor. Participants were given 15 min to consume the entire contents of the breakfast meal.

4.1.9.2 Mid-morning snacks

Ninety minutes following breakfast consumption, participants were issued with one of three mid-morning snack items, in a counterbalanced randomised manner. Snack items consisted of milk (< 2% fat, Tesco, UK), plain yogurt (Yeo Valley, UK), and orange fruit-juice (Pure orange juice smooth; control beverage) consumption (Tesco, UK). All snack items were isovolumetric (217 mL) and isoenergetic (427 kJ), yet differed according to macro-nutrient composition (**Table 4.0**). Items were made isovolumetric through the addition of water and were ingested separately to the snack foods, as implemented previously (Dougkas et al., 2012) All packaging labels were removed and snack items served in appropriate opaque serving containers.

Table 4.0 Nutritional composition of the snack items

	Milk	Yogurt	Fruit-Juice Control
Serving Size (+ mL water)	207 (10)	125 (92)	217
Energy content (kJ)	427	427	427
Carbohydrate (g)	10.2	8.4	32.0
Fat (g)	3.7	5.3	0.0
Protein (g)	7.5	5.9	1.1

Note: milk (< 2% fat, Tesco, UK), plain yogurt (Yeo Valley, UK), and fruit-juice (Pure orange juice smooth; control beverage) consumption (Tesco, UK).

4.1.9.3 *Ad-libitum* pasta meal

Lunchtime food intake was evaluated by means of a homogenous pasta meal and comprised pasta (Tesco, UK), tomato sauce (Tesco, UK), cheddar cheese (Tesco, UK) and olive oil (Tesco, UK) and provided 859 kJ of energy per 100 g of food (205 kcal; energy contributions 14% protein, 52%

carbohydrate and 34% fat). The test lunch was distributed 90 min following snack consumption (180 min following breakfast) and offered *ad-libitum*. Adolescent volunteers were initially provided with 400 g of the pasta meal. Research staff continuously refilled volunteers bowls before the dish became empty (ensuring the cue of an empty dish did not prompt termination), and the participants continued to eat. This process was repeated until volunteers indicated that they were comfortably full and wished to terminate the meal. Detailed information concerning the nutrient composition of the pasta meal and the method of cooking has been reported in previous studies (Javier T Gonzalez et al., 2015), and a similar pasta meals have been used successfully in adolescent populations (Rumbold et al., 2013). An example of the pasta procedure and data collection sheet is given in Appendix E (**see page 243**).

4.1.10 Energy intake assessment

Energy intake was assessed at two occasions. Firstly, at 180 min, via a homogenous pasta meal that was offered *ad-libitum*. Participants were requested to consume the pasta meal until comfortably full. Energy intake from the pasta meal was calculated based on the amount consumed and nutritional composition as indicated by the manufacturer. To facilitate this, research staff covertly weighed the meal prior to serving, and immediately following meal termination. Secondly, participants recorded all food and drink items consumed for the remainder of the trial day. This was completed utilising a combined weighed self-reported food record and 24 h dietary recall technique, used previously with adolescent populations (Rumbold, St Clair Gibson, Stevenson, et al., 2011). Participants were requested to give full comprehensive recordings of all food and drink items consumed, weighing all items prior to and following consumption (if leftovers were present). Information deemed necessary included methods of preparation and cooking, names of branded products and condiment use. For homemade dishes, participants were asked to record individual ingredients and quantities for the whole dish, along with a brief description of cooking method and

how much of the dish they consumed. Following each study day, research staff visited the adolescent participants separately on school premises and completed 24 h recall interviews. Interviews exercised a two-pass approach (Ashley et al., 2003), and lasted approximately 15 min per participant. Initially, participants were requested to recollect all eating episodes of the preceding day, highlighting the main food and drink items consumed. Researchers encouraged participants to elaborate on information relating to: brand names, condiment use, forgotten food or drink items, portion size and food handling. All interviews took place at the same time each day and were conducted by the same researcher. Trained research staff examined all food records utilising the nutritional software package Nutritics (Nutritics Professional v3.09, Nutritics, Ireland).

4.1.11 Electrochemiluminescence and glucose oxidase method

Quantitative assessments of GLP-$1_{7\text{-}36}$ (pg·mL), glucagon (pg·mL), leptin (pg·mL) and insulin (pmol·L) were simultaneously determined in 40 µL of plasma by electrochemiluminescence using a human hormone multiplex assay kit (Sector Imager 2400, MesoScale Discovery, Maryland, USA). To eliminate inter-assay variation, samples from each participant were analysed within the same run. Intra-assay coefficients of variation were determined by the repeated measurement of a single baseline fingertip-capillary blood sample 3 times. Average inter-assay coefficients of variation were 11%, 10%, 12% and 12% for GLP-$1_{7\text{-}36}$, glucagon, leptin and insulin, respectively.

Concentrations of blood glucose were quantified instantaneously by glucose oxidase method using an automated glucose analyser (BiosenC_line, EKF Diagnostics), based on an electro-chemical measuring principle following the conversion of β-D-glucose to gluconic acid. Prior to use, the analyser was calibrated with a solution of known concentration (12 mmol·L), provided by the manufacturer.

4.1.12 Statistical analysis

Computer software package Microsoft Excel (Microsoft, version 2010) was used for statistical analysis. For absolute data, values are presented as means and standard error of the mean ($_{SEM}$). Shapiro-Wilk tests were employed to check for normality, and data was log transformed if necessary. Mean differences ± 90% confidence intervals (CI) were used to determine differences at baseline for fingertip-capillary variables and measure of subjective appetite and are expressed relative to the control (fruit-juice) or yogurt trial (e.g. milk-yogurt, milk-control and yogurt-control). Fingertip-capillary variables and measures of subjective appetite were converted and computed into time-average AUC, using the trapezoidal rule. As the time points after the breakfast meal and mid-morning snack may influence the effect of a particular satiety-related components (e.g. hormonal, metabolic, physical or cognitive) (J. Blundell et al., 2010) the postprandial period was split into 0-90 min and 90-180 min. Data concerning postprandial time-averaged AUC estimates of fingertip-capillary variables, subjective appetite, energy intake (*ad libitum* and free-living) and metabolic variables (energy expenditure, substrate oxidation) are expressed as mean differences ± 90% confidence intervals relative to the control (fruit-juice) or yogurt trial (e.g. milk-yogurt, milk-control and yogurt-control).

4.2 Results

4.2.1 Subjective appetite profile

There were no differences in fasting subjective sensations of hunger, prospective food consumption, and satisfaction between trials. Fasting subjective fullness, however, was greater in the yogurt trial compared with the milk (-11.2 mm; 90% CI; -4.6, -17.9) and fruit-juice control (12.4 mm; 90% CI: 20.0, 4.8). Time-averaged AUC (0-90 min) subjective measures of hunger, fullness, prospective food consumption, and satisfaction (mean difference ± 90% CI) did not differ between trials (**Table 4.1**). Relative to the yogurt trial, time-averaged AUC (90-180 min) measures of subjective hunger

were greater following mid-morning milk consumption (6.3 mm; 90% CI: 0.5, 12.0, **Figure 4.1 panel A**). Measures of time-averaged AUC (90-180 min) subjective fullness were lower following mid-morning milk consumption (-6.0 mm; 90% CI: -10.2, -1.9, **Figure 4.1 panel B**) relative to the fruit-juice drink. This was also evident relative to the yogurt trial (-5.3 mm; 90% CI: -0.3, -10.3, **Figure 4.1 panel B**). Consistent with an increase and reduction in subjective hunger and fullness respectively, time-averaged AUC (90-180 min) sensations of prospective food consumption was greater following mid-morning milk consumption relative to the fruit-juice (4.9 mm; 90% CI: 2.6, 7.1, **Figure 4.1 panel C**) and yogurt trial (4.1 mm; 90% CI: 7.2, 1.1, **Figure 4.1 panel C**). No differences in time-averaged AUC (90-180 min) subjective hunger, fullness, prospective food consumption and satisfaction were present following yogurt consumption relative to fruit-juice (**Table 4.1**).

Table 4.1. Appetite-related peptides and subjective appetite time-averaged AUC, dichotomised according to 0-90 min and 90-180 min postprandial. Values are expressed as mean (SEM). * indicates a difference at the same time point relative to the fruit-juice (control), whereas ¥ indicates a difference relative to yogurt at the same time point. N = 11.

	Milk		Yogurt		Fruit-juice	
	0-90 AUC	90-180 AUC	0-90 AUC	90-180 AUC	0-90 AUC	90-180 AUC
	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)
Fingertip-capillary variables						
Plasma GLP-$1_{7\text{-}36}$ (pg·mL)	10.9	10.9	9.6	11.3*	10.3	10.1
	(1.0)	(0.8)	(0.5)	(0.7)	(0.6)	(0.6)
Plasma Glucagon (pg·mL)	79.0	95.5*	75.7	80.3	93.0	76.7
	(6.4)	(6.4)	(7.2)	(7.6)	(10.7)	(7.2)
Plasma Insulin (pmol·L)	299.9	173.2	285.4	155.2	302.3	166.9
	(39.0)	(38.3)	(29.2)	(23.3)	(29.6)	(26.9)
Plasma Leptin (pg·mL)	4888.1	4361.4	4184.2	3793.5	4903.1	4371.8
	(1943.7)	(1705.9)	(1270.3)	(1164.1)	(2310.6)	(1950.6)
Blood Glucose (mmol·L)	5.1	4.3	5.2	4.1*	5.1	4.5
	(0.1)	(0.1)	(0.2)	(0.2)	(0.2)	(0.1)
Subjective Appetite Sensations	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)
Hunger (mm)	54.8	69.5 ¥	54.6	63.3	57.4	67.4
	(4.3)	(4.2)	(3.0)	(5.9)	(4.8)	(3.1)
Fullness (mm)	35.0	20.8*¥	41.1	26.1	31.7	26.8
	(5.1)	(2.6)	(3.8)	(3.1)	(4.3)	(4.0)
Prospective Food Consumption (mm)	66.0	76.9*¥	64.5	72.8	63.4	72.1
	(3.3)	(3.5)	(3.5)	(4.0)	(4.6)	(3.2)
Satisfaction (mm)	37.4	25.3	38.9	26.3	32.3	28.2
	(4.1)	(3.5)	(2.7)	(2.4)	(3.8)	(3.4)

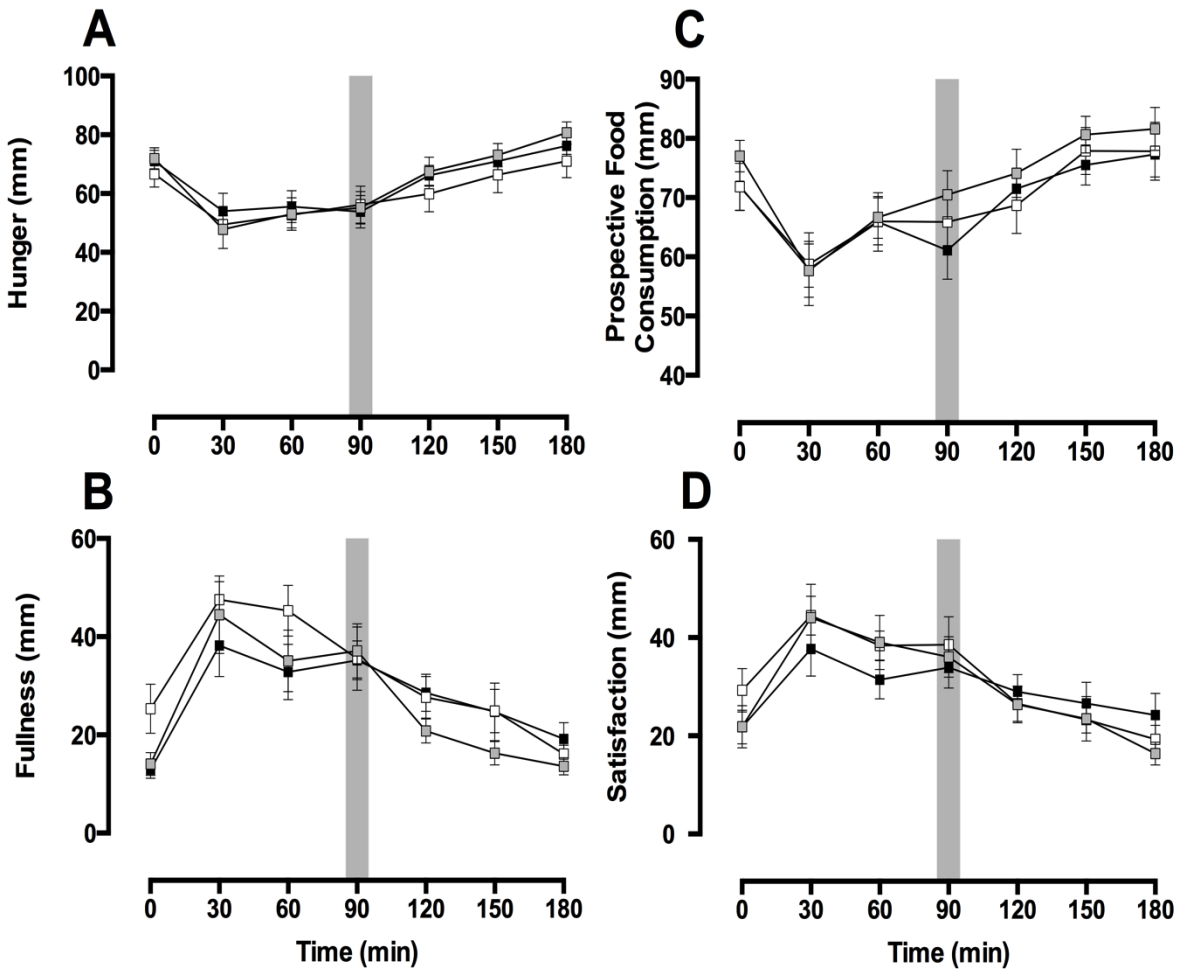

Figure 4.1 Mean ± $_{SEM}$ subjective measures of hunger (mm; panel A, n = 11), fullness (mm; panel B, n = 11), prospective food consumption (mm; Panel C, n = 11), and satisfaction (mm; Panel D, n = 11), obtained utilising validated paper-based VAS. Grey shaded boxes (-▒-) represent values obtained during the milk trial, whereas white shaded boxes (-□-) and black shaded boxes (-■-) represent concentrations obtained during the yogurt and fruit-juice trials, respectively. Snack items were distributed at 90 min, as represented by the grey shaded area.

4.2.2 Fingertip-capillary plasma variables

Baseline fingertip-capillary plasma concentrations of GLP-$1_{7\text{-}36}$, glucagon, insulin, leptin and blood glucose were comparable between trials (**Figure 4.1**). Time-averaged AUC (0-90 min) estimates of GLP-$1_{7\text{-}36}$, glucagon, insulin, leptin and blood glucose (mean difference ± 90% CI) did not differ between mid-morning milk and fruit-juice trials (**Table 4.1**), suggesting that the postprandial concentrations of these peptides were comparable following breakfast. Relative to the fruit-juice, time-averaged AUC (90-180 min) estimates of plasma glucagon displayed a greater expression following mid-morning milk consumption (16.8 pg·mL; 90% CI: 27.5, 6.1, **Figure 4.1 panel C**). Following yogurt consumption, time-averaged AUC (90-180 min) estimates of GLP-$1_{7\text{-}36}$ were increased (1.2 pg·mL; 90% CI 2.3, 0.2 **Figure 4.1 panel A**) and blood glucose lower (-0.4 mmol·L; -0.1, -0.7, **Figure 4.1 panel E**) relative to the fruit-juice.

4.2.3 Energy intake

Energy intake at the *ad libitum* pasta meal was lower following mid-morning milk consumption relative to the fruit-juice drink (-596.4 kJ: 90% CI: -105.7, -1087.1). No difference in *ad libitum* energy intake was observed following mid-morning milk consumption relative to the yogurt snack (-437.7 kJ; 90% CI: 191.4, -1066.8), or following the yogurt snack relative to the fruit-juice drink (-158.7 kJ: 90% CI: 431.7, -749.0). For free-living energy intake, no differences were observed following mid-morning milk (-815.1 kJ; 90% CI: 996.0, -2626.2) or yogurt consumption (-871.4 kJ; 90% CI: 558.3, -2301.1) relative to the fruit-juice drink. This was also evident concerning free-living energy intake following mid-morning milk consumption relative to the yogurt trial (56.3 kJ; 90% CI: 1049.7, -937.2).

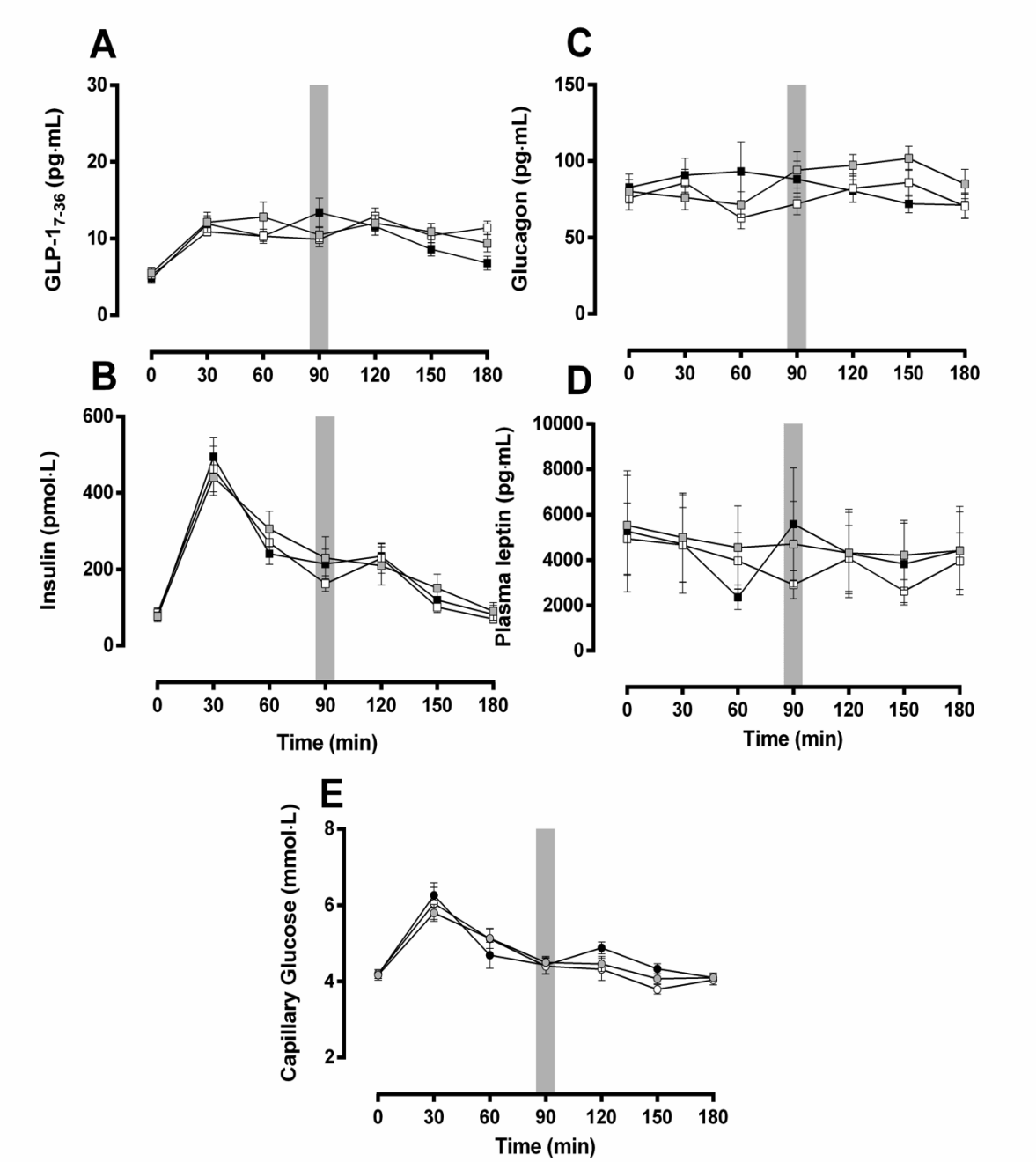

Figure 4.2 Mean ± $_{SEM}$ concentrations of plasma GLP-$1_{7\text{-}36}$ (pg·mL; panel A, n = 11), plasma insulin (pg·mL; panel B, n = 11), plasma glucagon (pmol·L; panel C, n = 11), plasma leptin (pg·mL; panel D, n = 11), and blood glucose (mmol·L; panel E, n = 11) obtained from fingertip-capillary blood samples. Grey shaded boxes (-▒-) represent values obtained during the milk trial, whereas white shaded boxes (-□-) and black shaded boxes (-■-) represent concentrations obtained during the yogurt and fruit-juice trial, respectively. To convert GLP-$1_{7\text{-}36}$ (pg·mL) and plasma glucagon (pg·mL) to their corresponding SI units multiply values by 0.298 and 0.287, respectively. Snack items were distributed at 90 min, as represented by the grey shaded area.

4.2.4 Gas analysis

Baseline energy expenditure, carbohydrate and fat oxidation were comparable between trials. There were no differences in total energy expenditure (kJ, 0-90 min), carbohydrate (g) or fat (g) oxidation between trials (**Table 4.2**). Following mid-morning milk consumption, total energy expenditure (kJ, 90-180 min) was greater relative to the fruit-juice (109.2 kJ; 90% CI: 197.2, 21.6). No differences in total energy expenditure (kJ, 90-180 min), carbohydrate or fat oxidation were present between the yogurt and fruit-juice, or between the milk relative to yogurt (**Table 4.2**).

Table 4.2. Average rates of energy expenditure and substrate metabolism, dichotomised according to 0-90 min and 90-180 min postprandial periods. Values are expressed as mean (± $_{SEM}$). * indicates a difference at the same time point relative to the fruit-juice control, whereas ¥ indicates a difference relative to yogurt at the same time point. N = 11.

	Milk		Yogurt		Fruit-juice	
	0-90 min	90-180 min	0-90 min	90-180 min	0-90 min	90-180 min
	Mean ($_{SEM}$)	Mean ($_{SEM}$)	Mean ($_{SEM}$)	Mean ($_{SEM}$)	Mean ($_{SEM}$)	Mean ($_{SEM}$)
Metabolic Variables						
Energy Expenditure (kJ)	861.6	935.1*	831.0	838.4	784.0	825.7
	(43.7)	(56.3)	(43.1)	(33.0)	(48.0)	(52.7)
Carbohydrate Oxidation (g)	11.3	9.8	17.6	16.8	8.6	7.5
	(3.8)	(3.3)	(3.3)	(4.7)	(5.1)	(4.2)
Fat Oxidation (g)	18.1	20.6	14.7	15.3	17.2	18.8
	(1.7)	(2.0)	(1.5)	(1.6)	(2.2)	(1.9)

4.3 Discussion

An emerging body of epidemiological evidence exists showing a relationship between milk-based dairy food consumption and body mass regulation in child and adolescent populations (Abreu et al., 2013; Barba et al., 2005; Moore et al., 2008). Accumulating evidence suggests that milk-based dairy foods may facilitate body mass regulation through actions on appetite and feeding behaviour (Anderson et al., 2004; Bowen et al., 2006; Luhovyy et al., 2007; Schneeman et al., 2003), yet research concerning the physiological mechanisms impacting on energy regulation among children and adolescents is sparse. The present study therefore aimed to assess the differential effects of milk-based dairy foods (milk and yogurt) on subsequent appetite, energy intake and metabolism in normal weight adolescent males relative to an isoenergetic and isovolumetric serving of fruit-juice. The observations arising from this study indicate that mid-morning milk consumption influences short-term feeding behaviour, reducing energy intake at an *ad libitum* pasta meal compared to the fruit-juice drink in adolescent males. Mid-morning milk consumption also elicited a greater postprandial (90-180 min) plasma glucagon response and led to an increased rate of energy expenditure compared to the fruit-juice drink. In addition, mid-morning yogurt consumption elicited a greater postprandial plasma GLP-$1_{7\text{-}36}$ response and attenuated blood glucose relative to the fruit-juice drink. Relative to the yogurt trial, time-averaged AUC (90-180 min) measures of subjective hunger were greater following mid-morning milk consumption. Measures of time-averaged AUC (90-180 min) subjective fullness were lower following mid-morning milk consumption relative to the fruit-juice drink. This was also evident relative to the yogurt trial. Consistent with an increase and reduction in subjective hunger and fullness respectively, time-averaged AUC (90-180 min) sensations of prospective food consumption were greater following mid-morning milk consumption relative to the fruit-juice and yogurt trial.

A key observation arising from the present study was that mid-morning milk consumption resulted in a reduction of energy intake at the *ad libitum* pasta meal relative to the fruit-juice drink.

This was, however, short-lived and did not continue into the free-living environment. This finding corresponds with previous observations in children and adolescents (Birch et al., 1993; Mehrabani et al., 2014; Vien et al., 2014; Zandstra et al., 2000), and contributes further to the understanding that milk-based dairy foods exert the potential to influence feeding behaviour in an acute setting. The mean reduction in energy intake between the milk and fruit-juice trial was -596.4 kJ, equating to -142.5 kcal, and is larger than previously reported (Birch et al., 1993; Mehrabani et al., 2014). In this sense, the consumption of low-fat milk with breakfast (Mehrabani et al., 2014) or ice cream (Birch et al., 1993) early morning (0930 h) reduced energy intake at an *ad libitum* assessment (120-300 min after dairy consumption) by approximately 250 kJ (in both investigations) relative to control trials. Nonetheless, methodological disparities between these and the present study may explain differences in feeding behaviour. With specific regard to the difference found in this study, Wang and colleagues (2006) suggest sustained reductions in daily energy intake averaging 110 to 165 kcal (460 to 690 kJ) may offer an effective approach for preventing excess weight accumulation in children and adolescents. Thus the finding that milk consumption imparts a metabolic advantage by favourably impacting on feeding behaviour relative to a control fruit-juice drink may convey a possible application for appetite control and food intake in overweight and obese persons. It would, however, be prudent for future investigations to assess whether milk-based dairy foods actually provide application to overweight and obese populations.

While there is formative evidence to suggest that milk-based dairy consumption influences feeding behaviour acutely among children and adolescents, the present study provides some mechanistic evidence that may have contributed to these observations. There are numerous ways milk consumption may have affected feeding behaviour relative to the fruit-juice drink in the present study. For example, it has been postulated that alterations in appetite- and metabolism-related peptide expression and metabolic responses (e.g. energy expenditure) may influence appetite, feeding behaviour and metabolism. In the majority of the aforementioned paediatric

studies, however, no quantitative measures of subjective appetite and/or appetite- and metabolism-related peptides were included which may have provided valuable insights concerning the mechanisms impacting on appetite and feeding behaviour. The acute effect of milk-based dairy food consumption on measures of subjective appetite and appetite-related peptides has received little attention in children and adolescents. Only one investigation (comprising two experiments) has sought to establish the effect of dairy food consumption on appetite and feeding behaviour in children (9-14 y), where measures of subjective appetite and appetite-related peptides were quantified and the results are ambivalent (Vien et al., 2014). As previously alluded to this study comprised two experiments, the first experiment included measures of subjective appetite, whereas the second experiment included measures subjective appetite together with appetite-related peptides (serum glucose, insulin and plasma GLP-1 and peptide YY). In the first experiment, Vein et al. (2014) illustrated reduced energy intake (amount not given) at a pizza meal ($p < 0.01$) offered 60 min following chocolate milk and yogurt consumption compared to a water drink. Consistent with a reduction in energy intake, measures of subjective appetite were significantly lower following 2% fat milk consumption compared with the yogurt drink only ($p < 0.01$). No additional effects were observed concerning energy intake following the consumption of 2% fat milk and fruit punch or on subjective measures of appetite after 1% fat chocolate milk, 1.5% fat yogurt drink, fruit punch or water. In the second experiment, identical procedures were followed, however antecubital-venous concentrations of serum glucose and insulin (baseline, 30, 60, 85, 115 and 145 min after consumption of the pre-meal preload) and plasma GLP-1 and peptide YY (baseline, 60 and 115 min after consumption of the pre-meal preload) were collected. Compared with the fruit punch preload, milk consumption resulted in a significantly greater GLP-1 AUC ($p < 0.03$). Nonetheless, *ad libitum* energy intake, insulin and glucose AUC were comparable between trials (Vien et al., 2014).

To some degree the results of the present study corroborate with the findings of the abovementioned study, considering we observed a reduction in energy intake at the *ad libitum* pasta

meal following milk consumption. Further, relative to the fruit-juice, time-averaged AUC (90-180 min) estimates of plasma GLP-$1_{7\text{-}36}$ displayed a significantly greater expression and blood glucose a significantly lower expression following yogurt intake. In contrast, we observed no capacity of milk to impact on concentrations of GLP-$1_{7\text{-}36}$, insulin, leptin and blood glucose relative to the yogurt or fruit-juice drink. Milk consumption did, however, stimulate the release of plasma glucagon. Furthermore, and somewhat unexpectedly, mid-morning milk-consumption appeared to have an opposite effect on subjective perceptions of appetite compared with energy intake at the *ad libitum* pasta meal, with participants demonstrating decreased fullness and increased hunger and prospective food consumption relative to the yogurt and fruit-juice trials. The implementation of VAS to quantify sensations of hunger, satiation and satiety have been employed in studies concerning older children and adolescents across a variety of experimental conditions (Bellissimo et al., 2007; Rumbold, St Clair Gibson, Allsop, et al., 2011; Rumbold et al., 2013; Thivel et al., 2012). Tracking changes in subjective appetite profiles over time provides important information in relation to the structure of the effects of feeding events, for instance the effect of diet composition on feeding behaviour or the effects of physiological variables on the appetite control system. However, alterations in subjective perceptions of food-related emotions do not always translate and reflect actual feeding behaviour (Thivel et al., 2012; Thivel et al., 2011). It is therefore reasonable to suggest that alterations in appetite- and metabolism-related peptides and metabolic responses may supersede subjective perceptions of food-related emotions to influence appetite, feeding behaviour and metabolism. Indeed this may be the case in the present study. This is certainly an area where further research is required and the identification that fingertip-capillary blood sampling offers and appropriate methodological and reproducible approach to systematically quantify appetite- and metabolism-related peptides (chapter two) provides a platform to aid this.

From a physiological perspective, accumulating evidence has confirmed the control of appetite and feeding behaviour to be heavily, yet not exclusively, governed by multiple hormonal

peptides of gastrointestinal, pancreatic and adipose tissue origin. In humans, hormonal peptides including GLP-1 (Verdich et al., 2001), glucagon (Chan et al., 1984), insulin (Air et al., 2002) and leptin (Schwartz et al., 2000), among others, act to influence the physiological mechanisms controlling energy intake and expenditure, and are produced and secreted into the circulation in response to the ingestion of a meal. The postprandial response of these peptides is profoundly influenced by ingested macro- (and micro-) nutrient composition, and also energy content. The appearance of GLP-$1_{7\text{-}36}$, for example, rises immediately following meal consumption in proportion to energy content (Huda et al., 2006), but also when carbohydrates and lipids are present (Layer, Juul Holst, Grandt, & Goebell, 1995). Glucagon concentrations are more variable, but increase with fasting and protein ingestion (Acheson et al., 2011). In the present study, the experimental preloads were isoenergetic (427 kJ) and isovolumetric (217 mL) yet differed according to macro-nutrient composition (**Table 4.0**). It may therefore be unsurprising that mid-morning milk consumption resulted in a greater plasma glucagon AUC response considering its macronutrient composition relative to the fruit-juice control. Glucagon has been shown to potently increase satiety and acutely reduce food intake in humans (Flint et al., 2007; Parker et al., 2013; Woods et al., 2006). Additionally, there is evidence suggesting that glucagon exerts properties that may stimulate energy expenditure and thermogenesis (Heppner et al., 2010; Marroquí et al., 2014). Consistent with this, milk consumption increased energy expenditure in the present study and is in agreement with adolescent literature (Apolzan et al., 2006). Apolzan et al. (2006) evaluated energy expenditure (kcal·min) for 240 min after a low calcium non-dairy control, supplemental calcium or a dairy-based product in overweight adolescent male and females. They observed a greater rate of energy expenditure following the consumption of the dairy-based product compared with the low calcium non-dairy control, but only in adolescent males. No differences were recorded following supplemental calcium ingestion, which may suggest additional constituents housed within milk-based dairy foods act to impact on metabolism. It is also well established, for example, that protein elicits a greater effect on diet induced thermogenesis (20-35% of energy consumed) compared to

calorie matched intakes of carbohydrate (5-15% of energy consumed) or fat (0-3% of energy consumed) (Halton et al., 2004) albeit in adults. Thus, considering the higher protein content and time-averaged AUC glucagon response following the milk drink (compared with yogurt and fruit-juice), a reduction of energy intake and an elevated rate of energy expenditure may not be surprising.

Considered together, the findings presented throughout this study indicate that mid-morning milk consumption influences short-term feeding behaviour, suppressing energy intake at the *ad libitum* pasta meal, but did not impact on energy intake for the remaining hours of the study day. Alongside a reduction in feeding behaviour, the consumption of milk stimulated postprandial energy expenditure compared with servings or fruit-juice and yogurt. It is reasonable to suggest this was facilitated by means of an increased postprandial glucagon expression. The consumption of milk stimulated postprandial glucagon expression and energy expenditure compared with an isoenergetic and isovolumetric serving of fruit-juice and yogurt. Yogurt consumption stimulated the secretion of GLP-$1_{7\text{-}36}$ and attenuated blood glucose, however, we observed no capacity for this to influence appetite, feeding behaviour or metabolism. Caution should be observed when extrapolating the results of this study, as the effect of dairy snack consumption was only examined in an acute setting (24 h). Consequently, the observations may not be applicable to longer durations. Further investigations might explore the longer-term implications of dairy food consumption for the regulation of food intake and control of body mass. In particular, the measurement of appetite-related peptides would be advantageous over longer time periods, providing valuable insights concerning the mechanisms impacting on appetite and feeding behaviour.

CHAPTER FIVE

MODERATE-TERM MILK CONSUMPTION IN ADOLESCENT MALES: IMPACT ON APPETITE, FEEDING BEHAVIOUR AND METABOLISM

5.0 Introduction

It is well established that feeding behaviour is a fundamental component in the development of adiposity such that the onset of weight gain is primarily driven by a caloric imbalance (Moinuddin et al., 2012). This may be facilitated following an increase in energy intake, decreased energy expenditure, or certainly a combination of both (Moinuddin et al., 2012). While numerous aspects of the diet have been implicated in the development of obesity, this relationship is complex and poorly understood. One potential dietary behaviour frequently cited as a potential avenue within the field of weight management and/or weight loss is to consume foodstuff that offer a metabolic advantage (Hollis & Mattes, 2005). Such advantages may transpire if the foodstuff raises postprandial metabolism, is improperly absorbed and digested or attenuates appetite (subjectively and objectively) and feeding behaviour at the next available opportunity (Hollis & Mattes, 2005). It is relevant to note that milk-based foodstuff purportedly convey a metabolic advantage as illustrated in chapter four of this thesis, reducing energy intake at the *ad libitum* pasta meal. This study provided evidence that mid-morning milk consumption influenced short-term feeding behaviour in adolescent males (15-18 y), suppressing energy intake at the *ad libitum* pasta meal, but did not impact on energy intake for the remaining hours of the study day. Coinciding with a reduction in feeding behaviour, the consumption of milk stimulated postprandial energy expenditure compared with an isoenergetic and isovolumetric serving of fruit-juice and yogurt. It is reasonable to suggest these observations were modulated following the increased postprandial secretion of plasma glucagon. Secondly, the consumption of yogurt stimulated postprandial GLP-$1_{7\text{-}36}$ and attenuated concentrations of blood glucose. Irrespective of this, we observed no capacity for yogurt to influence measures of subjective appetite, feeding behaviour or metabolism. Taken together, milk appears to convey a metabolic advantage and impacts favourably on appetite, feeding behaviour and metabolism among adolescents in an acute setting.

Efforts to establish the underlying relationship between milk-based dairy foods and adiposity have highlighted several plausible mechanisms. As previously introduced throughout

chapter four, one proposed mechanism of action through which milk-based dairy foods may protect against obesity is through favourable actions on appetite and feeding behaviour (Aziz et al., 2007). Indeed, it appears that acute consumption of milk-based dairy foods in children and adolescents is associated with reduced energy intake (Birch et al., 1993; Mehrabani et al., 2014; Vien et al., 2014; Zandstra et al., 2000), increased concentrations of appetite- and metabolism-related peptides (Vien et al., 2014) and increased postprandial metabolism (Apolzan et al., 2006). To date, only one study has evaluated moderate-term (3-week) calcium carbonate ($CaCO_3$) or dairy calcium consumption on modulation of energy metabolism in overweight adolescent boys (13-15 y) and girls (12-14 y) (C. M. Weaver et al., 2011). Exercised in a randomised crossover fashion, adolescents participated in two 3-week metabolic sessions and received 650 mg/d (control) or 1300 mg/d (one half of participants achieved this through $CaCO_3$, and the other through dairy foods. During the supplementation periods participants followed a strictly controlled diet, and observations for energy expenditure, substrate oxidation and energy balance were conducted. The experimental data from the cited study demonstrated no capacity of $CaCO_3$ or dairy calcium to alter energy balance under controlled conditions relative to baseline observations (Weaver et al., 2011).

Considering the possible mechanisms of milk consumption on appetite, feeding behaviour and metabolism, no studies have explored the impact of moderate-term dairy consumption on both appetite regulation and feeding behaviour in adolescents. In overweight and obese adults, during energy-restricted weight-loss interventions, a dietary pattern high in dairy and calcium for 3-months (Jones et al., 2013) or supplementation with milk for 6-months relative to an isoenergetic placebo (Gilbert et al., 2011) reduced feeding behaviour and attenuated appetite regulation. Both interventions resulted in similar weight loss, however, milk-based dairy food consumption attenuated the orexigenic effect of weight loss, brought about by suppressed subjective sensations of appetite (e.g. hunger) and increases in plasma GLP-1 and PYY (Gilbert et al., 2011; Jones et al., 2013). Taken together, supplementation with milk appears to favourably impact on appetite, appetite- and metabolism-related peptide expression and feeding behaviour. Nonetheless, it remains

difficult to comment on the potential impact of milk supplementation on energy regulation in adolescents. In this context, understanding the relationship between snacking, feeding behaviour and appetite regulation is of great importance, providing insight concerning the potential differential effects of snack items on appetite, feeding behaviour and metabolism in adolescent populations. Accordingly, the current study aimed to compare the effect of daily milk or fruit-juice supplementation as a mid-morning snack for 4-weeks on appetite, feeding behaviour and metabolism in free-living adolescents. A secondary aim of this study was to evaluate the impact of milk or fruit-juice supplementation on measures of body mass, body composition and blood lipids.

5.1 Materials and methods

5.1.1 Study design

This study employed a parallel design with two intervention groups. Participants were randomly allocated to groups, and received either a daily mid-morning milk drink (n = 10) or an isocaloric and isovolumetric fruit-juice drink (n = 9) for 28 days (4-weeks). Participants were matched according for age, body mass, BMI and habitual calcium intake. In this sense, age, body mass, BMI and habitual calcium intake for those allocated to the milk drink were: mean ± $_{(SD)}$ 16.0 (1.1) y, 69.4 (17.3) kg, 22.0 (4.8) kgm^2 and 773.3 (171.2) mg·d respectively, and 16.2 (0.8) y, 68.2 (9.8) kg, 21.6 (2.4) kg/m^2 and 808.9 (268.9) mg·d for the fruit-juice group, respectively.

5.1.2 Study population

In total, 19 male adolescents participated in the study. Participants were recruited from a local secondary school in the North-East of England, after attendance at an initial advertisement seminar. For the purpose of this study, sample size assessment was computed based on previous observations that mid-morning milk consumption elicits a 16.8 pg·mL greater plasma glucagon response relative to a serving of fruit-juice (chapter two), and presents a between-day typical error of 8.2% (B. P.

Green et al., 2014). Consequently, it was estimated that 18 participants (nine per group) would provide > 80% chance of statistically detecting a difference with $p < 0.05$. The study was conducted according to the guidelines laid down in the 2013 Declaration of Helsinki (WMA, 2013), and all procedures involving human participants were approved by the Faculty of Health and Life Sciences Ethics Committee of the University of Northumbria. Adolescent ascent and written informed parental consent was obtained from all participants prior to data collection.

5.1.3 Familiarisation

Approximately 1 week preceding the start of the supplementation periods, all participants attended the clinical testing laboratories for an orientation visit. The aims of the orientation visit were threefold. Firstly, participants were habituated with the equipment and methodological procedures that were intended to be employed in the study. In this sense, participants were familiarised with the blood sampling equipment, gas collection techniques, subjective appetite scales and test foods that were employed throughout. Secondly, as recommended by Livingstone et al. (1992), participants were trained and educated on the procedures required to appropriately document free-living feeding behaviour. Finally, rates of habitual calcium intakes were estimated through the use of a validated food frequency questionnaire for determining calcium and vitamin D intake in adolescents (C. Taylor et al., 2009).

5.1.4 Study protocol

During the supplementation periods, participants were instructed to maintain their usual feeding behaviour and physical activity patterns. On day 0 (baseline) and 28 (endpoint) of the intervention period, participants attended study days at the clinical testing laboratory where measures of appetite, feeding behaviour and metabolism were collected. In addition, measures of anthropometry and blood lipid profile were also determined. For the 24 h preceding each participant's initial visit

food and fluid consumption was recorded using self-reported, weighed food diaries. Participants were requested to replicate these dietary behaviours for the 24 h before their final visit. To facilitate dietary replication, photocopies of the self-reported weighed food diaries were distributed to each participant. Participants refrained from caffeine and alcohol consumption (≥ 12 h) and avoided any form of strenuous activity (≥ 24 h) before data collection. Upon waking and until arrival at the clinical testing laboratory the consumption of water was only permitted. Participants were requested to record, document and replicate morning water consumption (if any) for subsequent study days. The protocol implemented during study days was identical to that reported in chapter four (**Section 4.1.5**).

Briefly, on study days participants reported to the clinical testing laboratory at 0830 h, after a 12 h overnight fast. Within 30 min of arrival, participants provided a baseline fingertip-capillary blood sample, expired gas sample (300 sec resting sample) and completed a series of VAS. Following the completion of baseline measurements (t = 0), participants were provided with a standardised cereal and milk breakfast. Participants remained in the laboratory for 180 min. This period started upon the first mouthful of the breakfast meal. Further samples of fingertip-capillary blood and VAS were collected at 30, 60, and 90 min before semi-skimmed milk or fruit-juice consumption, and again at 30, 60 and 90 min (120, 150 and 180 min) following this. The intervals between test meals were selected to be representative of a typical school day. Expired gas samples (300 sec) were collected at 25-30, 55-60, 85-90, 115-120, 145-150, and 175-180 min throughout the experimental period. Additional food and fluid consumption was prohibited until trial termination, apart from water that was offered *ad libitum*. In trial *ad libitum* fluid consumption (if any) was documented and matched during subsequent trials. At 180 min, a homogenous *ad libitum* pasta meal was provided. Throughout test periods, participants remained sedentary in an environment free from food cues. All testing took place during school term-time. On completion of the *ad libitum* pasta meal, participants were returned to the school campus. For the remainder of the study day, participants recorded any additional food and drink items consumed utilising a combined weighed

self-reported food record and 24 h dietary recall technique, used previously with adolescent populations (Rumbold, St Clair Gibson, Stevenson, et al., 2011). Following each study day, research staff visited the adolescent participants separately on school premises and completed 24 h recall interviews. The reader is directed to **Figure 4.0** within chapter four (**Section 4.1.5**) whereby a schematic representation of the study day protocol is presented.

5.1.5 Anthropometry

Measures of anthropometry (body mass, stature, body composition) were determined on day 0 and 28 of the intervention period. Measures of body mass were determined to the nearest 0.1 kg, using portable scales (Seca, Birmingham, UK) shoeless and wearing lightweight clothing. Stature and seated height were measured to the nearest 0.01 m using a portable stadiometer (Holtain Ltd, UK). From these parameters a measure of BMI (kg/m^2) was computed. Body densitometry was determined through whole body air displacement plethysmography (Bod Pod; Life Measurement, Inc, Concord, CA), and converted to percentage body fat according to age-specific equations (Lohman, 1986).

5.1.6 Supplementation

A serving of semi-skimmed milk (Tesco, UK) or fruit-juice (Pure orange juice smooth; control beverage, Tesco, UK), was provided daily to participants. Supplements were matched for energy content (427 kJ) and volume (217 mL), yet differed according to macro-nutrient composition. The energy content and volume of the preloads was previously utilised in chapter four of this thesis and was sufficient to favourably impact on feeding behaviour and appetite- and metabolism-related peptide expression. An overview of the compositional make-up is given in chapter four (**Table 4.0, section 4.1.9.2**). A member of the research team visited the school campus daily, and distributed the supplements between 11:00-11:15 h. This time represented the students break time at school.

Supplements were provided in identical 250 mL clear bottles. Participants were registered on a daily basis to monitor compliance, and supplements were consumed in the presence of the visiting researcher (during weekdays only). For weekend periods, participants were provided with the supplements to consume once daily between 11:00-11:15 h. Supplements were again provided in identical 250 mL clear bottles. Participants were requested to return empty bottles. Compliance with weekend consumption was evaluated by returned items to the study staff.

5.1.7 Subjective appetite profile

Subjective measures of appetite (hunger, gut fullness, satisfaction and prospective food consumption) were assessed using validated (A. Flint et al., 2000) 100 mm, paper based, VAS as described in chapter four (**Section 4.1.5**).

5.1.8 Fingertip-capillary blood sampling

Fingertip-capillary blood samples (0.3 mL) were obtained from a pre-warmed fingertip pierced with a sterile automated lancet (Accu-Check, Mannhein, Germany) for the determination of plasma GLP-$1_{7\text{-}36}$, glucagon, insulin, leptin. Additional fingertip-capillary (0.02 mL) blood samples for concentrations of blood glucose were drawn into sodium heparinised capillary tubes and transferred into eppendorfs containing 1 mL haemolysis solution (EKF Diagnostics). Briefly, samples were collected at baseline (t = 0 min) and at 30, 60, 90, 120, 150 and 180 min following breakfast consumption. At baseline, an additional fingertip-capillary blood sample (0.3 mL) was obtained for the determination of total cholesterol, low-density lipoprotein, high-density lipoprotein and triglyceride concentrations. Methodological approaches concerning pre-analytical (specimen collection, sample treatment) and analytical procedures (sample handling) were followed as described in chapter four (**section 4.1.7**).

5.1.9 Gas analysis

Rates of substrate metabolism and energy expenditure (kJ) were estimated according to the procedures implemented in chapter four (**Section 4.1.7**). Briefly, expired gas samples (300 sec) were collected at 25-30, 55-60, 85-90, 115-120, 145-150, and 175-180 min.

5.1.10 Test meals

5.1.10.1 Breakfast meal

Following baseline measures, participants consumed a standardised breakfast meal consisting of semi-skimmed milk (Sainsbury, UK) and Kellogg's Rice Krispies (Kelloggs, Manchester, UK). The quantity issued was estimated as described in chapter four (**Section 4.1.8.1**).

5.1.10.2 Mid-morning snack

Ninety min following breakfast consumption, participants were issued with the mid-morning snack item they had been assigned to. Snack items consisted of milk (< 2% fat, Tesco, UK) or fruit-juice (Pure orange juice smooth; control beverage, Tesco, UK). As implemented in chapter four, snack items were matched for energy content (427 kJ) and volume (217 mL). Snack items, however, differed according to macro-nutrient composition (**chapter four, Table 4.0**). All packaging labels were removed and snack items served in appropriate serving containers.

5.1.10.3 *Ad-libitum* pasta meal

Lunchtime food intake was evaluated at 180 min, as described in Chapter four (**Section 4.1.8.3**). Briefly, the homogenous pasta meal comprised of pasta (Tesco, UK), tomato sauce (Tesco, UK), cheddar cheese (Tesco, UK) and olive oil (Tesco, UK) and provided 859 kJ of energy per 100 g of food (205 kcal; energy contributions 14% protein, 52% carbohydrate and 34% fat). The test lunch

was distributed 90 min following snack consumption (180 min following breakfast) and offered ad-libitum. Detailed information concerning the nutrient composition of the pasta meal and on the method of cooking has been reported in previous studies (Gonzalez et al., 2015), and a similar pasta meals have been used successfully in adolescent populations (Rumbold et al., 2013).

5.1.11 Energy intake assessment

Energy intake was assessed at two occassions. Firstly, at 180 min, via a homogenous pasta meal that was offered *ad-libitum*. Secondly, participants recorded all food and drink items consumed for the remainder of the study day. This was completed utilising a combined weighed self-reported food record and 24 h dietary recall technique, used previously with adolescent populations (Rumbold, St Clair Gibson, Stevenson, et al., 2011). Methodological procedures to estimate energy intake from the pasta meal and on leaving the laboratory environment were followed as described in chapter four (**Section 4.1.9**).

5.1.12 Electrochemiluminescence, reflectance photometry and glucose oxidase method

Quantitative assessments of GLP-$1_{7\text{-}36}$ (pg·mL), glucagon (pg·mL), leptin (pg·mL) and insulin (pmol·L) were simultaneously determined in 40 µL of plasma by electrochemiluminescence using a human hormone multiplex assay kit (Sector Imager 2400, MesoScale Discovery, Maryland, USA). To eliminate inter-assay variation, samples from each participant were analysed within the same run. Intra-assay coefficients of variation were determined by the repeated measurement of a single baseline fingertip-capillary blood sample three times. Average inter-assay coefficients of variation were 9.7%, 8.2%, 6.4% and 8.1% for GLP-$1_{7\text{-}36}$, glucagon, leptin and insulin, respectively. For the determination of total cholesterol, low-density lipoprotein, high-density lipoprotein and triglyceride concentrations, 30 µL of whole blood was applied to specific test strips (Reflotron® HDL

Cholesterol; Reflotron® total Cholesterol; Reflotron® Triglyceride) using an automated pipette and analysed immediately using reflectance photometry (Reflotron® Plus Sprint System, Roche Diagnostics, F. Hoffman-La, Roche Ltd., Switzerland).

Concentrations of blood glucose were quantified instantaneously by glucose oxidase method using an automated glucose analyser (BiosenC_line, EKF Diagnostics), based on an electro-chemical measuring principle following the conversion of β-D-glucose to gluconic acid. Prior to use, the analyser was calibrated with a solution of known concentration (12 mmol·L), provided by the manufacturer.

5.1.13 Statistical analysis

Computer software package Microsoft Excel (Microsoft, version 2010) was used for statistical analysis. For absolute data, values are presented as means and standard error of the mean ($_{SEM}$). To investigate the effect of daily mid-morning milk or fruit-juice supplementation on appetite, feeding behaviour, metabolism and body mass, values obtained during baseline (day 0) study days were compared with endpoint (day 28) study day values. Differences concerning baseline and endpoint fasting fingertip-capillary variables, subjective appetite, energy intake (*ad libitum* and free-living) and metabolic variables (energy expenditure, substrate oxidation) are expressed as mean differences ± 90% confidence intervals relative to baseline observations. Fingertip-capillary variables and measures of subjective appetite were converted and computed into time-average area under the curve (AUC), using the trapezoidal rule. As the time points after breakfast meal and mid-morning snack consumption may influence the effect of a particular satiety-related component (hormonal, metabolic, physical or cognitive) (Blundell et al., 2010) the postprandial period was split into 0-90 min and 90-180 min. Data concerning postprandial time-averaged AUC estimates of fingertip-capillary variables, subjective appetite, and metabolic variables are expressed as mean differences ± 90% confidence intervals relative to baseline observations.

5.2 Results

Nineteen participants were screened and entered the supplementation intervention, however, one participant from each group failed to attend the endpoint study day. Consequently, data are presented for 17 participants [milk drink (n = 9) fruit-juice drink (n = 8)]. Measures of anthropometry (body mass, BMI, and body composition), and markers of blood lipid profile (cholesterol and triglycerides) determined at baseline (day 0) and endpoint (day 28) are presented in **Table 5.0**. Relative to baseline observations, measures of body mass, BMI, body composition and blood lipid profile were comparable following fruit-juice supplementation. This was also evident following milk supplementation with regard to body mass, BMI and blood lipid profile, however, measures concerning body composition (although percentage body fat remained comparable), in particular lean weight (-0.9 kg; 90% CI: -0.1, -1.6) increased and fat weight (0.9 kg; 90% CI: 1.7, 0.1) decreased.

Table 5.0 Baseline and endpoint participant characteristics and blood lipid profiles Values are expressed as mean (± $_{SEM}$). * indicates a difference at endpoint relative to baseline observations.

	Milk (n = 9)			Fruit-juice (n = 8)		
	Baseline	Endpoint	Difference	Baseline	Endpoint	Difference
	Mean ($_{SEM}$)	Mean ($_{SEM}$)	Mean $_{(90\% CI)}$	Mean ($_{SEM}$)	Mean ($_{SEM}$)	Mean $_{(90\% CI)}$
Participant Characteristics						
Body mass (kg)	69.4	69.5	-0.1	68.3	68.4	-0.2
	(6.1)	(6.2)	(0.4, -0.7)	(3.7)	(3.8)	(0.2, -0.5)
BMI ($kg \cdot m^2$)	22.0	22.1	0.0	21.6	21.8	-0.2
	(1.7)	(1.7)	(0.2, -0.2)	(0.9)	(1.0)	(0.0, -0.5)
Body fat (%)	15.5	15.1	0.3	9.3	9.8	-0.5
	(4.1)	(3.7)	(1.7, -1.0)	(2.1)	(1.7)	(0.9, -1.9)
Lean weight (kg)	56.1	57.0	-0.9*	58.1	58.5	-0.4
	(2.1)	(2.4)	(-0.1, -1.6)	(1.8)	(1.7)	(0.4, -1.2)
Fat weight (kg)	13.8	12.9	0.9*	9.6	9.9	-0.3
	(5.1)	(4.9)	(1.7, 0.1)	(2.6)	(2.6)	(0.6, -1.3)
Blood lipid profile						
Low-Density Lipoprotein (mmol)	1.7	1.8	-0.1	2.0	1.9	0.1
	(0.1)	(0.1)	(0.0, -0.2)	(0.2)	(0.1)	(0.1, 0.0)
High-Density Lipoprotein (mmol)	0.8	0.7	0.1	0.9	0.9	0.0
	(0.1)	(0.1)	(0.2, 0.0)	(0.1)	(0.1)	(0.0, 0.0)
Total cholesterol (mmol)	2.7	2.7	0.0	3.0	3.0	0.1
	(0.1)	(0.1)	(0.1, -0.1)	(0.2)	(0.2)	(0.1, 0.0)
Triglyceride (mmol)	0.9	1.0	-0.1	0.9	0.9	0.0
	(0.0)	(0.1)	(0.1, -0.2)	(0.1)	(0.1)	(0.0, -0.1)

5.2.1 Subjective appetite profile

Relative to baseline (week 0) observations, fasting measures of subjective appetite (hunger, fullness, prospective food consumption and satisfaction) did not differ following fruit-juice consumption. A similar pattern emerged among the milk group, however, measures of fasting prospective food consumption were greater during endpoint observations (-16.2 mm; 90% CI: -5.2, -27.2). Time-averaged AUC (0-90 min) subjective measures of hunger, fullness, prospective food consumption, and satisfaction (mean difference ± 90% CI) did not differ relative to the baseline (week 0) observations between supplement groups (**Figures 5.0 & 5.1, Table 5.1**). Furthermore, no differences in time-averaged AUC (90-180 min) subjective hunger, fullness, prospective food consumption and satisfaction were observed following fruit-juice supplementation, relative to baseline (week 0) observations (**Figures 5.0 & 5.1, Table 5.1**). This was also evident for the milk group, however, time-averaged AUC (90-180 min) subjective measures of prospective food consumption were greater following mid-morning milk supplementation (-4.2 mm; 90% CI: -0.4, -8.0, **Figure 5.1, panel D**).

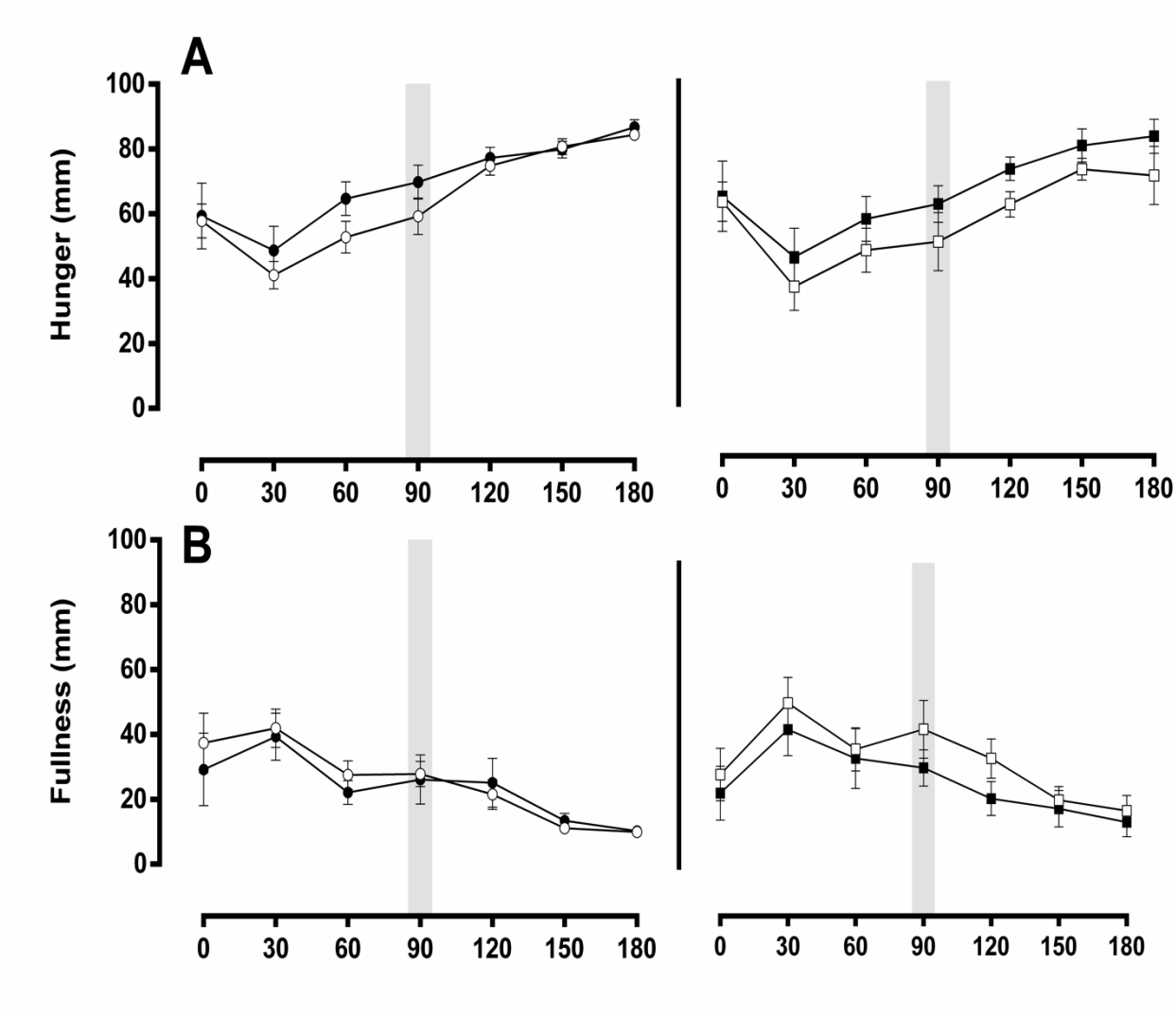

Figure 5.0 Mean ± $_{SEM}$ subjective measures of hunger (mm; panel A) and fullness (mm; panel B), obtained utilising validated paper-based VAS. Graphs depicted on the left are from the milk group (n = 9) whereas graphs depicted on the right are from the fruit-juice group (n = 8). For the left sided graphs, white shaded circles (-○-) represent values obtained during baseline observations, whereas black shaded circles (-●-) represent values obtained during endpoint observations. For the right sided graphs, white shaded boxes (-□-) represent values obtained during baseline observations, whereas black shaded boxes (-■-) represent values obtained during endpoint observations. Snack items were distributed at 90 min, as represented by the grey shaded area.

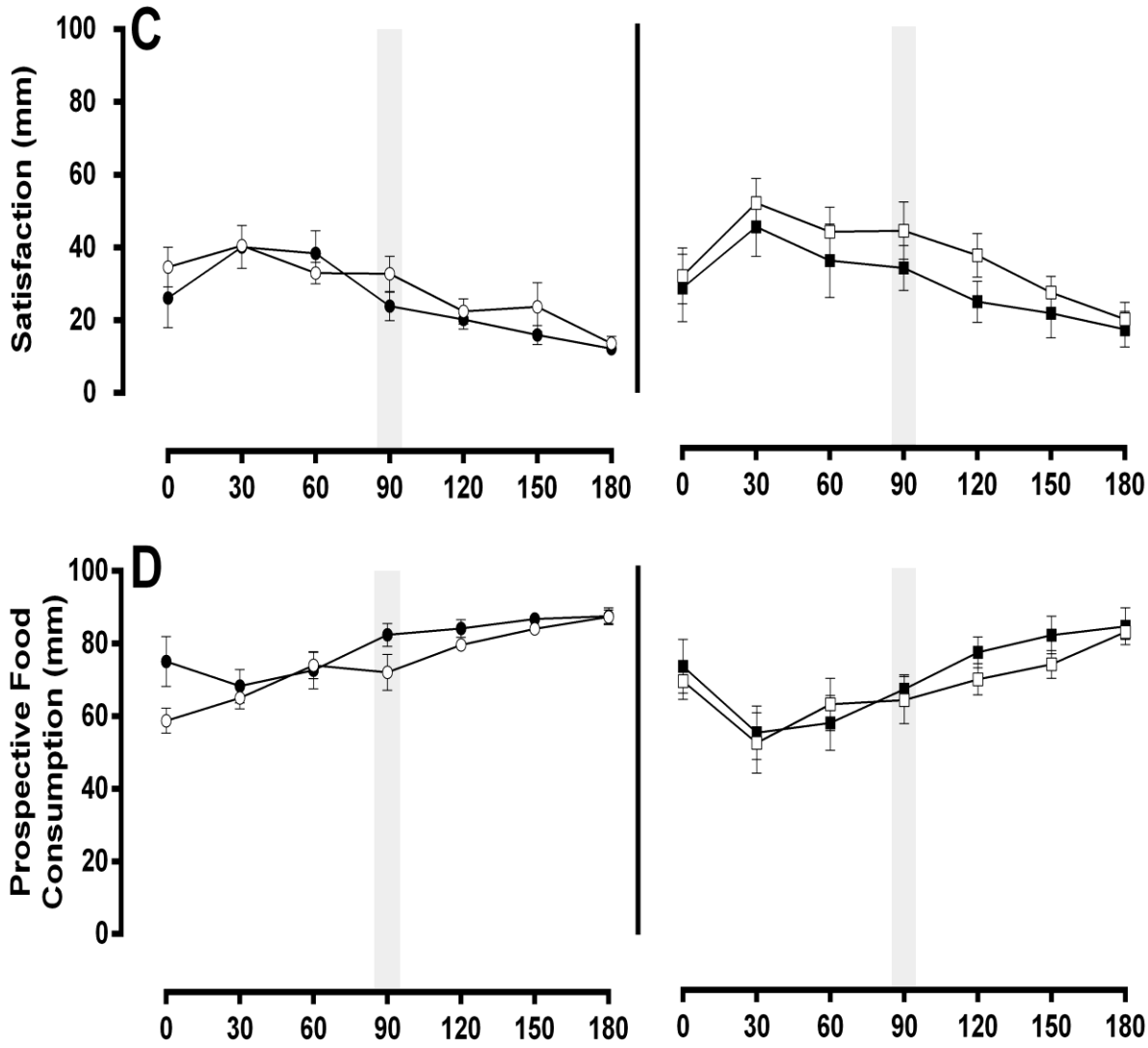

Figure 5.1 Mean ± $_{SEM}$ subjective measures of satisfaction (mm; Panel C) and prospective food consumption (mm; Panel D), obtained utilising validated paper-based VAS. Graphs depicted on the left are from the milk group (n = 9) whereas graphs depicted on the right are from the fruit-juice group (n = 8). For the left sided graphs, white shaded circles (-○-) represent values obtained during baseline observations, whereas black shaded circles (-●-) represent values obtained during endpoint observations. For the right sided graphs, white shaded boxes (-□-) represent values obtained during baseline observations, whereas black shaded boxes (-■-) represent values obtained during endpoint observations. Snack items were distributed at 90 min, as represented by the grey shaded area.

Table 5.1 Baseline and endpoint 0-90 min time-averaged AUC fingertip-capillary variables and subjective appetite. Values are expressed as mean ± $_{SEM}$, and mean difference with 90% confidence intervals relative to baseline observations. * indicates a difference relative to baseline observations in the milk supplementation group. ¥ indicates a difference relative to baseline observation in the fruit-juice supplementation group.

	Milk (n = 9)			Fruit-Juice (n = 8)		
	Baseline	Endpoint	Difference	Baseline	Endpoint	Difference
	Mean (SEM)	Mean (SEM)	Mean (90% CI)	Mean (SEM)	Mean (SEM)	Mean (90% CI)
Fingertip-capillary variables 0-90 min						
Plasma GLP-1_{7-36} (pg·mL)	9.5	10.7	-1.2	9.0	10.2	-1.3
	(2.3)	(2.3)	(2.0, -4.5)	(1.5)	(1.2)	(2.0, -4.5)
Plasma Glucagon (pg·mL)	134.9	134.9	-10.6	90.9	79.3	-1.4
	(25.7)	(25.7)	(43.5, -64.8)	(6.7)	(13.3)	(19.8, -22.6)
Plasma Insulin (pmol·L)	277.5	356.9	-79.4*	353.6	352.0	1.6
	(48.8)	(54.6)	(-29.5, -129.3)	(74.2)	(34.3)	(85.6, -82.5)
Plasma Leptin (pg·mL)	3496.6	3919.3	-422.7	3502.0	3135.9	366.1
	(1706.6)	(1700.1)	(356.9, -1202.4)	(1690.6)	(1238.2)	(1223.1, -491.0)
Blood Glucose (mmol·L)	5.4	5.1	-0.3	5.2	5.2	0.0
	(0.1)	(0.2)	(0.0, -0.7)	(0.1)	(0.2)	(0.2, -0.2)
Subjective Appetite Sensations	Mean (SEM)	Mean (SEM)	Mean (90% CI)	Mean (SEM)	Mean (SEM)	Mean (90% CI)
Hunger (mm)	50.8	59.3	-8.5	46.9	46.9	-8.5
	(3.3)	(4.9)	(0.8, -17.9)	(6.0)	(6.0)	(7.3, -24.3)
Fullness (mm)	34.0	29.7	4.3	43.6	36.6	7.0
	(5.1)	(5.2)	(11.7, -3.1)	(5.9)	(7.8)	(18.7, -4.6)
Prospective Food Consumption (mm)	68.2	73.3	-5.1	60.4	60.9	-0.4
	(2.6)	(4.3)	(2.0, -12.2)	(6.1)	(6.0)	(8.4, -9.3)
Satisfaction (mm)	35.7	34.5	1.2	44.2	37.1	7.1
	(1.8)	(4.2)	(8.8, -64)	(6.1)	(7.3)	(17.6, -.3.4)

Table 5.2 Baseline and endpoint 90-180 min time-averaged AUC fingertip-capillary variables and subjective appetite. Values are expressed as mean ± $_{SEM}$, and mean difference with 90% confidence intervals relative to baseline observations. * indicates a difference relative to baseline observation in the milk supplementation group. ¥ indicates a difference relative to baseline observation in the fruit-juice supplementation group.

	Milk (n = 9)			Fruit-Juice (n = 8)		
	Baseline	Endpoint	Difference	Baseline	Endpoint	Difference
	Mean (SEM)	Mean (SEM)	Mean (90% CI)	Mean (SEM)	Mean (SEM)	Mean (90% CI)
Fingertip-capillary variables 90-180 min						
Plasma GLP-1_{7-36} (pg·mL)	9.2	11.6	-2.3	7.7	7.0	0.6
	(1.8)	(3.4)	(3.3, -7.9)	(1.5)	(1.3)	(2.3, -1.0)
Plasma Glucagon (pg·mL)	120	165.6	-45.4*	47.0	61.9	-14.9
	(24.0)	(21.2)	(-1.2, -89.6)	(9.1)	(14.7)	(11.8, -41.5)
Plasma Insulin (pmol·L)	164.7	197.1	-32.4*	199.3	203.6	-4.2
	(29.0)	(38.4)	(-5.3, -59.6)	(37.4)	(38.1)	(47.1, -55.5)
Plasma Leptin (pg·mL)	2976.4	3360.7	-384.3	3033.2	3088.5	-55.3
	(1319.3)	(1449.2)	(0.1, -768.7)	(1448.7)	(1347.1)	(274.0, -384.7)
Blood Glucose (mmol·L)	4.5	4.2	0.3*	4.6	4.5	0.1
	(0.1)	(0.1)	(0.4, 0.1)	(0.1)	(0.1)	(0.2, -0.1)
Subjective Appetite Sensations	Mean (SEM)	Mean (SEM)	Mean (90% CI)	Mean (SEM)	Mean (SEM)	Mean (90% CI)
Hunger (mm)	75.8	78.4	-2.6	64.9	75.1	-10.3
	(2.3)	(2.8)	(2.7, -8.0)	(4.3)	(4.2)	(0.6, -21.1)
Fullness (mm)	17.1	18.9	-1.8	30.5	21.9	8.6
	(2.6)	(3.8)	(2.6, -6.1)	(5.3)	(4.9)	(18.1, -0.9)
Prospective Food Consumption (mm)	81.1	85.3	-4.2*	71.5	78.1	-6.6
	(1.8)	(1.8)	(-0.4, -8.0)	(3.7)	(4.4)	(0.6, -13.9)
Satisfaction (mm)	23.0	18.0	5.0	32.1	23.5	8.6¥
	(3.8)	(2.1)	(11.2, -1.2)	(4.9)	(5.4)	(16.3, 1.0)

5.2.2 Fingertip-capillary plasma variables

Relative to baseline (day 0) observations, fasting fingertip-capillary concentrations of GLP-$1_{7\text{-}36}$, glucagon, insulin, leptin and blood glucose were comparable following milk and fruit-juice supplementation. Time-averaged AUC (0-90 min) estimates of GLP-$1_{7\text{-}36}$, glucagon, insulin, leptin and blood glucose (mean difference ± 90% CI) did not differ following fruit-juice supplementation (**Table 5.1**). This was also evident for time-averaged AUC (90-180 min) estimates of GLP-$1_{7\text{-}36}$, glucagon, insulin, leptin and blood glucose (**Table 5.2**). Relative to baseline observations (day 0), fingertip-capillary concentrations of insulin displayed a greater time-averaged AUC (0-90 min) following milk supplementation (-79.3 pmol·L; 90% CI: -29.5, -129.3, **Figure 5.4 panel B left graph**). This was also evident for time-averaged AUC (90-180 min) (-32.4 pmol·L; 90% CI: -5.3, -59.6, **Figure 5.4 panel B left graph**). Consistent with the insulinotropic effect of milk, endpoint time-averaged AUC (90-180 min) estimates of blood glucose were lower relative to baseline observations. Time-averaged AUC (90-180 min) estimates of glucagon (mean difference ± 90% CI) were greater after mid-morning milk consumption following milk supplementation (-45.4 pg·mL; 90% CI: -1.2, -89.6, **Figure 5.3 panel B left graph**).

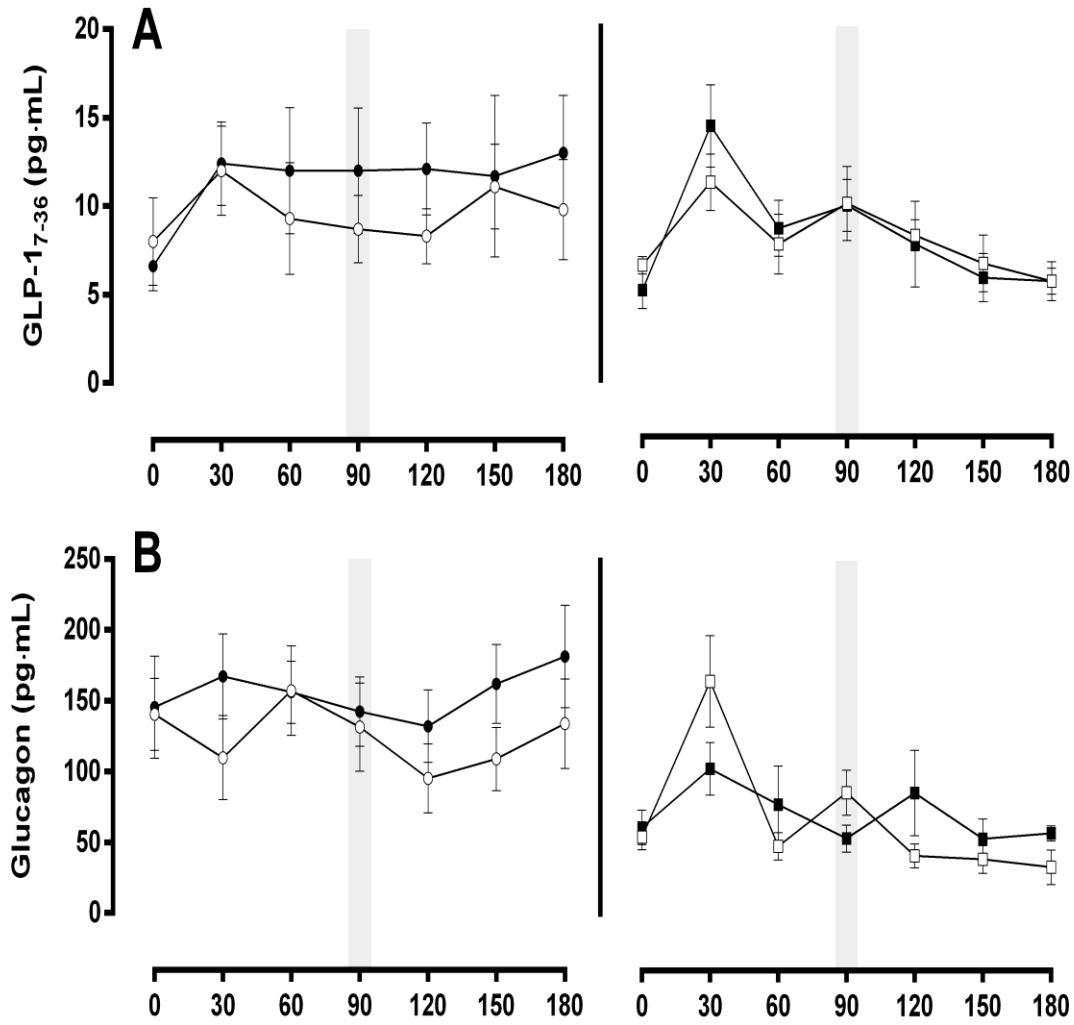

Figure 5.2 Mean ± $_{SEM}$ concentrations of plasma GLP-1_{7-36} (pg·mL; panel A) and plasma glucagon (pg·mL; panel B), obtained from fingertip-capillary blood samples. Graphs depicted on the left are from the milk group (n = 9) whereas graphs depicted on the right are from the fruit-juice group (n = 8). For the left sided graphs, white shaded circles (-○-) represent values obtained during baseline observations, whereas black shaded circles (-●-) represent values obtained during endpoint observations. For the right sided graphs, white shaded boxes (-□-) represent values obtained during baseline observations, whereas black shaded boxes (-■-) represent values obtained during endpoint observations. To convert GLP-1_{7-36} (pg·mL) and plasma glucagon (pg·mL) to their corresponding SI units multiply values by 0.298 and 0.287, respectively. Snack items were distributed at 90 min, as represented by the grey shaded area.

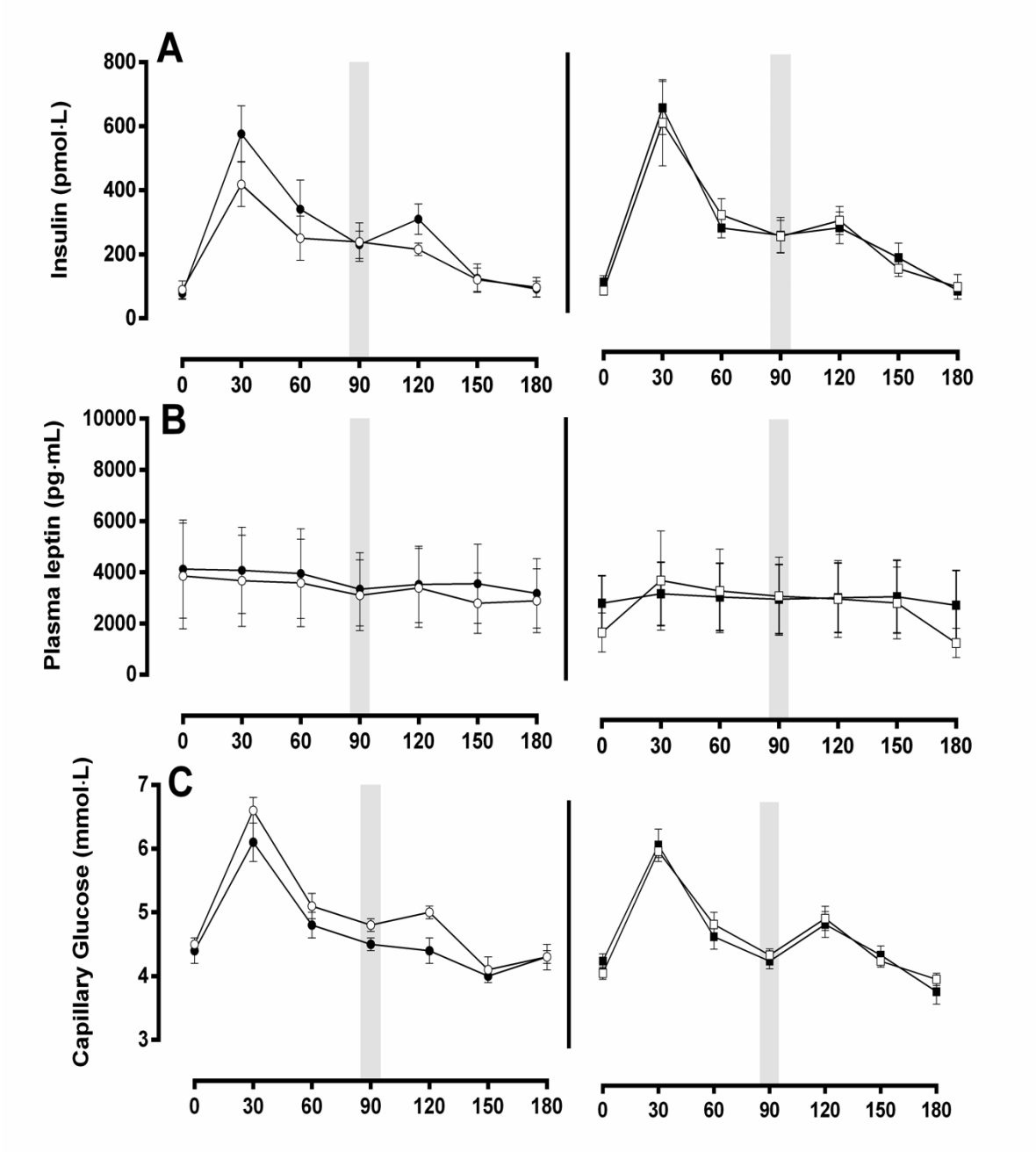

Figure 5.3 Mean ± $_{SEM}$ concentrations of plasma insulin (pmol·L; Panel A), plasma leptin (pg·mL; Panel B) and capillary blood glucose (mmol·L; Panel C), obtained from fingertip-capillary blood samples. Graphs depicted on the left are from the milk group (n = 9) whereas graphs depicted on the right are from the fruit-juice group (n = 8). For the left sided graphs, white shaded circles (-○-) represent values obtained during baseline observations, whereas black shaded circles (-●-) represent values obtained during endpoint observations. For the right sided graphs, white shaded boxes (-□-) represent values obtained during baseline observations, whereas black shaded boxes (-■-) represent values obtained during endpoint observations. Snack items were distributed at 90 min, as represented by the grey shaded area.

5.2.3 Energy intake

Following fruit-juice supplementation, energy intake at the *ad libitum* pasta meal was greater at the endpoint (6272.0 kJ) study day relative to baseline observations (-887.8 kJ; 90% CI:-244.1, -1531.5). Nonetheless, this was transient, as no differences were observed following fruit-juice supplementation for free-living energy intake. Milk supplementation appeared to have an opposite effect on feeding behaviour. Energy intake at the *ad libitum* pasta meal remained comparable at the endpoint study day (4994.4 kJ) relative to baseline observations (4792.5 kJ), yet were reduced under free-living conditions relative to baseline observations (1882.8 kJ; 90% CI: 2706.0, 1059.6).

5.2.4 Gas analysis

Baseline energy expenditure, carbohydrate and fat oxidation was comparable at the endpoint study day relative to baseline observations following both mid-morning fruit-juice and milk supplementation. There were no differences in total energy expenditure (kJ, 0-90 min), carbohydrate (g) or fat (g) oxidation following mid-morning fruit-juice and milk supplementation at endpoint study day relative to baseline observations (**Table 5.3**). No differences in total energy expenditure (kJ, 90-180 min), carbohydrate or fat oxidation were present following mid-morning milk supplementation relative to baseline observations.

Table 5.3. Baseline and endpoint average rates of energy expenditure and substrate metabolism, dichotomised according to 0-90 min and 90-180 min postprandial periods. Values are expressed as mean (± $_{SEM}$). * indicates a difference relative to baseline observation in the milk supplementation group. ¥ indicates a difference relative to baseline observation in the fruit-juice supplementation group.

	Milk (n = 9)			Fruit-Juice (n = 8)		
	Baseline	Endpoint	Difference	Baseline	Endpoint	Difference
	Mean (SEM)	Mean (SEM)	Mean (95% CI)	Mean (SEM)	Mean (SEM)	Mean (95% CI)
Metabolic variables 0-90 min						
Energy expenditure (kJ)	1063.0	971.4	91.6	1175.5	1177.6	-2.1
	(73.4)	(60.4)	(185.9, -2.6)	(223.7)	(246.8)	(136.3, -140.5)
Carbohydrate oxidation (g)	34.3	24.6	9.7	33.1	36.5	-3.4
	(6.9)	(5.8)	(25.6, -6.3)	(6.3)	(13.4)	(11.4, -18.2)
Fat oxidation (g)	13.8	15.0	-1.2	17.5	16.1	1.4
	(2.7)	(2.1)	(5.6, -7.9)	(5.0)	(2.6)	(6.7, -4.0)
Metabolic variables 90-180 min						
Energy expenditure (kJ)	1028.2	961.3	66.8	1071.9	1089.9	-18.0
	(34.6)	(47.1)	(138.9, -5.2)	(133.9)	(100.1)	(91.1, -127.2)
Carbohydrate oxidation (g)	28.4	24.9	3.5	34.3	30.5	3.8
	(6.2)	(4.9)	(18.0, -11.0)	(4.8)	(6.8)	(9.0, -1.3)
Fat oxidation (g)	15.4	14.2	1.1	14.1	16.0	-1.9
	(2.7)	(2.0)	(8.2, -6.0)	(3.6)	(3.8)	(0.6, -4.5)

5.3 Discussion

The main aim of this study was to establish the effect of milk or fruit-juice supplementation on appetite, feeding behaviour, metabolism and body mass in adolescent males. In the present free-living study we show that daily supplementation with milk as a mid-morning snack four 4-weeks reduces feeding behaviour, and it is reasonable to suggest this was facilitated through integrated endocrine responses to food consumption. In this sense, changes in appetite- and metabolism-related peptide expression following the breakfast meal and milk consumption indicate that milk supplementation conveys a metabolic advantage, influencing feeding behaviour (suppressing energy intake under free-living conditions) but failed to influence postprandial metabolism. Relative to baseline observations, daily milk supplementation increased endpoint postprandial (0-90 min) plasma insulin following a standardised breakfast meal. Furthermore, daily milk supplementation increased endpoint postprandial (90-180 min) plasma insulin and glucagon and attenuates endpoint postprandial (90-180 min) blood glucose following mid-morning milk consumption. Furthermore, although measures of body mass and percentage body fat remained comparable following milk supplementation, we observed milk to elicit favourable outcome on measures lean and fat mass. In contrast, supplementation with fruit-juice increased energy intake at the *ad libitum* pasta meal.

The mechanism by which milk consumption affects feeding behaviour under free-living conditions is not properly understood, however, there are several possible explanations and constituents of milk that may act synergistically to elucidate its actions. From a physiological perspective, the consumption of milk reportedly lowers postprandial secretion of blood glucose (Panahi et al., 2013). It is suggested this modulation is facilitated through an insulinotropic effect of milk (Nilsson et al., 2004; Östman et al., 2001). The insulinotropic response to milk consumption is not solely the influence of one individual nutritional component. Instead, milk-based dairy foods and their properties work in a synergistic manner to stimulate the secretion and activity of insulin. High-quality proteins, namely casein and whey, constitute approximately 82 and 18% respectively

of the total protein found in milk and provide an abundance of essential amino acids. The protein induced insulinotropic effect may involve digestion and the release of plasma amino acids (valine, isoleucine and leucine) of which are known to mediate insulin secretion (Schmid et al., 1989; van Loon et al., 2000b). In addition, the insulinotropic and glucose lowering effect of milk consumption may involve appetite- and metabolism- related hormone secretion. Consumption of milk-based dairy foods is associated with increased concentrations of plasma appetite-regulating peptides in adults (Anderson et al., 2004; Bowen et al., 2006; Luhovyy et al., 2007; Schneeman et al., 2003) and also children and adolescents (Vien et al., 2014), stimulating the expression of GLP-1 a potent incretin peptide (lowering blood glucose and stimulating insulin) (Asmar & Holst, 2010). Furthermore, the consumption, injection or infusion of glucose in humans and animal models has demonstrated to suppress the expression of glucagon, while reduced levels of glucose are associated with heightened glucagon secretion (Ohneda et al., 1969; Unger, Aguilar-Parada, Müller, & Eisentraut, 1970). Considered together, the finding that milk supplementation elicited an insulinotropic effect and increased the postprandial response of plasma glucagon and attenuated postprandial blood glucose may have been expected. As mentioned in chapter four, the postprandial response of these peptides is profoundly influenced according to ingested macro- (and micro-) nutrient composition, and also energy content. Insulin and glucagon for example rise in response to protein feeding (Acheson et al., 2011). In the present study, the experimental preloads were isoenergetic (427 kJ) and isovolumetric (217 mL), yet differed according to macronutrient composition (**Table 4.0**). In this sense, the milk supplement contained considerably more protein than the fruit-juice drink (7.5 g vs. 1.1 g). It may therefore be unsurprising that milk supplementation resulted in a greater time-averaged AUC plasma glucagon and insulin response considering its macro-nutrient composition relative to the fruit-juice. Indeed, greater responses of insulin and glucagon have been related to anorexigenic behaviours in the adult literature including increased satiety and acutely reduce food intake (Anne Flint et al., 2007; J. A. Parker et al., 2013; Penick et al., 1961; Stephen C Woods et al., 2006).

In the present study, relative to baseline observations daily milk supplementation failed to impact on energy intake at the *ad libitum* pasta meal, yet reduced feeding behaviour in the free-living environment. The finding that milk and milk-based dairy foods have the potential to influence feeding behaviour favourably corresponds with our previous observations presented in chapter four and with other child and adolescent literature (Birch et al., 1993; Mehrabani et al., 2014; Vien et al., 2014; Zandstra et al., 2000). The mean reduction in energy intake between the endpoint and baseline observations was -1882.8 kJ, equating to -450 kcal. As previously introduced in chapter four, Wang and colleagues (2006) suggest sustained reductions in daily energy intake averaging 110 to 165 (460 to 690 kJ) kcal may offer an effective approach for preventing excess weight accumulation in children and adolescents. Thus, the finding that milk supplementation resulted in a 450 kcal reduction in free-living energy intake may convey a possible application for appetite regulation and feeding behaviour in overweight and obese persons, yet this remains to be examined. In contrast, albeit short-lived, supplementation with fruit-juice resulted in an increased energy intake at the *ad libitum* pasta meal. On average, participants consumed 892.9 kJ (213.4 kcal) more energy at the endpoint relative to baseline observations. Based on the abovementioned approach for preventing weight accumulation, fruit-juice supplementation appears ineffective as a potential supplemental strategy to aid weight management in adolescents. Indeed, recent evidence has emerged illustrating that replacement of sugar-sweetened beverages with milk or water, but not fruit-juice, illustrated an inverse association with body fatness development throughout the transition from childhood to adolescence (Zheng et al., 2015). As previously alluded to, the experimental preloads were isoenergetic and isovolumetric, yet differed according to macronutrient composition (the milk supplement contained considerably more protein than the fruit-juice drink). In the scientific literature it is widely recognised that protein is more satiating than isoenergetic servings of carbohydrate or fat (Astrup, 2005; Westerterp-Plantenga, Rolland, Wilson, & Westerterp, 1999). Thus, considering milks higher protein content a reduction of energy intake may not be surprising.

Although we present evidence that milk supplementation reduced feeding behaviour under free-living conditions, and that this may have been facilitated through alterations in the postprandial response of plasma glucagon, insulin and blood glucose, milk supplementation failed to impact on measures of subjective appetite. Consequently, this highlights the necessity to quantify appetite- and metabolism-related peptides in younger populations in order to identify potential mechanisms influencing body mass maintenance and/or loss. It is important to note that subjective perceptions of food-related emotions in response to a number of stimuli are inconsistent and do not always translate to reflect actual feeding behaviour in children and adolescents (Thivel et al., 2012; Thivel et al., 2011). It is reasonable to suggest that alterations in appetite- and metabolism-related peptides may supersede subjective perceptions of food-related emotions to influence appetite, feeding behaviour and metabolism. In agreement with our earlier findings (chapter four), the consumption of milk appeared to have an opposite effect on subjective perceptions of appetite. In particular, participants in the milk group demonstrated increased prospective food consumption following milk consumption relative baseline observations.

There is evidence in the adolescent literature that single milk constituents or whole milk-based dairy food consumption exert the ability to affect postprandial metabolism (Apolzan et al., 2006). In an acute setting (chapter four) we recently showed the consumption of milk stimulated postprandial energy expenditure (109 kJ; 90% CI: 197.2, 21.6) compared with isocaloric and isovolumetric servings of fruit-juice and yogurt. In addition, Apolzan et al. (2006) evaluated energy expenditure (kcal·min) for 240 min after a low calcium non-dairy control, supplemental calcium or a dairy-based product in overweight adolescent males and females. They observed a greater rate of energy expenditure (0.4 kJ·min) following the consumption of the dairy-based product compared with the low calcium non-dairy control, but only in adolescent males. No differences were recorded following supplemental calcium ingestion, which may suggest additional constituents housed within milk-based dairy foods act to impact on metabolism. To expand, in adults high rates of calcium consumption have been linked to greater postprandial energy metabolism and fat oxidation

(Cummings, James & Soares, 2006; Teegarden et al., 2008; Zemel et al., 2008) . In the present study, however, we did not observe any capacity of milk or fruit-juice supplementation to impact on measures of energy expenditure or substrate metabolism relative to baseline observations. The statistical approach utilised in this study may explain why the milk and fruit-juice supplementation failed to impact on measures of energy expenditure or substrate metabolism. In chapter four, data was expressed as mean differences ± 90% confidence intervals relative to the control (fruit-juice) or yogurt trial (e.g. milk-yogurt, milk-control and yogurt-control), whereas in the present study data are expressed as mean differences ± 90% confidence intervals for baseline observations relative to endpoint observations. Furthermore, it may be reasonable to suggest the supplemental protocol (volume and supplemental duration) and dietary restriction (or lack of) confounded any effects on measures of postprandial metabolism, however this is speculative. Indeed, Melanson and colleagues (2005) illustrated increased intakes of calcium (~1400 mg/d) daily for 7 days led to an approximately 30% greater rate of fat oxidation compared with low intakes of calcium (~500 mg/d), when under energy restriction. For this reason, the level of calcium contained within the supplemental beverages (257 mg/d for milk) may have been insufficient to elicit an effect on postprandial metabolism, and therefore may demonstrate a dose response especially considering Melanson and colleagues (2005) found no differences under energy balance conditions.

It is interesting to note the aforementioned changes occurred independent of changes in body mass and percentage fat, however, it is important to consider that the study population were healthy free-living adolescents and the intervention was only conducted over 4-weeks (28 days). The observation that milk failed to impact on body mass, percentage body fat or blood lipids in free-living conditions is consistent with some existing literature in children and adolescents from randomised clinical trials who have reported no effect of milk or dairy supplementation on body mass or body composition (Cadogan, Eastell, Jones & Barker, 197; Chan et al., 1995; Merrilees et al., 2000). However, Arnberg et al. (2012) recently reported milk supplementation to increase body

mass in overweight adolescents. This may be unsurprising, as the aforementioned studies did not include energy-restriction with milk supplementation and participants were free-living. In the present study, although measures of body mass and percentage body fat remained comparable following milk supplementation, we observed milk to elicit favourable outcome on measures of lean and fat mass and corroborates findings from an earlier study in Chilean children (Albala et al., 2008). During the supplementation periods of this study, participants were instructed to maintain their usual feeding behaviour and physical activity patterns. No objective measures of feeding behaviour and physical activity were collected so differences between groups cannot be ruled out. In a supplementation study, overweight and obese Chilean children (8-10 y) who habitually consumed sugar-sweetened beverages were randomly assigned to a control group (followed habitual beverage consumption) or an intervention group (3 servings of milk daily) for 16-weeks. On completion of the supplemental protocol no differences in percentage body fat were observed between the groups, however, the milk supplemented group displayed a significant increase in lean body mass (Albala et al., 2008). Together, these findings may suggest that milk supplementation without energy-restriction is insufficient to bring about weight loss, but can alter the composition of fat and lean tissue.

Considered together, the findings presented throughout this study indicate that milk supplementation for 4-weeks (28 days) impacts favourably on feeding behaviour under free-living conditions, and does not adversely affect body mass or body composition. Relative to baseline observations, milk elicits an increased postprandial glucagon and insulin secretion and attenuates blood glucose concentrations albeit with disagreement from subjective measures of appetite. Milk supplementation failed to impact on measures of postprandial metabolism, and reasons for this may involve the lack of dietary restriction during supplemental periods. In contrast, supplementation with fruit-juice resulted in increased energy intake at the *ad libitum* pasta meal. Nonetheless, we observed no capacity for fruit-juice supplementation to influence measures of subjective appetite,

appetite and metabolism-related peptides or postprandial metabolism relative to baseline observations. Caution should be observed when extrapolating the results of this study, because supplementation periods were relatively modest (4-weeks). Consequently, the longer-term implications of daily milk supplementation on appetite, feeding behaviour, metabolism and body mass in free-living adolescent remain to be determined. Nonetheless, the results of this study (and chapter four) provide evidence that mid-morning milk consumption impacts favourably on feeding behaviour in adolescent males (15-18 y), and it is reasonable to suggest this is facilitated through integrated endocrine responses (plasma glucagon, insulin and blood glucose) to food consumption.

CHAPTER SIX

GENERAL DISCUSSION

6.0 Introduction

This chapter will collate and consider the findings of the experimental chapters of this thesis. The present body of work was designed to establish the acute- and moderate-term impact of milk-based dairy food consumption on subsequent appetite, feeding behaviour and metabolism in healthy adolescent males. To elaborate further, the studies presented within this thesis aimed to examine the effects of mid-morning milk-based dairy consumption on measures of subjective appetite, appetite- and metabolism- related peptides (GLP-$1_{7\text{-}36}$, glucagon, insulin, leptin and blood glucose), feeding behaviour and postprandial metabolism (energy expenditure and substrate metabolism). To accomplish this, four studies were conducted. Studies one (chapter two) and two (chapter three) were categorised as methodological studies, whilst studies three (chapter four) and four (chapter five) were considered intervention studies. Accordingly, the thesis aims were fourfold:

- Chapter two: Establish the potential of an alternative methodological approach to quantify appetite- and metabolism-related peptide expression for use with paediatric populations. For this, the agreement of GLP-$1_{7\text{-}36}$, glucagon, insulin and leptin between fingertip-capillary and antecubital-venous blood sampling was assessed. In addition, this chapter explored the between day test-retest reproducibility of the aforementioned peptides in a resting state utilising fingertip-capillary blood.

- Chapter three: Compare patterns of dairy food consumption among a sample of children (9-11 y) and adolescents (15-18 y) from the North-East of England. In particular, to establish dairy food popularity (types, frequencies and amounts) and identify potential populations (sex & age) to target within the intervention-based sections of this thesis.

- Chapter four: Examine the acute effects of mid-morning dairy consumption on subsequent appetite, feeding behaviour and metabolic responses relative to an isoenergetic and isovolumetric serving of fruit-juice in healthy adolescent males.

- Chapter five: Examine the effect of milk or fruit-juice supplementation (4-weeks) on appetite, feeding behaviour and metabolism in healthy, free-living adolescent males. Secondly, to evaluate the impact of milk or fruit-juice supplementation on measures of body mass, body composition and blood lipid profiles.

6.1 Agreement and reproducibility of appetite- and metabolism- related peptides under resting conditions

Multiple hormonal peptides of gastrointestinal, pancreatic and adipose tissue origin pertinent to the regulation of energy homeostasis are produced and secreted into the circulation in response to the ingestion of a meal. Furthermore, postprandial responses of these peptides are profoundly influenced according to ingested macro- (and micro-) nutrient composition. Accurate quantification of these peptides is therefore essential when exploring hormonal responses in studies concerning appetite, feeding behaviour and metabolism. In clinical and research practice, quantitative measures of these peptides have commonly been assessed utilising venepuncture or antecubital-venous catheterisation. Obtaining intravenous access, however, is often problematic in certain exercise and field settings and also within vulnerable populations, where alternative techniques to blood sampling may prevail. Indeed, this is an important aspect influencing study design especially where vulnerable populations are concerned, such as children and adolescents. Methods including saliva or capillary samples may help overcome such issues associated with venous blood collection and consequently provide an alternative approach to quantify measures of appetite- and metabolism-

related peptides. The nature of saliva sampling offers a feasible alternative to quantify measures of appetite-related peptides, however, contamination from eating episodes is likely to impact on concentrations especially within nutritional interventions. For this reason, the first study of this thesis (chapter two) was therefore designed to establish the potential of an alternative methodological approach to quantify appetite- and metabolism-related peptide expression for use with vulnerable populations. For this, the agreement of GLP-$1_{7\text{-}36}$, glucagon, insulin and leptin between fingertip-capillary and antecubital-venous blood sampling was assessed. In addition, this chapter explored the between day test-retest reproducibility of the aforementioned peptides in a resting state utilising fingertip-capillary blood. Establishing the test-retest reproducibility of these peptides was of great importance, making certain that the intervention(s) or variable(s) were responsible for any observed differences and were not brought about by random variability or measurement error. Quantifying the level agreement and reproducibility between methods and days would subsequently inform researchers and practitioners whether appropriate comparisons between studies using venous and capillary blood sampling could be made. This was deemed particularly important given that blood obtained from antecubital-venous and fingertip-capillary blood is characteristically dissimilar.

The results presented throughout this chapter demonstrated, for the first time, that at rest fingertip-capillary blood sampling offers an appropriate methodological and reproducible approach to systematically assess concentrations of appetite- and metabolism- related peptides. Specifically for fingertip-capillary plasma glucagon, reproducibility was strong between visits (CV_r = 8.2%). Plasma GLP-$1_{7\text{-}36}$ and leptin demonstrated modest reproducibility (CV_r = 22.7 and 25.0%, respectively). Plasma insulin exhibited the greatest variability and indicated a large degree of random error between visits (CV_r = 36.0%), indicating the two methods should not be employed interchangeably. When considering our findings, the results of this chapter hold important implications for researchers and practitioners who wish to quantify and compare the expression of these appetite- and metabolism-related peptides in various populations or field-based scenarios

where antecubital-venous blood sampling might be contraindicated (e.g. paediatrics, adolescents, and elderly). Indeed, this study provided valuable information regarding the utility of fingertip-capillary blood sampling for use throughout the subsequent series of studies within this thesis. For example, knowledge concerning the typical error (reported as a coefficient of variation) for the aforementioned peptides [in particular fingertip-capillary plasma glucagon (8.2%)] were subsequently applied to facilitate study design, where sample size estimations were based on these findings (chapter four and five).

6.2 Patterns of dairy food consumption among children and adolescents

Considering the overall purpose of this thesis was to establish the appetite and metabolic responses to acute and moderate-term dairy consumption, it was necessary to assess patterns of dairy food consumption in populations relevant to the thesis. In particular, it was of importance to identify dairy food popularity (types, frequencies and amounts) and potential populations to target within the intervention-based sections of this thesis. To date, much of our knowledge concerning dairy consumption is centred on national surveillance data. Accordingly, data indicate that milk-based dairy food consumption has fallen in recent years and declines further with age, especially from childhood through to adolescence. Nonetheless, dietary surveys typically rely on retrospective assessment methods, which pose complications of misreporting error and are therefore not entirely robust. Furthermore, the wide-ranging age groupings (e.g. 4-10 y; 11-18 y) make it difficult to differentiate between consumption in middle-childhood and adolescence. Consequently, the second methodological study of this thesis (chapter three) aimed to examine and compare patterns of free-living dairy food consumption among a sample of children and adolescents exercising more finite age boundaries and more robust dietary assessment tools. Accordingly, the present study comprised a convenience sample of participants aged 9-11 y (n = 40; 15 boys and 25 girls) and 15-18 y (n = 40; 20 males and 20 females). In this study, two methodological approaches were exercised to

explore patterns of milk-based dairy food consumption, which the authors' believe to be a novel aspect of the study. Briefly, adolescent (15-18 y) free-living dietary intake was evaluated utilising a combined weighed self-reported food record and 24 h dietary recall technique, used previously with adolescent populations (Rumbold, St Clair Gibson, Stevenson, et al., 2011). For children (9-11 y), free-living dietary intake was evaluated through parental weighed food records. Dietary intakes were evaluated during school term-time over four consecutive days, including two weekdays and two weekend days and food records were subsequently explored to determine types, amounts and frequency of dairy food consumption.

Following dietary data collection, analysis revealed a main effect for sex on overall milk consumption ($F_{1,71} = 7.07$, $p = 0.010$) and daily milk portions ($F_{1,71} = 6.79$, $p = 0.011$), indicating that independent of age, boys consumed greater amounts of milk compared to girls. Furthermore, findings reported throughout this chapter indicated that milk was the most favorable dairy food consumed among children and adolescents, consumed by 91% of participants (across all ages and sexes). No interaction or main effect for any other variable (cheese, yogurt, butter, ice cream, cream and custard) was evident. Although we sought no statistical evidence that milk-based dairy food consumption differed between middle-childhood and adolescence, for boys and adolescent males, a downward trend of total milk and dairy food consumption with increasing age was noted whereas patterns of milk and dairy food consumption remained widely stable among girls and female adolescents. As dietary habits shaped throughout this phase may ultimately track into adulthood (Lake et al., 2006), continual dairy food avoidance and low dairy consumption could be disadvantageous, particularly among adolescents, leading to lasting nutritional and health-related implications. Additionally, continual dairy food avoidance and low dairy consumption may present increased risk of developing overweight and obesity as demonstrated throughout chapter one of this thesis. For this reason, it was deemed necessary to target the intervention-based studies on adolescent males (15-18 y). Taken together, the establishment of fingertip-capillary blood sampling as a less-invasive approach to quantify measures of appetite- and metabolism-related peptides

(chapter two) and identification of patters of milk-based dairy food consumption (in particular milk representing the most popular consumed item) enabled the main aim of the thesis to be investigated.

6.3 Milk-based dairy food consumption and feeding behaviour

Scientific literature supports the hypothesis that milk-based dairy food consumption may provide a protective effect against adiposity in adolescents (Dror, 2014). Efforts to establish the underlying relationship between milk-based dairy foods and adiposity have highlighted several plausible mechanisms. One proposed mechanism of action through which dairy confers anti-obesity properties is through favourable actions on appetite and feeding behaviour (Aziz et al., 2007). Indeed, this was illustrated throughout chapters four and five of this thesis. In chapter four, acute mid-morning milk consumption reduced energy intake at the *ad libitum* pasta meal relative to a fruit-juice drink, whereas we observed no capacity of yogurt to influence feeding behaviour relative to milk and fruit-juice. The finding that mid-morning milk consumption impacted favourably on feeding behaviour was further replicated in chapter five. In this sense, moderate-term milk supplementation impacted on feeding behaviour in the free-living environment, reducing energy intake relative to baseline observations, yet not at the *ad libitum* pasta meal. In contrast, supplementation with fruit-juice resulted in an increased energy intake at the *ad libitum* pasta meal relative to baseline observations (chapter five). Considering the effect of milk consumption on feeding behaviour, there are several possible factors and nutritional components which may have contributed to these findings. Probably the most well established contributing factor concerns protein. In the scientific literature there is evidence that protein is more satiating than isocaloric equivalents of carbohydrate and fat. In the present thesis, the experimental preloads were isoenergetic (427 kJ) and isovolumetric (217 mL), yet differed according to macronutrient composition (**Table 4.1**). In this sense, the milk drink contained considerably more protein than the fruit-juice drink (7.5 g vs. 1.1 g, respectively) and yogurt (5.9 g). For this reason, it may therefore

be unsurprising that milk consumption influenced feeding behaviour relative to caloric equivalents of fruit-juice and yogurt.

In chapter four, the mean reduction in energy intake between milk and the fruit-juice was -596.4 kJ, equating to -142.5 kcal and following milk supplementation the mean reduction in energy intake between the endpoint and baseline observations was -1882.8 kJ, equating to -450 kcal. As alluded to throughout these chapters, Wang and colleagues (2006) suggest sustained reductions in daily energy intake averaging 110 to 165 kcal may offer an effective approach for preventing excess weight accumulation in children and adolescents. Thus the finding that acute- and moderate-term milk consumption imparts a metabolic advantage by favourably impacting on feeding behaviour may convey a possible application for appetite regulation and feeding behaviour in overweight and obese persons. Nonetheless, it would prudent for future investigations to assess whether milk-based dairy foods actually provide application to overweight and obese metabolically diseased populations.

Together, the observation that milk consumptions reduces energy intake corresponds with previous observations in children and adolescents (Birch et al., 1993; Mehrabani et al., 2014; Vien et al., 2014; Zandstra et al., 2000), and contribute further to the understanding that milk-based dairy foods exert the potential to influence feeding behaviour in an acute- and moderate-term setting. While there is indeed formative evidence to suggest that milk-based dairy consumption influences feeding behaviour it remains challenging to comment on the physiological mechanisms that may facilitate these actions, considering only few have included subjective measures of appetite, appetite- and metabolism-related peptides, and postprandial metabolism. Chapters four and five therefore explored the appetite and metabolic responses to acute and moderate-term dairy consumption in healthy adolescent males, whereby measures of subjective appetite, appetite- and metabolism- related peptides (GLP-$1_{7\text{-}36}$, glucagon, insulin, leptin and blood glucose), feeding behaviour and postprandial metabolism (energy expenditure and substrate metabolism) were determined in an attempt to understand the physiological mechanisms facilitating changes.

6.4 Milk-based dairy food consumption on measures of subjective appetite and appetite- and metabolism-related peptides

As previously alluded to, one proposed mechanism of action through which dairy confers anti-obesity properties of milk-based dairy foods is through favourable actions on appetite and feeding behaviour (Aziz et al., 2007). In relation to subjective measures of appetite, the findings presented throughout chapters four and five do not support the hypothesis that milk-based dairy consumption has a favourable effect on appetite. Somewhat unexpectedly, acute mid-morning milk consumption supplementation failed to impact favourably on measures of subjective appetite and appeared to have an opposite effect whereby measures of time-averaged AUC subjective fullness were lower following mid-morning milk consumption relative to the fruit-juice drink. This was also evident relative to the yogurt trial. Consistent with an increase and reduction in subjective hunger and fullness respectively, time-averaged AUC sensations of prospective food consumption was greater following mid-morning milk consumption relative to the fruit-juice and yogurt trial (chapter four). Interestingly, this was further replicated in chapter five where time-averaged AUC subjective measures of prospective food consumption were greater following mid-morning milk supplementation. Based on the summary provided in section 6.3, it is important to note the observations concerning subjective appetite appeared to have a negligible effect on feeding behaviour, especially considering the fact that milk-based dairy consumption reduced energy intake. Of course, the sensory characteristics of foods play a vital role in feeding behaviour (Hogenkamp, Stafleu, Mars, Brunstrom & de Graaf, 2011; Yoemans, Blundell & Leshem, 2004), and include factors such as texture and flavour. Recently, Hogenkamp et al. (2011) investigated the role of texture and flavour attributes on expectations concerning the satiating capacity of dairy products. In general, study findings demonstrated a rise in anticipated satiation with increased thickness of the milk-based dairy food provided, while flavour characteristics failed to influence the anticipated level of satiation. It could therefore be speculated that milk, of which is much less viscous than

yogurt, in part contributed to the lower perceived satiety. Tracking changes in subjective appetite profiles over time provides important information in relation to feeding events. However, there is evidence in the adolescent literature that subjective perceptions of food-related emotions in response to a number of stimuli are inconsistent and do not always translate to reflect actual feeding behaviour in children and adolescents (Thivel et al., 2012; Thivel et al., 2011). Consequently, this highlights the necessity to include measures of appetite- and metabolism-related peptides in younger populations. It is therefore reasonable to suggest that alterations in appetite- and metabolism-related peptides and metabolic responses may supersede subjective perceptions of food-related emotions to influence appetite, feeding behaviour and metabolism.

In chapters four and five measures of appetite- and metabolism-related peptides were quantified utilising the fingertip-capillary blood sampling technique established in chapter two of this thesis. These were included alongside measures of subjective appetite in an attempt to provide evidence from a physiological standpoint. From a physiological perspective, the consumption of milk reportedly lowers postprandial secretion of blood glucose (Panahi et al., 2013), and it is suggested this modulation is facilitated through an insulinotropic effect of milk (Nilsson et al., 2004; Östman et al., 2001). This was confirmed in chapter five with moderate-term milk supplementation and has added to the knowledge regarding the insulinotropic effects of milk within adolescent populations. Although we found no evidence that milk supplementation altered fasting concentrations of insulin, relative to baseline observations, milk supplementation for 4-weeks increased endpoint postprandial plasma insulin following a standardised breakfast meal. Furthermore, milk supplementation increased endpoint postprandial plasma insulin and attenuated endpoint postprandial blood glucose following mid-morning milk consumption. We observed no capacity for fruit-juice supplementation to impact on measures of subjective appetite, appetite- and metabolism-related peptide expression, postprandial metabolism, body composition or blood lipid profile relative to baseline observations. As previously alluded to, postprandial responses of these peptides are profoundly influenced according to ingested macro- (and micro-) nutrient composition.

Considering milk offered considerably more protein than fruit-juice this may, in part, explain the greater insulin response. The protein induced insulinotropic effect may involve digestion and the release of plasma amino acids (valine, isoleucine and leucine) of which are established as mediators of insulin secretion (Schmid et al., 1989; van Loon et al., 2000b). It is therefore possible that the potentiation of postprandial insulin following milk supplementation is likely to be as a result of elevated plasma amino acids concentrations. However, as no attempt to measure the release of plasma amino acids was included in chapter five it remains difficult to explain whether this may have contributed to the insulinotropic effect of milk and thus warrants further investigation.

In an acute setting (chapter four), mid-morning milk consumption elicited a greater time-averaged AUC expression of plasma glucagon relative to an isoenergetic and isovolumetric serving of fruit-juice. This initial finding was surprising, and was the first study to report an elevated glucagon response to milk consumption. Interestingly, this finding was further replicated in chapter five. In this sense, daily milk supplementation increased endpoint postprandial plasma glucagon following mid-morning milk consumption relative to baseline observations. In humans, deceased blood glucose levels are correlated with enhanced glucagon release (Ohneda et al., 1969; Unger et al., 1970). Consequently, as milk supplementation provided a glucose lowering effect, an increased postprandial response of plasma glucagon may have been expected. Indeed, in the adult literature greater insulin and glucagon responses have been related to anorexigenic behaviours including increased satiety and acutely reduce food intake (Flint et al., 2007; Parker et al., 2013; Penick et al., 1961; Woods et al., 2006). This may therefore offer justification for the favourable effects of milk consumption on feeding behaviour in chapters four and five.

6.5 Comparisons with adult literature

As cited previously in the general introduction and literature review it appears that in young people milk-based dairy food consumption impacts favourably on feeding behaving, resulting in reduced

energy intake (Birch et al., 1993; Mehrabani et al., 2014; Vien et al., 2014; Zandstra et al., 2000), and increased concentrations of appetite-related peptides (Vien et al., 2014). Indeed, this was also illustrated throughout chapters four and five of the present thesis, whereby experimental studies highlighted mid-morning milk consumption reduced feeding behaviour in adolescent males and may have been brought about through integrated endocrine responses of appetite- and metabolism-related peptides. The findings concerning milk and milk-based dairy food consumption on subsequent feeding behaviour (Dougkas et al., 2012; Dove et al., 2009) and appetite-related peptides (Soenen & Westerterp-Plantenga, 2007) corroborate to some extent with data from adult studies, yet findings concerning subjective appetite data do not correspond (Almiron-Roig & Drewnowski, 2003; Dougkas et al., 2012; Dove et al., 2009; Harper, James, Flint, & Astrup, 2007; Soenen et al., 2007; Tsuchiya, Almiron-Roig, Lluch, Guyonnet, & Drewnowski, 2006). As with young people, there are relatively few studies that have examined the effect of milk or milk-based dairy products as whole food on appetite and energy intake regulation (table 6.0). Consequently, the effect of milk-based dairy food consumption on measures of appetite and energy intake in adults is equivocal. The succeeding paragraphs of this section will comment only on adult studies utilising only milk or milk products as whole food and not individual dairy components or fortified products (e.g. dairy proteins, fats and carbohydrates) as this does not give a true representation of milk or individual milk products as whole food on appetite and energy intake regulation.

In the studies presented in table 6.0 the effect of milk and milk-based dairy food consumption on *ad libitum* energy intake relative to isoenergetic or isovolumetric servings of fruit-juice or sugar-sweetened beverages was explored. The energetic content provided by the test beverages being between 814 kJ and 1.5 MJ, and subsequent energy intake being assessed between 30 min and 4 h after milk consumption. In addition, measures of subjective appetite were collected throughout, and only two studies quantified measures of appetite-related peptides. In the majority of these studies milk-based dairy consumption increased subjective perceptions of satiety relative to

isoenergetic or isovolumetric servings of fruit-juice or sugar-sweetened beverages was assessed, yet failed to translate and impact on subsequent feeding behaviour (table 6.0) whereas the opposite was apparent throughout chapters four and five of the present thesis. To the knowledge of the author, only Dove et al. (2009) and Dougkas et al. (2012) identified that, in overweight men and women, milk-based dairy food consumption increased perceptions of satiety that later transpired in a reduction in *ad libitum* energy intake relative to an isoenergetic and isovolumetric serving of fruit-juice drink and an isovolumetric serving of water, respectively. In the studies conducted by Dougkas et al. (2012) and Dove et al. (2009) energy intake was significantly lower 90 min and 4 h following dairy snack and skimmed milk consumption relative to water and fruit-juice, respectively. Concluding remarks from the latter cited study indicated that the period between the preload and subsequent *ad libitum* assessment of energy intake might be crucial since scientific evidence indicates protein and products of its digestion heighten satiety over several hours. Indeed, this may reign true considering no differences where noted in *ad libitum* energy intake assessments which took place 30-50 min following milk-based dairy food consumption. While in the majority of the aforementioned investigations no differences in later feeding behaviour was witnessed, not all were principally powered to uncover differences in feeding behaviour and energy intake. Again, only Dove et al. (2009) and Dougkas et al. (2012) adequately powered their studies with energy intake as the primary measure.

Table 6.0 Adult studies assessing milk and milk-based dairy food consumption on appetite regulation and feeding behaviour

Study	Participants	Study details	Energy intake, subjective appetite, and appetite-related peptides
Dougkas et al. (2012)	Overweight males, N = 40.	Randomised, crossover trial. Participants attended 4 sessions, 1 week apart, and received one of three isoenergetic (814 kJ) and isovolumetric (410 mL) servings of dairy snacks [milk, yogurt and cheese or water (control)] 120 min following a standardised breakfast. Ninety min following snack consumption *ad libitum* energy intake was assessed. Subjective appetite was determined throughout, and measures of plasma amino acids, glucose, insulin, ghrelin and PYY were quantified at 0 min and 80 min.	Yogurt had the greatest suppressive effect on subjective appetite, whereby perceived hunger was 8, 10 and 24% ($p < 0.001$) lower compared with cheese, milk and water, respectively. Energy intake was significantly ($p < 0.02$) lower following all dairy snacks compared to water. No differences were recorded between dairy snacks. There were no differences in the postprandial response of appetite-related peptides.
Dove et al. (2007)	Overweight females, N = 21. Overweight males, N = 13.	Randomised, crossover trial. Participants attended 2 sessions, 1 week apart, and received either a 600 mL serving of skimmed milk or an isovolumetric and isoenergetic (1062 kJ) serving of fruit-juice together with a fixed energy breakfast. Four h following breakfast *ad libitum* energy intake was assessed. Subjective satiety was determined throughout the morning.	Subjective perceptions of satiety were greatest throughout the morning after skimmed milk, compared with fruit juice ($p < 0.05$) with the differences increasing over the 4 h postprandial period ($p < 0.05$). In accordance with this, participants consumed significantly less energy at lunch after skimmed milk compared with fruit juice ($p < 0.05$). Measures of appetite-related peptides were not quantified.
Almiron-Roig & Drewnowski (2003)	Healthy volunteers, N = 14 males and N = 18 females.	Randomised, crossover trial. Participants attended 4 sessions, 1 week apart, and received one of three isoenergetic (1036 kJ) and isovolumetric (590 mL) servings of energy-containing beverages [cola, skimmed milk, fruit-juice or sparkling water (control)] alongside a slice of toast. Two and a half h following consumption an *ad libitum* lunch was provided. Subjective ratings of hunger, fullness and desire to eat were determined at 20 min intervals throughout.	In comparison to sparkling water, all beverage items reduced subjective hunger, desire to eat and increased ratings of fullness. No differences were recorded in *ad libitum* energy intake across all conditions. Measures of appetite-related peptides were not quantified.

Harper et al. (2007)	Healthy volunteers, N = 22 males.	Randomised, crossover trial. Participants attended 2 sessions whereby participants received either cola (500 mL, 900 kJ) or an isoenergetic and isovolumetric serving of chocolate milk. The preloads were provided 3 h following standardized breakfast consumption, and 30 min preceding *ad libitum* energy intake assessment. Subjective ratings of appetite were determined at 30 min intervals from baseline (t = 0 min) until 30 min after meal termination (t = 240 min).	Subjective perceptions of satiety and fullness were greater following chocolate milk consumption compared with cola (p = 0.0007 and p = 0.0004, respectively). Cola consumption resulted in an increased perception of prospective food consumption and hunger (p = 0.004 and p = 0.01, respectively). Nonetheless, no significant differences in energy intake at the *ad libitum* assessment were observed. Measures of appetite-related peptides were not quantified.
Soenen & Westerterp-Plantenga (2007)	Study 1, N = 30 (15 males and 15 females). Study 2, N = 40 (20 males and 20 females)	Randomised, crossover trial. Participants attended 4 sessions, 1 week apart, and received either an isoenergetic (1500 kJ) and isovolumetric (800 mL) serving of a sucrose-containing preload, high fructose corn syrup-containing preload, milk or a no energy diet preload. Preloads were distributed following baseline blood samples and VAS ratings, and the entire contents had to be consumed within 10 min. Further blood samples were collected again at 15, 30, 60 and 120 min following preload consumption to determine postprandial plasma concentrations of GLP-1, ghrelin, insulin and glucose. Further VAS ratings were collected at 20, 50, 80, 110 and 140 min after preload consumption. *Ad libitum* energy intake assessment took place 50 min following preload consumption.	In comparison to the no energy diet preload, all energy-containing beverages increased satiety. Glucagon-like peptide 1 (GLP-1) (p < 0.001) and ghrelin (p < 0.05) concentrations changed accordingly. No differences in insulin and glucose were observed. No differences in *ad libitum* energy intake were observed 50 min after consumption of the sucrose-containing preload, high fructose corn syrup-containing preload or milk. Furthermore, no differences in VAS ratings or appetite-related peptides were observed after consumption of the sucrose-containing preload, high fructose corn syrup-containing preload or milk.
Tsuchiya et al. (2006)	Healthy volunteers, N = 16 males and 16 females	Randomised, crossover trial. Participants attended 4 sessions, 1 week apart, and received either one of three fruit-flavoured yogurts (semisolid yogurt containing peach, identical yogurt in a drinkable homogenized form, a peach-flavored milk-based beverage) or a serving of fruit-juice. All test preloads were isoenergetic (836.8 kJ). The preloads were distributed 90 min following a light breakfast, and 90 min preceding *ad libitum* energy intake assessment. Ratings of hunger, fullness and desire to eat were collected at baseline (t = 0 min) and at regular 20 min intervals until lunch.	Dairy preloads increased ratings of satiety greater than the fruit-juice and milk-based beverage (p < 0.05). No significant differences in energy intake at the *ad libitum* assessment were observed between all four conditions. Measures of appetite-related peptides were not quantified.

Information concerning postprandial responses of appetite-related peptides to milk and milk-based dairy food consumption in adults is limited (table 6.0); consequently, it remains increasingly difficult to discuss the implications of milk and milk-based dairy consumption on postprandial concentrations of appetite-related peptides. In the two available studies (Dougkas et al., 2012; Soenen & Westerterp-Plantenga, 2007) that quantified appetite-related peptides following milk and milk-based dairy consumption findings are equivocal. In the study conducted by Dougkas et al. (2012) no differences in the postprandial response of plasma glucose, insulin, ghrelin and PYY were observed. However, this may be unsurprising considering the minimal time points in which appetite-related peptides were quantified (0 and 80 min). Including more time points would have indeed provided further insight into the actions of milk and milk-based dairy food consumption on the postprandial response of the appetite-related peptides. In contrast, an increased secretion of GLP-1 and suppression of ghrelin was observed following 800 mL of milk (Soenen & Westerterp-Plantenga, 2007). Nonetheless, postprandial responses of GLP-1 and ghrelin were identical after an isoenergetic (1500 kJ) and isovolumetric (800 mL) serving of a sucrose-containing preload, high fructose corn syrup-containing preload compared with milk consumption. It therefore makes it challenging to elucidate considering the appearance of both peptides occurs in the circulation in proportion to energy content (Huda et al., 2006). Findings related to chapters four and five of the present thesis indicated that mid-morning milk consumption elicited a greater postprandial plasma glucagon response relative to an isoenergetic and isovolumetric serving of fruit-juice drink. In addition, mid-morning yogurt consumption elicited a greater postprandial plasma GLP-$1_{7\text{-}36}$ response and attenuated blood glucose relative to the fruit-juice drink. Furthermore, daily milk consumption increased postprandial glucagon and insulin secretion and attenuates blood glucose concentrations relative to baseline observations, both of which influenced subsequent feeding behaviour at *ad libitum* assessments. It may therefore be suggested that adults might be less sensitive to the actions of appetite-related peptides compared with young people (Bellissimo et al., 2007; Zandstra et al., 2000). Currently, knowledge concerning postprandial responses of appetite-

related peptides to milk and milk-based dairy food consumption arises primarily from studies examining individual dairy components (e.g. dairy protein) as opposed to whole products. Although the effect of dairy protein on satiety or energy intake has been invested extensively studies have often used preloads of 50-70 g of protein, which is considerably higher than that found in standard dairy portions (Hollis & Mattes, 2007). Further research is therefore warranted to better establish the effect of commercially available milk and milk-based dairy foods consumed as whole foods at physiologically relevant intakes in both young people and adult populations on postprandial responses of appetite-related peptides.

6.6 Limitations and future directions

The studies contained within this thesis provide a baseline for further appetite- and metabolism-related research in adolescent populations. Though the work presented throughout this thesis has numerous strengths, the findings are not without limitation. Limitations confined to specific studies are addressed in corresponding experimental chapters, however, some general limitations warrant mention. Firstly, the results arising from this thesis are drawn from several acute- to moderate-term based studies. Consequently, observations require careful interpretation as study findings may not translate over extended periods. It would therefore be of great interest to lengthen observational periods in an attempt to determine the longer-term effect of milk-based dairy food consumption on appetite, feeding behaviour and metabolism in paediatric and adolescent populations. Secondly, a notable limitation of the findings presented throughout this thesis is that results may only be assumed for a relatively small population free-living adolescent males. Caution should therefore be observed when extrapolating the results of this thesis, as findings may not generalise to female adolescents or indeed younger populations. It would thus be advantageous to follow on from this body of work by investigating female adolescents and younger populations. If younger populations were to be studied, it would be prudent to include measures of appetite- and metabolism-related

peptides, especially considering this thesis provides support for the use of fingertip-capillary blood sampling within vulnerable populations. In addition, it would be of value to further examine the influence of this foodstuff utilising isoenergetic, isovolumetric and macronutrient matched items as it is currently unclear whether the aforementioned differential effects of milk-based dairy food consumption on measures of subjective appetite, appetite- and metabolism related peptides and postprandial metabolism are solely a product macronutrient discrepancies. Based on the available literature, increased milk-based dairy consumption and physical activity independently influence body mass, however, gaps remain in the literature concerning the combined effects of these behaviours on body mass and body composition. It would also therefore be of benefit to examine the combined effect of milk-based dairy consumption and physical activity on appetite, feeding behaviour and postprandial metabolism and the impact of this on body mass and composition in paediatric and adolescent populations.

It is important to note that techniques available to assess appetite in young people are not constrained to the methodological approaches utilised throughout this thesis. In the domain of appetite research there are a multitude of techniques that may be applied to assess human feeding behaviour and appetite regulation in young people, and their uses are fully dependant on the objective of the experimental study. For experimental investigations aimed at determining the impact of a food and/or beverage on subsequent feeding behaviour and appetite regulation, researchers to date have tended to use the volume of food consumed as their primary measure (Brunstrom, Shakeshaft, & Scott-Samuel, 2008). The volume of food consumed may be quantified following self-report weighed food records or at supervised eating events such as *ad libitum* meals (Brunstrom et al., 2008). When implementing this approach, the total volume of food consumed is influenced by a range of extrinsic factors including food palatability (Yeomans, Lee, Gray & French, 2001), food and serving size offered (Norton, Anderson, & Hetherington, 2006; Raynor & Epstein, 2001; Rolls, Roe, & Meengs, 2006; Wren et al., 2001), and by hedonic and physiological

factors that inhibit further food consumption (Kissileff & Van Itallie, 1982; Woods, Benoit, Clegg & Seeley, 2004). In an attempt to identify reasons facilitating the total volume of consumption researchers may seek to quantify expected satiation and measure the influence such consumption may have on subsequent feeding behaviour and appetite regulation (Brunstrom et al., 2008). There are numerous ways in which expected satiety may be quantified. Previous studies have implemented questionnaires to identify how filling participants expected certain food to be (Green & Blundell, 1996). Alternatively, the use of photographs have been utilised to estimate and rate how long certain illustrated images would attenuate hunger (Brunstrom et al., 2008; de Graaf, Stafleu, Staal, & Wijne, 1992). In addition to measures of expected satiety, researcher may seek to quantify dietary restraint (Savage, Hoffman, & Birch, 2009). In the scientific literature there are two questionnaires available to researchers to assess dietary restraint in young people, these include the Dutch Eating Behaviour Questionnaire and the Dutch Eating Behaviour Questionnaire-Children (Van Strien, Frijters, Bergers, & Defares, 1986). Although not a direct measure of appetite, a measure of dietary restraint may provide evidence for subsequent feeding behaviour. In this sense, Wardle et al. (Wardle et al., 1992) demonstrated that young (11.8-18.0 y) participants (n = 439 girls and 407 boys) classified with an increased level of dietary restraint had meaningfully lower rates energy, carbohydrate and fat intakes ($p < 0.001$). Indeed, including a measure of expected satiation and dietary restraint may have complimented the work presented throughout this thesis, and may have illustrated further why acute- and moderate-term milk consumption impacted favourably on feeding behaviour outside of the physiological environment.

6.7 Practical implications

The results presented throughout the present thesis may provide numerous important clinical and practical implications. Firstly, for the first time, chapter two demonstrated that at rest fingertip-capillary blood sampling offers an appropriate methodological and reproducible approach to

systematically assess concentrations of appetite- and metabolism- related peptides. Consequently, these findings hold valuable information regarding the utility of fingertip-capillary blood sampling for the quantification and comparison of appetite- and metabolism-related peptides in various populations or field-based scenarios where antecubital-venous blood sampling might be contraindicated. Secondly, although no statistical evidence was sought indicating a difference between milk-based dairy food consumption between middle-childhood and adolescence, for boys and adolescent males, a downward trend of total daily dairy food consumption with increasing age was noted. Considering the nutritional and health-related impact continual milk and dairy food avoidance may incur these results begin to highlight the importance of increasing dairy food consumption, especially in adolescent populations. In chapter four it was found that acute mid-morning milk consumption reduced energy intake at the *ad libitum* pasta meal relative to a fruit-juice drink. The finding that mid-morning milk consumption impacted favourably on feeding behaviour was further replicated in chapter five. To elaborate, daily mid-morning milk supplementation reduced feeding behaviour in the free-living environment, reducing energy intake relative to baseline observations, yet not at the *ad libitum* pasta meal. In contrast, daily supplementation with fruit-juice resulted in an increased energy intake at the *ad libitum* pasta meal relative to baseline observations (chapter five). Together, the findings of chapters four and five illustrate that mid-morning milk consumption may convey a metabolic advantage in free-living adolescent males under resting conditions. In this sense, mid-morning milk consumption impacts favourably on feeding behaviour and it is reasonable to suggest this is facilitated through integrated endocrine responses to food consumption. The wider implications of the evidence provided throughout this thesis need addressing. In particular, it is important to determine whether acute- and moderate-term diary consumption actually provides an application to overweight and obese metabolically diseased populations. Based on these findings, nonetheless, it seems appropriate to encourage the consumption of milk at mid-morning over fruit-juice drinks or other snacks in adolescents.

Indeed, the findings of chapters four and five indicate milk is an attractive alternative to fruit-juice and sugar-sweetened beverages and consequently may provide an important baseline for stakeholders to help shape the development of future nutrition provision for children and adolescents to positively influence young peoples eating habits, especially within the school environment (Gortmaker et al., 1999). The role of stakeholders is of great importance in terms of effectiveness of nutrition education and provision (Hughes, 2010), and the school environment is certainly an ideal setting considering children and adolescents spend much of their time there (Coleman, Shordon, Caparosa, Pomichowski, & Dzewaltowski, 2012). Stakeholders are individuals, groups and organisations who are interested in or affected by the issue under consideration, or have an influence on intervention implementation (Hughes, 2010). For primary and secondary schools, there are a host of stakeholders who can have an impact on school nutrition. These include teachers and staff, parents and/or guardians, pupils, health professional, school retailers and caterers and education authorities (Hughes, 2010). In this sense, it would be advantageous for stakeholders to offer and/or promote the consumption of milk during mid-morning breaks in secondary schools, replacing energy dense food choices that offer litter nutritional benefit. In theory, increasing the provision of milk could positively influence and promote healthy feeding behaviour and avoid food choices that could contribute to weight gain and obesity, reducing and even replacing consumption of sugar-sweetened beverages. Nonetheless, future investigation is certainly warranted to help further establish the longer-term health benefits of milk-based dairy food consumption in paediatrics and adolescents. To achieve this, future investment from public health sectors and collaboration with all related sectors would help form a solid evidence-base from which to help shape the development of future nutrition provision for children and adolescents.

Investment in this area of study is especially important considering the recent advice from the Scientific Advisory Committee on Nutrition (SACN), encouraging a reduction in consumption of ‘free sugars’ (SACN, 2015). In July 2015, SACN published a comprehensive report on

carbohydrates and health, which were last considered by the Committee on the Medical Aspects of Food Policy a few decades ago. The main conclusions emerging from this report detail that the recommended population average (for individuals aged 2 years and above) intake of 'free sugars' should be halved and not exceed 5% of total dietary energy (SACN, 2015). The SACN report also recommend that sugar-sweetened beverage intake should be minimised (SACN, 2015). The aforementioned recommendations are based on an in-depth review of the literature, which for children and adolescents demonstrated that the consumption of sugar-sweetened beverages, compared to non-sugar sweetened beverages results in greater weight gain and increases in BMI in children and adolescents due to increased energy intake and incomplete dietary compensation (SACN, 2015).

Evidence from the most recent National Diet and Nutrition Survey indicate that sugar intakes of all individuals are above recommendations, contributing between 12-15% of total dietary energy intake, with the consumption of sugar and sugar-sweetened beverages highest in school age children (Bates et al., 2014). Consequently, reducing free sugar intake to 5% will be a challenge but by meeting these recommendations within 10 years we could save the NHS approximately £500 million annually (Buttris, 2015). The definition of 'free sugar' set by SACN includes all sugars added to foods plus those naturally present in fruit-juices, syrups and honey. It does not, however, include the sugars naturally present and intact in fruit or vegetables, or milk and milk products (SACN, 2015). The Government and Department of Health have subsequently agreed the free sugar recommendations proposed by SACN. It is therefore of great importance that these recommendations are considered and integrated into official UK dietary advice, and should be reflected throughout official nutrition policy instruments, such as the eatwell plate and advice on institutional catering. Furthermore, additional proposed interventions and/or policies specifically targeting the school nutrition environment including warning labels on sugar-sweetened beverages (Capewell, 2014) and removal of such drinks from schools may offer significant opportunities to

help reduce consumption (Mâsse, de Niet-Fitzgerald, Watts, Naylor, & Saewyc, 2014). Based on these new recommendations, findings arising from the experimental studies contained throughout this thesis, and the fact that milk and milk products are not included under the SACN definition of free sugars provide further justification to promote milk products (in particular milk) as an attractive alternative to fruit-juices and sugar-sweetened beverages. It is important to note, however, that different sources of milk products (for example flavoured milks and ice-creams) may not offer equal merit. In this sense, it may be more appropriate for nutrition policy instruments to focus promotion on un-sweetened milk products as utilised in throughout this thesis. It would therefore be of great interest to establish if different sources of milk products impact on appetite and feeding behaviour is the same or a different manner, and thus provides a platform for future studies.

6.8 Conclusions

Considered together, the research presented in this thesis has built on existing knowledge and contributes to the understanding that milk-based dairy food consumption (in particular milk consumption) conveys a metabolic advantage in free-living adolescent males under resting conditions. In this sense, milk-based dairy consumption impacts favourably on feeding behaviour and it is reasonable to suggest this is facilitated through integrated endocrine responses to food consumption. To summarise, the findings in this thesis have demonstrated:

- At rest, fingertip-capillary blood sampling offers an appropriate methodological and reproducible approach to systematically assess concentrations of appetite- and metabolism-related peptides. These findings hold important implications for researchers and practitioners who wish to quantify and compare the expression of these appetite- and metabolism-related peptides in various populations or field-based scenarios where

antecubital-venous blood sampling might be contraindicated (e.g. paediatrics, adolescents, and elderly). Knowledge concerning the typical error (reported as a coefficient of variation) of the aforementioned [for example fingertip-capillary plasma glucagon (8.2%)] can subsequently be applied to facilitate future study design, where sample size estimations are based on these findings.

- Independent of age, boys consume greater amounts of milk compared to girls. Milk represents the most popular dairy food consumed among children and adolescents, consumed by 91% of participants (across all ages and sexes). Although non-significant, for boys and adolescent males, a downward trend of total milk and dairy food consumption with increasing age was noted whereas patterns of milk and dairy food consumption remained widely stable among girls and female adolescents.

- In an acute setting the consumption of milk influences short-term feeding behaviour, suppressing energy intake at an *ad libitum* pasta meal compared to the fruit-juice drink (-564.4 kJ). Mid-morning milk consumption elicited a greater postprandial (90-180 min) plasma glucagon response (16.8 pg·mL) and led to an increased rate of energy expenditure (109.2 kJ) relative to an isoenergetic and isovolumetric serving of fruit-juice drink. In addition, mid-morning yogurt consumption elicited a greater postprandial (90-180 min) plasma GLP-$1_{7\text{-}36}$ response and attenuated blood glucose relative to the fruit-juice drink (1.2 pg·mL), however, this appeared insufficient to influence measures of subjective appetite, feeding behaviour or energy expenditure relative to the fruit-juice preload.

- In a moderate-term setting, milk supplementation for 4-weeks (28 days) impacts favourably on feeding behaviour under free-living conditions, reducing energy intake (1882.8 kJ) relative to baseline observations. Milk supplementation elicits as increased postprandial glucagon (90-180 min, -45.4 pg·mL) and insulin (0-90 and 90-180 min, -79.4 and -32.4 pmol·L respectively) secretion and attenuates blood glucose concentrations (90-180 min, 0.3 mmol·L) relative to baseline observations. Daily milk supplementation, however, failed to impact on measures of postprandial metabolism, and reasons for this may involve the lack of energy-restriction during supplemental periods. In contrast, supplementation with fruit-juice resulted in increased energy intake at the *ad libitum* pasta meal (-887.8. kJ) Nonetheless, we observed no capacity for fruit-juice supplementation to influence measures of subjective appetite, appetite and metabolism-related peptides or postprandial metabolism relative to baseline observations.

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APPENDICES

APPENDIX A

EXAMPLE ELECTROCHEMILUMINESCENCE PROCEDURE

ELECTROCHEMILUMINESCENCE

PRINCIPLE OF THE ASSAY

Quantitative assessments of plasma GLP-$1_{7\text{-}36}$, glucagon, insulin and leptin were determined in duplicate by electrochemiluminescence (Sector Imager 2400, MesoScale Discovery [MSD], Maryland, USA) using 40µL of plasma in a human hormone multiplex assay kit (Sector Imager 2400, MSD, Maryland, USA). Briefly, the process of electrochemiluminescence detection utilises labels that emit light when electrochemically stimulated. The Sector Imager 2400 utilises custom designed optics and ultrasensitive photodetectors, which derives a quantitative measure of light emitted. By means of trademarked electronics and efficient signal procession algorithms, the intensity of light and signal emitted quickly translates to useful data. The succeeding paragraphs aim to provide an overview of the assay protocol utilised throughout to determine concentrations of GLP-$1_{7\text{-}36}$, insulin, glucagon and leptin.

PREPARATION OF BLOCKER A SOLUTION

To make up the **Metabolic Assay Working Solution** dispense and combine the following items for one plate use only.

Weigh out 1.25 g of Blocker A dry powder

Add 20 mL of distilled water and stir until all protein is resupended.

Add 5 mL of MSD Phosphate Buffer (5X)

PREPARATION OF METABOLIC ASSAY WORKING SOLUTION

To make up the **Metabolic Assay Working Solution** dispense and combine the following items in an appropriate sized vial. A 15 mL tube is recommended (per assay plate).

35 µL of Aprotinin

70 µL of Blocker E

6895 µL of Diluent 6

PREPARATION OF DETECTION ANTIBODY SOLUTION

To make up the **detection Antibody Solution** dispense and combine the following items in an appropriate sized vial. A 15 mL tube is recommended (per assay plate).

90 µL of 10% Blocker D-B

90 µL of 10% Blocker D-R

60 µL of

30 µL of 100X Anti-hInsulin Antibody

30 µL of 100X Anti-Glucagon Antibody

30 µL of 100X Anti-hLeptin Antibody

2700 µL of Diluent 12

PREPARATION OF READ BUFFER T

To make up **Read Buffer T**, dispense and combine the following items in an appropriate sized vial. A 20 mL tube is recommended (per assay plate).

5 mL of Stock read Buffer T (4X)

15 mL of Distilled water

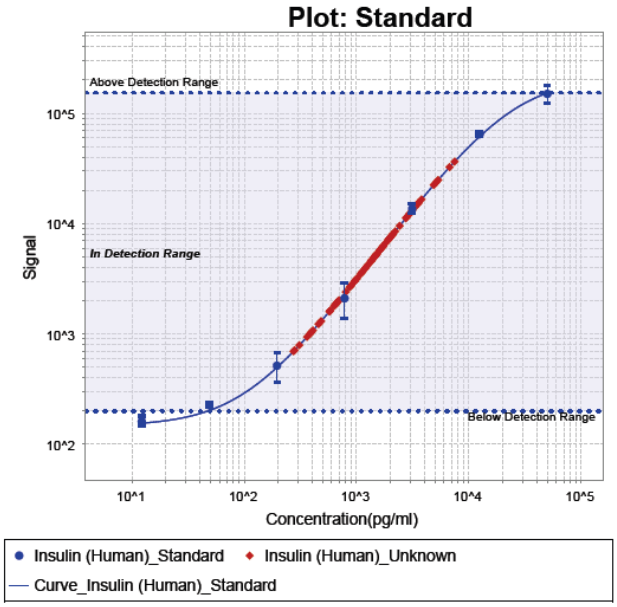
PREPARATION OF CALIBRATORS

STANDARD	GLP-1 ($_{7-36}$) con. (pg/mL)	Insulin conc. (pg/mL)	Glucagon con. (pg/mL)	Leptin conc. (pg/mL)	Dilution Factor
Stock conc.	1000000	5000000	1000000	10000000	-/-
STD-01	10000	50000	10000	100000	100
STD-02	2500	12500	2500	25000	4
STD-03	625	3125	625	6250	4
STD-04	156	781	156	1563	4
STD-05	39	195	39	391	4
STD-06	9.8	49	9.8	98	4
STD-07	2.4	12.2	2.4	24	4
STD-08	0	0	0	0	N/A

NOTE: To prepare this 8-point standard curve:

I. Prepare the highest Calibrator by adding 10 µL of 1 µg/mL GLP-1 (7-36)amide, 10 µL of 5 µg/mL Insulin, 10 µL of 1 µg/mL Glucagon and 10 µL of 10 µg/mL Leptin to 960 µL of Metabolic Assay Working Solution.

II. Prepare the next Calibrator by transferring 50 µL of the diluted Calibrator to 150 µL of Metabolic Assay Working Solution. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators. The recommended 8th Standard is Assay Diluent Working Solution (i.e. zero Calibrator). Calibrators should be kept on ice prior to addition to the plate



Example standard curve for insulin. Blue dots represent known standard concentrations. Blue line represents generated standard curve. Red dots represent unknown values (subject values).

ASSAY PROTOCOL

1. Take out appropriate plasma samples along with the MSD MULTIPLEX kit and allow thaw at room temperature.

2. Make up **Blocker A Solution**.

3. Each well of the plate requires 150 µL of the Blocker A solution. Once dispensed, seal the plate with an adhesive plate seal and incubate for 1 hour (room temperature) plate shaker (300-1000 rpm). Shaking the assay plate will…

4. Appropriately label eppendorf tubes for the formulation of the standards. Following this, make up the standards as per below.

5. Following shaking, wash the plate 3X with phosphate buffer solution + 0.05% Tween-20.

6. Dispense 20 µL of the **Metabolic Assay Working Solution** into all wells. Immediately pipette 40 µL of the plasma sample or calibrator in to appropriate wells. Once dispensed, seal the plate with an adhesive plate seal and incubate for 2 hour (room temperature) plate shaker (300-1000 rpm).

7. Following shaking, wash the plate 3X with phosphate buffer solution + 0.05% Tween-20.

8. Dispense 25 µL of the **1X Detection Antibody Solution** into all wells. Once dispensed, seal the plate with an adhesive plate seal and incubate for 2 hour (room temperature) plate shaker (300-1000 rpm).

9. Following shaking, wash the plate 3X with phosphate buffer solution + 0.05% Tween-20.

10. Dispense 150 µL of the **1X Read Buffer T** into all wells. The plate is ready to be analysed.

APPENDIX B

EXAMPLE FOOD DIARY TEMPLATE

FOOD AND

EXERCISE DIARY

Participant Code:……

TRIAL:……

You have been asked to record your food intake and activity patterns for the remainder of your trial date.

You will be asked to repeat this for the next two trials.

PLEASE COULD YOU COMPLETE THE FOLLOWING:

MEASURE	READING
Date of Birth	
Age (years)	
Gender (M/F)	
Weight (kg)	
Standing Stature (cm)	
Seated Stature (cm)	
Body Mass Index (kg/m^2)	

FAMILIARISATION	RECEIVED (Y/N)
Kitchen weighing scales – correct measuring technique	
Reporting food consumption	
Food recall interview	
Accelerometer placement (right hip)	

THANKS FOR TAKING THE TIME TO COMPLETE YOUR
FOOD AND

EXERCISE DIARY

INSTRUCTIONS

KEEPING A RECORD OF WHAT YOU NORMALLY EAT AND DRINK ENABLES THE DIETICIAN TO CALCULATE YOUR CURRENT DAILY FOOD INTAKE. SO THEREFORE SUBSEQUENT NUTRITIONAL INFORMATION MAY BE TAILORED TO YOUR SPECIFIC REQUIREMENTS

* RECORD **EVERYTHING** YOU EAT AND DRINK OVER A SEVEN DAY PERIOD

* DO NOT ALTER THE FOOD AND FLUID YOU NORMALLY CONSUME

* RECORD THE TIME AT WHICH THE FOOD OR FLUID WAS CONSUMED

* RECORD ALL FOOD EATEN **AS SOON AS POSSIBLE AFTER IT IS CONSUMED**

* IDEALLY AT ALL TIMES THE FOOD RECORD CHARTS SHOULD BE CLOSE AT HAND AS CONTINUAL RELIANCE ON MEMORY INCREASES ERROR

* LIST THE FOODS IN THE ORDER IN WHICH THEY ARE USUALLY EATEN. NOTE BEVERAGES ARE USUALLY STATED LAST AT THE END OF EACH MEAL

* NOBODY IS GOING TO CRITICISE WHAT YOU EAT, SO BE HONEST. DO NOT LEAVE OUT ANY WINE / BEER / SPIRITS OR ANY SWEET / SAVOURY SNACKS, AS THESE WILL CONTRIBUTE SIGNIFICANTLY TO YOUR OVERALL ENERGY INTAKE

* IT IS IMPORTANT TO RECORD ANY **SUPPLEMENTS** TAKEN i.e.: CREATINE, H5, VITAMIN/MINERAL COMPLEXES etc

* RECORD THE AMOUNT i.e. 1 TABLET / 200 MG, AND WHEN YOU TAKE THE SUPPLEMENT IN THE TABLE PROVIDED

INCLUDE AS MUCH DETAIL AS POSSIBLE ABOUT THE FOOD CONSUMED

* IT IS ESSENTIAL TO INCLUDE THE AMOUNTS/PORTION SIZE OF FOOD ACTUALLY EATEN. THIS CAN BE ACHIEVED BY USING HOUSEHOLD MEASURES.

  i.e.: TEASPOON / DESSERT SPOON / TABLESPOON / ICE-CREAM SCOOP
  SMALL / MEDIUM / LARGE PORTIONS / GLASS
  ¼, ½, 1 PINT
  THIN / THICK SPREADING

* IF YOU CANNOT MAKE A RELIABLE ESTIMATE OF A FOOD WEIGHT, DESCRIBE THE FOOD SIZE AS ACCURATELY AS POSSIBLE i.e.: 2 x LOW FAT PORK SAUSAGES 1 INCH DIAMETER, 3 INCHES LONG, RATHER THAN GUESSING THE WEIGHT

* IF CONSUMING PRE-PACKAGED FOODS i.e. PACKET OF CRISPS, IF AT ALL POSSIBLE, TRY TO LOOK AT THE PACKET AND RECORD THE ACTUAL WEIGHT

* RECORD ANYTHING ADDED TO DRINKS e.g. SUGAR, MILK etc

INSTRUCTIONS (CONTINUED)

* **SPECIFY THE TYPE OF FOOD**
  i.e. BREAD: SMALL / MEDIUM / LARGE LOAF
  THIN / MEDIUM / THICK SLICE
  WHITE / BROWN / GRANARY

  MILK: SKIMMED / SEMI-SKIMMED / FULL-FAT
  PASTEURISED / STERILISED / UHT

* IF KNOWN INCLUDE THE BRAND NAME i.e. KELLOGS CORNFLAKES, DEL MONTE FRESH UNSWEETENED ORANGE JUICE

* KEEP FOOD LABELS FOR REFERENCE IF POSSIBLE

* **SPECIFY THE COOKING METHOD**
  IT IS IMPORTANT TO RECORD THE COOKING METHOD AS THIS MAKES A DIFFERENCE TO THE OVERALL ENERGY CONTENT OF THE DIET

  i.e. RAW / BOILED / POACHED / SMOKED / GRILLED / SHALLOW FRIED / DEEP-FRIED / BRAISED / ROASTED

* TRY TO INCLUDE RECIPES FOR HOMEMADE DISHES IF AT ALL POSSIBLE

* IF ONE RECORDS A FOOD ITEM / MEAL BUT ONLY EATS A FRACTION, REMEMBER TO RECORD THE ACTUAL AMOUNT EATEN AND THE AMOUNT LEFT i.e. ¼, 1/3 etc

* NOTE ANYTHING YOU DO NOT EAT e.g. JACKET POTATO (NOT SKIN), CHICKEN (NOT SKIN)

RECORD ALL EXERCISE UNDERTAKEN:

* RECORD THE TIME YOU CARRY OUT THE EXERCISE

* RECORD THE DURATION OF YOUR EXERCISE SESSION e.g. 60 MINUTES

* SPECIFY THE INTENSITY OF YOUR EXERCISE SESSION:
  e.g. LEVEL 5 ON TREADMILL / 7 MIN PER MILE RUNNING PACE / 6 MPH ON TREADMILL / 15 MPH ON BICYCLE
  VERY LIGHT / MODERATE / HARD / VERY HARD
  COULD STILL TALK / SLIGHTLY OUT OF BREATH / BREATHING VERY HARD

TIME OF CONSUMPTION	DESCRIPTION OF FOOD OR FLUID	WEIGHT OF PRODUCT	COOKING METHOD
Breakfast			
6:00 am	Del Monte unsweetened fresh orange juice	250mL	
	Kellogs Cornflakes	med bowl (50g)	
	Granulated sugar	2 x tsp(15g)	
	Semi-Skimmed milk (pasteurised)	1 x sm glass 125mL	
9:30 am	Streaky bacon	3 x rashers	Grilled
	Pork sausages	2 x (4"x1") (80g)	Grilled
	Scrambled egg	med portion	Microwave
	Large, medium sliced wholemeal toast	2 x slices	Toasted
	Thin spreading anchor butter	1 x knob (7g)	
	Thin spreading seedless raspberry jam	1 x tsp (15g)	
	Mug of tea	x 1	
	Semi-skimmed milk (pasteurised)	1 x tblsp (20mL)	
	Granulated sugar	1 x tsp (7g)	
Mid Morning			
11 am	McVities large chocolate digestive biscuits	x 3 (45g)	
	Mug of coffee	x 1	
Lunch			
1 pm	Heinz country vegetable soup (tinned)	1 x med bowl (350g)	
	Crusty granary roll	2 x small (150g)	
	Cheese and ham sandwich containing:	x 1	
	-Large, medium sliced white bread	2 x slices	
	-Mild cheddar cheese	1 x matchbox size	
	-Thickly cut honey roast ham	1 x bread size	
	-Hellman's Mayonnaise	1 x tsp (30g)	
	Ski Bio (reduced fat) peach/mango yoghurt	1 x 150g pot	
2:30 pm	Banana	1 x med (80g)	Raw
	Blackcurrant squash made with soda water	1 x pint	
Mid Afternoon			
4 pm	Packet Seabrook cheese and onion crisps	1 x 30g pack	
	Kit-Kat (4 bar)	x 1	
	Mug of tea	x 1	
	Semi-skimmed milk (pasteurised)	1 x tblsp)	
Dinner			
6pm	Steak and kidney pie (individual, round with puff pastry top)	x 1 (6" x 6")	
	Jacket potato	1 x large	Baked
	Anchor butter	1 x knob	
	Carrots	med portion (45g)	Boiled
	Broccoli	med portion (120g)	Boiled
	Rhubarb and apple crumble	med portion	
	Bird's custard made with semi-skimmed milk	130 ml	
7:00 pm	Mug of tea	x 1	
Supper			
10:00	Jacob's cream crackers	x 4 (75g)	
	Mild cheddar cheese	30g	
	Flora Light	1 x knob (9g)	
	Mug of Cadbury's chocolate break	1 x 4 tsp	

APPENDIX C

EXAMPLE INFORMATION SHEET AND INFORMED CONSENT

Ben Green (M.Sc)
PhD Research Student
Department of Sport, Exercise and Rehabilitation
Northumberland Building
Newcastle upon Tyne
NE1 8ST
Tel: +44(0)191 2437018
Email: benjamin.green@northumbria.ac.uk

TITLE OF PROJECT:

Participant ID Number:

Principal Investigator: Mr. Benjamin Green

1. What is the purpose of the project?
Dairy products represent a nutrient dense foodstuff, and inclusion as part of a healthy balanced diet is widely recognised. An emergent body of literature exists concerning the potential role of dairy consumption and body mass, suggesting a modest inverse association. Several mechanisms concerning the underlying relation between dairy consumption and body mass have been proposed, and include: increased postprandial fat oxidation, manipulation of appetite-regulating gut peptides influencing subsequent energy intake.

In collaboration with Cardinal Hume we would like to explore the impact daily exposure to a commercially available dairy snack plays on metabolism and appetite regulation.

2. Why has my son been selected to take part?
Your son has been selected to participate in the abovementioned study because they are between the ages of 15-18 years old and does not hold an aversion/dislike to milk or milk based products.

3. What will my son and I have to do?
If your son decides to participate in the abovementioned investigation they will be entered into a 4-week intervention. Your child will be asked to visit the physiology laboratory within Northumbria University on two occasions, once preceding and once following the intervention. Laboratory visits will take place on a weekend. During the intervention itself, your son will attend a daily snack session. Daily snack sessions will take place at mid-morning break and on school campus, during which time they will be requested to consume a semi-skimmed milk dairy snack or control. Please take the time to read through this document thoroughly, detailing all the procedures that we intend to implement during testing periods.

Weekend Testing Sessions
Preceding and following the intervention, your child will be required to visit the clinical testing laboratory at Northumbria University on one weekend day. Each weekend testing session will last approximately 5-hours. Prior to your child's first weekend session we require your son to record all food/drinks items consumed for the 24 hours preceding the first treatment. This will be subsequently supplemented with a dietary recall interview on arrival at the lab. We request these habits are replicated for the 24 hours preceding the final weekend testing session (e.g. foods consumed, portion sizes). **It is imperative that your child replicates his dietary habits for the 24 hours prior to each visit, as this can dramatically influence food intake and appetite.**

Additionally, we request your son to record any fluids (water only) consumed upon waking to arrival at their first laboratory visit. We kindly ask this is replicated prior to their second laboratory visit. We ask your son to refrain from any vigorous exercise in the 24 hours preceding laboratory visits. On your child's first weekend session, they will undergo a number of preliminary laboratory measures.
Your child will need to be on University campus at approximately 0815am (City Campus, Sport Central). A member of the research team will be there to greet your child. If transport to and from the University is required please inform the lead researcher and this can be organised for you. Testing will commence at approximately 0845am.
During testing, your child is not permitted to eat or drink (except water and the foods given throughout each session) as this may interfere with study results. **Please note: It is very important your son/daughter does not consume breakfast/any food or drink items, apart from water, prior to each weekend visit.** (We will provide breakfast and a pasta lunch meal for your child throughout each testing day). Following test termination no further dietary restrictions will be in place, however, your son will be asked to refrain from exercise for the remaining hours of the day.

Preliminary Laboratory Measures:
These measures will include measuring height, body weight and percentage body fat (using a non-invasive machine called the Bod Pod). These measures will be collected in private, in the presence of two same sex researchers. The nature of the Bod Pod assessment is non-invasive. This process may result in some students feeling claustrophobic, but this is very rare. Your child will be made fully aware of this prior to entering the instrument. Additionally, we will obtain a fingertip capillary blood sample (0.6mL). This small blood sample will enable us to quantify levels of triglycerides and cholesterol. If they wish to withdraw from any specific measurement they are able to do so. To standardise food intake and energy expenditure we ask that your son replicate their dietary and physical activity habits in the 24 hours prior to each testing day. To facilitate this, photocopies of their food diary will be issued to enable replication (food types, portion sizes).

Test Meals:
Breakfast-

The breakfast meal will consist of semi-skimmed milk (Sainsbury, UK) and Kellogg's Rice Krispies (Kelloggs, Manchester, UK), designed to provide 10% of the participants daily estimated energy requirement at a cereal to milk ratio of 30g: 125mL.

Dairy snack-

Approximately 90-minutes following breakfast your child will be distributed one semi-skimmed milk dairy snack. The dairy snacks and portion sizes will be based on measures collected from our previous work.

Ad-libitum past lunch-

90-minutes following dairy snack consumption your son/daughter will be presented with a pasta meal. Served in a tomato based sauce (Dolmio express tomato and basil pasta sauce, Masterfoods, UK) the meal will consist of pasta shapes (farfalle pasta shapes, Tesco, UK) along with grated cheddar cheese and olive oil (Tesco, UK). Your child will be allowed to eat as much of this meal as they wish until they indicate they are comfortably full.

Blood Sampling:
At nine intervals throughout each condition, finger-prick capillary blood samples (0.3 mL) will be collected for the determination of GLP-$1_{7\text{-}36}$, glucagon, insulin, leptin and glucose. In total, this will

result in 2.7 mL of blood collected over each visit. Approximately 5 min preceding each capillary sample (parallel to gas analysis) participants will submerge the entire sample hand in warm-water to ensure an adequate flow of capillary blood. On removal the hand will be dried thoroughly and the identified site for puncture further cleansed with an aseptic alcohol wipe. On puncturing the fingertip, the first drop of blood will be removed before subsequent collection. This process will be followed at each sample point.

Subjective Appetite – Visual Analogue Scales (VAS):
Immediately following each capillary blood sample, your child will be asked to complete a series of VAS to determine subjective appetite (hunger, gut fullness and prospective food consumption). Anchored with feelings of extremity, your child will simply be asked to mark a vertical line on the 100mm horizontal line, between the anchored terms, which they feel fittingly, represents how they are feeling at that time.

Gas Analysis:
Throughout testing, 5 min samples of continuous expired air will be collected preceding each VAS and blood sample to determine energy expenditure, fat and carbohydrate usage. For this measure your son will be required to wear a lightweight face mask and breathe as normal. At first the mask may feel a little restrictive, however, this is short-lived and after approximately 5-10 seconds this sensation disappears.

Daily Snack Sessions
During the intervention itself, your son will attend a daily snack session. Daily snack sessions will take place at mid-morning break and on school campus, during which time they will be requested to consume a semi-skimmed milk dairy snack or control. Snack sessions will be fully supervised by a member of the research team. In total, testing will last 4-weeks.

4. What are the exclusion criteria (i.e. are there any reasons why my son/daughter should not take part)?
Your child should not take part in the present investigation if they:

1) Are outside of the stipulated age range (15-18 years old)
2) A diabetic
3) Hold an aversion/dislike to milk, milk based products and the test meals provided.
4) Are known to be lactose intolerant
5) Currently taking any form of medication known to affect taste, smell and appetite

5. Will my son‘s participation involve any physical discomfort?
If your son engages in this study they will be required to give a number of capillary blood samples, collected by finger lancing. The process of fingertip-capillary blood sampling may leave the tip of the punctured finger a little sensitive with the potential for bruising, although occurrence of this is minimal. This may feel uncomfortable, however, will be short-lived. Your child will verbally be made aware of this prior to their first sample and asked if they have preferred finger from which the sample will be drawn. In order to help minimise any discomfort, proper aseptic methods will be followed, and all sampling shall be conducted by a professionally trained researchers.

6. Will my son‘s participation involve any psychological discomfort or embarrassment?
No.

7. Will my son have to provide any bodily samples (i.e. blood, saliva)?
Your son will be subject to fingertip capillary blood sampling. Briefly, 0.3mL capillary blood

samples shall be collected at nine intervals throughout each testing day. The finger-prick blood samples will involve trained researchers taking a very small amount of blood from your child's fingertip, using a small finger-prick needle known as a disposable lancet. This is a routine procedure which we use in our laboratories on a day-to-day basis. As parents/guardians you are welcome to attend the test days and accompany your child during the blood sampling. If you wish to do, please inform the principal investigator if you would like to do this.

8. How will confidentiality be assured?
A number of procedures have been put in place in order to assure your child's confidentiality. Firstly, your child will be issued with a unique identification code that will always be used to identify any data they provide. This will prevent any association between participant and data set. Documents containing your child's name against ID numbers will be stored separately from those containing ID numbers and in a locked filing cabinet. All documents containing information about your child will be stored securely in a locked filing cabinet which will only be accessible to the Principal Investigator named above. Any information you or your child provide will be used only for the original intended purpose of the investigation unless express informed consent is granted by yourself and your child. All electronic data will be stored on password-protected computers.

9. Who will have access to the information that me and my son/daughter provide?
Any information and data gathered from you and your child will only be available to the Principal Investigators identified in the information sheet (Ben Green) and members of the research team.

10. How will my son's information be stored / used in the future?
All information and data gathered from your child during this investigation will be stored in line with the Data Protection Act and will be destroyed after a maximum of 10-years following the termination of the study. During that time the data may be used by members of the research team only for purposes appropriate to the research question, but at no point will your child's personal information or data be revealed.

11. Has this investigation received appropriate ethical clearance?
Yes, this study and its protocol have received full ethical approved from the School of Health and Life Sciences Ethics Committee. If you require confirmation of this please contact the Chair of this committee, stating the title of the research project and the name of the principle investigator.
Chair of the Faculty of Health and Life Science Ethics Committee (Dr Mic Wilkinson)
Northumberland Building
Northumbria University
Newcastle upon Tyne
NE1 8ST
Email: mic.wilkinson@northumbria.ac.uk

12. Will my son/daughter receive any financial rewards / travel expenses for taking part?
No financial rewards or expenses for travel will be given to your child in return for his/her participation.

13. How can I withdraw my child from the project?
You are reminded of your right to withdraw your child from the study at any time. If you choose to do so, please do not hesitate to contact the Principal Investigator (Ben Green) as soon as possible at benjamin.green@northumbria.ac.uk, giving your child's confidential participant number code (on top of this sheet) and all your data will be deleted. The research team will facilitate your request and discuss with you how you would like your data to be treated in the future. If, for any reason after

participating you wish to withdraw your child's data from the study please do not hesitate to contact the Principle Investigator within a month of your participation in the study. After this date, it may not be possible to withdraw your individual data as the results may already have been published. As all data are anonymised, your individual data will not be identifiable in any way.

14. If I require further information who should I contact and how?

At any time, leading up to or throughout the investigation, you wish to speak to one of our research members please do not hesitate to contact the Principal Investigator (Ben Green) at Northumbria University who is the lead researcher of this study.

Address: **Mr. Ben Green**
PhD Research Student
School of Life Sciences
Room 431 Northumberland Building
Northumbria University
NE1 8ST
Email: benjamin.green@northumbria.ac.uk

Participant Identification number: _____

please where applicable	*tick*
I have read and understood the Participant Information Sheet.	☐
I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.	☐
I understand I am free to withdraw my child's data from the study at any time, without having to give a reason for withdrawing, and without prejudice.	☐
I agree to let my son/daughter take part in this study.	☐
I would like to receive feedback on the overall results of the study at the email address given below. Email address.....	☐
I am aware that primary data will be kept secure and the location of such data will only be known to the University. I agree to such data being held for a period of ten years after the completion of the research project, as required by several Research Councils.	☐

Signature of participant..... Date.....……..

(NAME IN BLOCK LETTERS).....…….

Signature of Parent / Guardian in the case of a minor

.....

APPENDIX D

EXAMPLE VISUAL ANALOGUE DIARY

Visual Analogue Diary

Name ……

Subject Code ……..

Trial Code ……

Date of Visit …….

You have been asked to keep a visual analogue diary during each day of your study trials. Please bring this diary with you to your next trial date or return to the researchers as soon as possible. On each scale please specify how you feel by marking a small vertical line between the anchored terms, which represents how you are feeling at that time.

Visual Analogue Scale Appetite

Participant: Date:

Trial: Time point: Baseline

How hungry do you feel?

I am not hungry at all. ——— I have never been more hungry.

How full do you feel?

Not at all full. ——— Totally full.

How satisfied do you feel?

I am completely empty. ——— I cannot eat another bite.

How much do you think you can eat?

Nothing at all. ——— A lot.

How tired do you feel?

Not at all tired. ——— Extremely tired.

Do you have any stomach discomfort?

No discomfort ——— Extremely uncomfortable

APPENDIX E

EXAMPLE AD LIBITUM PASTA MEAL MAKE UP AND RECORDING SHEET

Making the ad-lib pasta meal

The meal is started once the participant has had the porridge in the morning.

Process

1. Weigh mixing spoon and record exact weight on sheet
2. Weigh small serving bowl and record exact weight on sheet
3. Place a bowl on balance, tare balance and weigh out 400 g fusilli pasta
4. Record exact weight of pasta on sheet
5. Boil water in kettle and add 1500 ml boiling water to the pasta in bowl
6. Microwave at 1000 W for 7 min
7. Stir pasta gently
8. Microwave at 1000 W for 7 min
9. Stir pasta gently
10. While pasta is in microwave prepare cheese, pasta sauce and oil:
    a. Place a small plate on balance, tare balance and add 125 g grated cheese
    b. Record exact weight of cheese on sheet
    c. Weigh a large mixing bowl and record exact weight on sheet
    d. Tare balance with large mixing bowl and add 350 g pasta sauce
    e. Record exact weight of sauce on sheet
    f. Place a small container on balance, tare balance and add 40 g olive oil
    g. Record exact weight of oil on sheet
11. Drain pasta in a colander for 2 min (total)
    a. Transfer from bowl to colander three times shaking each time
    b. Leave in colander for rest of 2 min
    c. Dry bowl and return pasta to bowl at 2 min
12. Add cheese to the pasta slowly, mixing as you go
13. Pour pasta and cheese into the large mixing bowl with the pasta sauce and mix until homogenous
14. Add 40 g olive oil to large mixing bowl and mix until homogenous
15. Weigh bowl, contents and spoon together and record exact weight on sheet
16. Before serving warm mixing bowl for 3 min at 1000 W and record post-heating weight (bowl, contents and spoon) on sheet
17. Serve in the small serving bowl and continuously refill bowl before participant has finished and until they are comfortably full
18. Record the time the participant eats for
19. Weigh the serving bowl (without cutlery) and contents and record exact weight on sheet
20. Weigh the mixing bowl and remaining contents with the spoon and record exact weight on sheet

Subject: **Trial:** **Date:**

Preparation weights

	Mixing bowl	Serving bowl	Mixing spoon	Dry pasta
Weight (g)				

	Cheese	Pasta sauce	Olive Oil	Total weight pre heating (cooked pasta + ingredients + mixing bowl + spoon)
Weight (g)				

Total Energy (kcal) of ingredients = 2493kcal

Pasta= 1440kcal (360/100g) Sauce= 168kcal (48/100g) Cheese= 525kcal (420/100g) Oil= 360kcal (900/100g)

Serving weights

Pasta (g) pre heating *(total weight pre heating – mixing bowl - spoon)*	Total weight (g) post heating *(pasta + bowl + spoon)*	Pasta (g) post heating *(total weight post eating – mixing bowl – spoon)*	Total weight (g) of serving bowl (and left pasta)	Total weight (g)of mixing bowl + spoon (and left pasta)

Total end weight (g) *(serving and mixing bowl and contents)*	Pasta (g) left uneaten *(total end weight – mixing bowl – serving bowl – spoon)*	Pasta (g) eaten *(pasta (g) post heating – pasta (g) left uneaten)*

Energy Intake

Pasta (g) post heating	Energy (kcal) per gram of pasta	Energy Intake (kcal) from pasta eaten

Time started eating: Time finished eating: Total time eating: