Physiological studies of whey protein in older people, with a focus on feeding behaviour, nutrition, obesity, type 2 diabetes mellitus and post-prandial blood pressure

A thesis by
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Jis ke sir upar tu swami so dukh kaisa pave

O Lord, one who is under your protection, one who considers you to be above himself

He will not experience any suffering in life

Guru Granth Sahib, 473

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THESIS ABSTRACT

Australia's population is ageing. Health problems are more prevalent in elderly than younger people, and lead to much of the health expenditure and health resource allocation in this and other countries. Nutritional problems are common in the elderly and often contribute to the development and worsening of health problems in older people. A particular problem is the interacting effects of under-nutrition, reduced dietary protein intake, anabolic resistance to dietary protein (the need for greater intakes to produce the same benefits, particular on muscle mass and function) and consequent muscle loss, reduced function, and in some cases the development of sarcopaenia. Both type 2 diabetes (T2D) and obesity are prevalent in older people – and often coexist. Dietary measures are key to the management of both conditions, in older as in younger adults. Protein supplements either alone or in combination with other macronutrients are increasingly recommended to, and used by, older people, to prevent and/or manage these problems. Whey protein is often used alone or as part of these supplements, as it is has particularly beneficial effects on muscle anabolism. There remain gaps in our knowledge of the effects of whey protein ingestion in older people. This thesis comprises clinical studies designed to fill some of those gaps.

The studies in this thesis from five clinical trials involved assessing responses to oral whey protein ingestion particularly whey protein concentrate on feeding behaviour, nutrition, obesity, T2D and blood pressure (BP) in older and younger people with a focus on older people. Dietary protein supplementation may play a role in the management of these conditions. The principal outcomes assessed were appetite; food intake; gut hormone release; gastric emptying rate; circulating glucose,

glucagon and insulin concentrations; BP and heart rate (HR).

Chapter 1: Introduction focusses on rising burden of ageing in Australia and other developed countries; nutritional problems and adverse body composition changes associated with ageing i.e. undernutrition and sarcopaenia; nutritional management of under-nutrition and sarcopaenia with a focus on the effects of whey protein; rising rates of obesity, T2D and BP effects in older people and whey protein as a management strategy; gastric emptying and its effects in older people.

Chapter 2 is a review of the current literature relating to the use of protein supplements in the elderly, dietary protein requirements in older people, and the effects of ageing on the gastrointestinal responses to protein ingestion.

Chapter 3 describes the effect of differing whey protein loads (control, 30 g whey protein and 70 g whey protein) before breakfast on appetite and food intake at subsequent breakfast, lunch and dinner in healthy younger and older men. Energy intake was suppressed by whey protein drinks in a protein load-responsive fashion at breakfast and particularly, at lunch, but not at dinner, and suppression of energy intake by protein was less in healthy older than younger men. Cumulative protein intake was increased in a protein load responsive fashion. These findings support the use of whey-protein drink supplements in healthy older patients who aim to increase their protein intake without decreasing their overall energy intake (ACTRN12618000881235).

Chapter 4 describes the effect of whey protein load on energy intake, appetite and gastric emptying in young and older, obese men. The 30 g whey protein drink did not suppress appetite or energy intake in obese younger or older men suggesting that

obesity may blunt/abolish the age-related effect of whey protein on suppression of energy intake (ANZCTR12616001216404).

Chapter 5 describes the effects of co-ingesting whey protein and glucose alone and combined (a drink containing either 30 g glucose, 30 g whey protein, 30 g whey-protein plus 30 g glucose or control) on blood glucose, plasma insulin and glucagon concentrations in healthy younger and older men. The addition of 30 g of whey protein to 30 g of glucose in drink form substantially attenuated the increase in blood glucose concentrations induced by glucose alone; the magnitude of the whey-induced reduction in blood glucose was not affected by age, with comparable reductions in older to those in younger adult men; the stimulation of plasma insulin concentrations by whey protein was not reduced by ageing, unlike the insulin response to glucose; whey protein suppressed hunger less in older than younger men. Glucagon concentrations were unaffected by age. These results demonstrate that the ability of whey-protein to reduce carbohydrate-induced postprandial hyperglycaemia is retained in older men and that protein supplementation may be a useful strategy in the prevention and management of T2D in older people (ACTRN12619000420145).

Chapter 6 describes the effects of co-ingesting whey protein and glucose alone and combined (a drink containing either 30 g glucose, 30 g whey protein, 30 g whey-protein plus 30 g glucose or control) on blood glucose, plasma insulin and glucagon concentrations in older men with (not on injectable treatment) and without T2D. The addition of 30 g of whey protein to 30 g of glucose in drink form substantially attenuated the increase in blood glucose concentrations induced by glucose alone; the magnitude of the whey-induced reduction in blood glucose was not affected by the presence of T2D and the stimulation of plasma insulin concentrations by whey

protein. The ability of whey-protein to reduce carbohydrate-induced postprandial hyperglycaemia is retained in men with T2D (ACTRN12619000420145).

Chapter 7 describes the effects of age on BP and heart rate responses to whey protein in healthy younger and older men after oral ingestion of 0 g and 70 g whey protein. The older men exhibited a greater fall in SBP after whey-protein versus control than the younger men, with no BP change after the two drinks in younger men. The nadir in SBP occurred later in the older than younger men with SBP still apparently declining 180 min after whey-protein ingestion in the older men. The magnitude of the rise in HR was greater in the younger men indicating that following ingestion of 70 g whey protein, healthy older men exhibited a sustained fall in BP, despite an increase in HR, whereas in younger men there was no change in BP. BP may need to be monitored after high protein meals in older people at risk of postprandial hypotension (ACTRN12612000941864 and ACTRN12614000846628).

Chapter 8 describes the BP and heart rate responses to oral protein intake in healthy older men after ingestion of 30 g and 70 g of whey protein. The older men exhibited a decrease in systolic blood pressure (SBP) after ingestion of 30 g and 70 g of whey protein to a similar degree after both the drinks and greatest between 120 and 180 mins after ingestion. HR was increased maximally after 70 g particularly in the third hour (hr) and diastolic blood pressure (DBP) decreased non significantly after protein drinks. Even modest protein loads in older men can result in postprandial hypotension (PPH) and care must be taken (ACTRN12612000941864).

Chapter 9 describes the acute effects of whey protein, alone and mixed with other macronutrients in varying amounts such as 70 g whey-protein (P_{280}); (ii) 14 g whey-

protein, 28 g carbohydrate, 12.4 g fat (M_{280}); (iii) 70 g whey-protein, 28 g carbohydrate, 12.4 g fat (M_{504}); or (iv) a non-caloric control drink (C) on BP and heart rate in healthy older men. SBP decreased after all three nutrient drinks with the greatest reduction after the M_{504}. Maximal decreases in SBP occurred about 2 hr after drink ingestion and were sustained thereafter. Maximum DBP decreases and HR increases occurred after M_{504}, with no differences between the effects of the P_{280} and M_{280} drinks, thereby demonstrating that the effects of whey-protein containing drinks to lower BP and increase HR appear to be primarily dependent on their energy content rather than macronutrient composition and may persist for at least 3 hr after ingestion. Pure whey-protein drinks may represent the best approach to maximize protein intake without increasing the potential for deleterious BP falls in older people (ACTRN12614000846628).

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Avneet Kaur Oberoi October, 2022 BSc, MSc

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PUBLICATIONS ARISING FROM THIS THESIS

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2. **Oberoi AO**, Giezenaar C, Clames A, Bøhler K, Lange K, Horowitz M, Jones KL, Chapman I, Soenen S. *Whey protein drink ingestion before breakfast suppressed energy intake at breakfast and lunch, but not during dinner, and was less suppressed in healthy older than younger men.* Nutrients 2020; 12: 3318. doi: 10.3390/nu12113318 **(Chapter 3).**

3. **Oberoi AO**, Giezenaar C, Jensen C, Lange K, Hausken T, Jones KL, Horowitz M, Chapman I, Soenen S. *Acute effects of whey protein on energy intake, appetite and gastric emptying in younger and older, obese men*. Nutrition & Diabetes 2020; 10: 37. doi.org/10.1038/s41387-020-00139-8 **(Chapter 4).**

4. **Oberoi AO**, Giezenaar C, Rigda RS, Lange K, Horowitz M, Jones KL, Chapman I, Soenen S. *Comparative effects of co-ingesting whey protein and glucose alone and combined on blood glucose, plasma insulin and glucagon concentrations in younger and older men.* Nutrients 2022; 14: 3111. doi: 10.3390/nu14153111 **(Chapter 5).**

5. **Oberoi AO**, Giezenaar C, Rigda RS, Horowitz M, Jones KL, Chapman I, Soenen S. *Effects of co-ingesting glucose and whey protein on blood glucose, plasma insulin and glucagon concentration, and gastric emptying, in older men with and without type 2 diabetes men.* Submitted to Diabetes, Obesity and Metabolism 2022 **(Chapter 6).**

6. Giezenaar C, **Oberoi AO**, Jones KL, Horowitz M, Chapman I, Soenen S. *Effects of age on blood pressure and heart rate responses to whey protein in younger and older men*. Journal of the American Geriatrics Society 2021; 69:1291–1299. doi: 10.1111/jgs.17083 **(Chapter 7).**

7. **Oberoi AO**, Giezenaar C, Lange K, Jones KL, Horowitz M, Chapman I, Soenen S. *Blood pressure and heart rate responses following dietary protein intake in older men.* Nutrients 2022; 14: 1913. doi:10.3390/nu14091913 **(Chapter 8).**

8. **Oberoi AO**, Giezenaar C, Lange K, Jones KL, Horowitz M, Chapman I, Soenen S. *Acute effects of whey protein, alone and mixed with other macronutrients, on blood pressure and heart rate in older men.* BMC Geriatrics 2022; 22: 535. doi:10.1186/s12877-022-03213-1 **(Chapter 9) (**Work published on Central Adelaide Local Health Network and Royal Adelaide Hospital website- **https://centraladelaide.health.sa.gov.au/protein-and-blood-pressure/**).

ABSTRACTS AND PRESENTATIONS

1. **Oberoi AO**, Giezenaar C, Rigda R.S, Lange K, Horowitz M, Jones KL, Chapman I, Soenen S. *Effects of whey protein on blood glucose concentrations with oral glucose intake in younger and older men*. Australasian Diabetes Congress (ADC), Brisbane, August, 2021 **(Poster)**.

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3. **Oberoi AO**, Giezenaar C, Jensen C, Lange K, Hausken T, Jones KL, Horowitz M, Chapman I, Soenen S. *Effects of whey protein on energy intake, gastric emptying and blood glucose in obese, older and younger men*. ESNM NeuroGastro, September, 2021 **(Poster)**.

4. **Oberoi AO**, Giezenaar C, Rigda R.S, Lange K, Horowitz M, Jones KL, Chapman I, Soenen S. *Effects of whey protein on blood glucose concentrations with oral glucose intake in younger and older men.* 49th American Aging Association, Virtual 2021 **(Poster)**.

5. **Oberoi AO**, Giezenaar C, Jensen C, Lange K, Hausken T, Jones KL, Horowitz M, Chapman I, Soenen S. *Effects of whey protein on energy intake, gastric emptying and blood glucose in obese, older and younger men.* 4th Meeting of the Federation of Neurogastroenterology and Motility (FNM), Adelaide, 2021 **(Poster)**. **Recipient of AMS HDR Travel Research Support Award, Round 1 2021.**

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8. **Oberoi AO**, Giezenaar C, Jensen C, Lange K, Hausken T, Jones KL, Horowitz M, Chapman I, Soenen S. *The effect of a whey protein drink on energy intake, appetite and gastric emptying in younger and older, obese men.* The Medical Staff Society Research Prize, Royal Adelaide Hospital, Adelaide, 2020 **(Oral)**.

9. **Oberoi AO,** Giezenaar C, Rigda R.S, Lange K, Horowitz M, Jones KL, Chapman I, Soenen S. *Effects of whey protein and glucose intake on glycaemia and energy intake in older men.* Australian Diabetes Congress (ADC), Brisbane, 11-13 November, 2020 **(Poster)**.

10. **Oberoi AO**, Giezenaar C, Jensen C, Lange K, Hausken T, Jones KL, Horowitz M, Chapman I, Soenen S. *The effect of a whey protein drink on energy intake, appetite and gastric emptying in younger and older, obese men*. 58th Australian Society of Medical Research National Scientific Conference (ASMR), Adelaide, 2019 **(Oral)**.

CHAPTER 1: INTRODUCTION

INTRODUCTION

Our research group has a longstanding interest in undernutrition in older people, its possible causes, adverse consequences and management strategies. One of the more widely used management strategies is protein or protein-enriched nutritional supplements. These have been shown to help preserve muscle mass and function in older people (1-4). Whey protein has particular anabolic properties which make it a favourable choice as a protein supplement in older people and such supplements are being used increasingly by older people (5-8). Our group has conducted studies assessing appetite and feeding responses to whey protein in older people and the effect of healthy ageing on these responses (9-18). These studies have largely involved assessment of appetite ratings from the time of whey intake to a single later test meal and of food intake at that meal. More recently, we have expanded the scope of these studies to examine the effects of whey on feeding behaviour not just at the next meal but throughout the study day (chapter 3) and in obese as well as non-obese participants (chapter 4). We have also now studied the effects of whey in older people on outcomes that are possibly both beneficial (blood glucose lowering: chapters 5 and 6) and harmful (excessive BP lowering: chapters 7-9). These studies on additional effects of whey protein form the basis of this thesis.

The following sections of the introduction provides a background to and rationale for the studies described in this thesis.

1.1 The rising burden of ageing in the population of Australia and other developed countries

The populations of developed countries, including Australia, are ageing. Worldwide, the percentage of populations over 60 years increased from 8% in 1950 to 12% in 2014 and is expected to reach 21% by 2050 (19). Global life expectancy increased from 47 years to about 70 years from 1950 to 2014 and will increase to 75 years by 2050 (20, 21). The proportion of Australia's population over 65 years of age increased from 5% in 1937 to 9% in 1976 and 15% in 2016, and is expected to rise to more than 22% by 2056 (22). These increases impose a significant personal and national economic burden, as many disease conditions and disabilities become more common with increasing age, adversely impacting quality of life (22). The percentage of Australian health spending expended on people over 65 years of age is expected to increase from 71% in 2012 to 84% by 2035 (23). Therefore, optimising the health of older people is crucial for individual and societal benefits.

1.2 Nutritional problems and adverse body composition changes associated with human ageing

1.2.1 Undernutrition and sarcopaenia

Ageing is associated with changes in appetite, feeding behaviour, gut function and body composition (2).With healthy ageing, there is reduction in appetite and food intake, which has been termed the 'anorexia of ageing' (1, 24). Between 20 and 80 years, the average daily energy intake decreases by up to 30%. Older people have reduced hunger ratings, and increased fullness and satiety compared to younger adults (25-30). In the American National Health and Nutrition Examination Survey,

NHANES III, 1989, for example, there was a reduction in the average daily energy intake of about 1321 calories/day in men and 629 calories/day in women between the ages of 20-80 years (31, 32). The mechanisms responsible for reduced appetite and food intake in older people are multiple and complex, and include both central and peripheral (gastrointestinal) factors.

Often not appreciated is that weight loss is more common than weight gain in older people (33, 34). Prospective and cross-sectional studies demonstrate that body weight and body mass index (BMI) tends to increase up until 50-60 years of life and decline thereafter (1, 35). In one study older American men > 65 years of age lost 0.5% of their body weight per year on an average over 2 years and 13.1% had involuntary weight loss of 4% per annum or more (36). The prevalence of nutritional risk in older people as per the Mini Nutritional Assessment varies from 45% to 100% (community, domiciliary settings, hospitals and aged care facilities) (37-41), and when marked this can result in nutritional problems, particularly undernutrition which can have deleterious effects on the function and quality of life of older people (33).

Weight loss, especially if involuntary, is a predictor of poor outcomes in older people (42) with an approximately two-fold increased risk of mortality with a weight loss of 4-5% in about 1-3 years (34). In a longitudinal observation study known as the cardiovascular health study conducted in 1989 on 4714 community dwelling people aged 65 years and above showed that a weight loss of 5% in 17% participants resulted in a significant increase in the mortality rate (crude mortality rate- 6 in 100 person; age and gender adjust hazard ratio was 2.09; 95% confidence interval (CI), 1.67–2.62 and risk-adjusted was 1.67; 95% CI, 1.29–2.15) (34). Furthermore, in the

systolic hypertension in the elderly program from 1984-1990, an average weight loss of 1.6 kg/year was associated with all-cause mortality (odds ratio- 4.9, 95% CI, 3.5–6.8) as compared to a weight stable group used as a reference (43).

The age-associated weight loss is disproportionately due to the loss of lean rather than fat tissue. On average, body fat increases and lean muscle mass (fat free mass) decreases throughout adult life. With ageing, there are morphological and (44) physiological changes in skeletal muscle such as reduced number and size of skeletal muscle fibres along with infiltration of adipose cells into skeletal muscle. Skeletal muscle atrophy begins in the third or fourth decade of life, with a rate of muscle loss of about 3-8% per decade after 30 years of age (45, 46). This muscle loss is chiefly due to an imbalance between muscle protein synthesis and muscle protein breakdown, due to increased oxidative stress, inflammation and reduced mitochondrial capacity (47).

Loss of muscle mass contributes to reduced physical strength. The rate of decline in muscle strength is about 2-4% per year after 50-60 years of age (48). This further results not only in the functional impairments such as reduced grip strength and gait but speed but also disability (49). Age-related reductions in muscle mass, strength and function are associated with increased rates of falls, fractures and frailty (2, 50-52). These adverse outcomes are even more likely when muscle mass is excessive, a condition that has been termed sarcopaenia (53). Age related changes in body composition are chiefly responsible for sarcopaenia when it develops, but environmental factors such as reduced physical activity due to chronic diseases such as obesity, and medical conditions leading to fatigue, pain, inflammation and inadequate calorie and protein intake can also contribute (54). These changes are

associated with adverse health outcomes such as poor cognitive function, immunodeficiency, increased rates of hospitalisation and admission to nursing homes and ultimately increased mortality (32).

1.2.1.1 Nutritional management of under-nutrition and sarcopaenia

A recognition of the adverse outcomes associated with age-related weight loss and sarcopaenia has led to attempts to prevent and treat it. Nutritional measures and exercise are commonly used strategies and usually first line (55, 56). For example, the Food First approach (an evidence based strategy enabling individuals to consume a variety of food for good health and performance and also eat intuitively, promoting social and emotional wellbeing) is one such first line of treatment to maintain weight, prevent frailty and sarcopaenia (57). This approach combines strategies aimed at increasing food intake (consuming large amounts of usual foods to maintain body weight i.e. increasing the nutrient content of the food particularly energy and protein rich or cooking nutritious meals for the elderly or proving home meal services like meals on wheels especially in aged care setting; fortification with additional nutrients) and strategies aimed at improving knowledge and behaviour (provide one on one counselling, tailored diet plan, a resource kit by a professional and increased encouragement to eat) and often helps to counteract weight loss (58-62). However, the successful implementation of such strategies is not always achievable due to a various reasons such as affordability, convenience and staffing issues and variable adherence (58).

The next step in improving the nutrition of older people is often the use of nutritional supplements, particularly protein supplements, which are increasingly recommended

to and used by older people to achieve increased energy and protein intake (63). Supplements provide the opportunity to tailor the timing and composition of added protein for maximum benefit (63).

Protein is considered to be the most satiating of all macronutrients, at least in non-elderly people. High protein diets are associated with increased satiety, thermogenesis and improved insulin sensitivity (64-66). They have shown to assist in weight loss in young adults with obesity and improve oral glucose tolerance, and cardiovascular outcomes in young adults with T2D (67-69). In non-elderly adults, dietary protein facilitates weight loss by suppression of energy intake and appetite (9, 15, 25-28, 70-72), preservation of lean mass (73-76) and has greater satiating effect than carbohydrates or fats (77, 78). A classic study by Weigle et al. showed that a diet containing 30% of protein versus an iso-caloric diet with 15% of protein was more satiating and associated with greater weight loss over time and decreased energy intake (79). McAuley et al. showed greater insulin sensitivity in obese women with 30% protein diet at 8 weeks as compared to high fat or carbohydrate diets (80). A diet containing 30% protein showed ~ 0.5 % reductions in HbA1c in patients with T2D (66).

The ability of ingested protein to stimulate muscle protein synthesis decreases with ageing, a change that has been termed anabolic resistance (6, 81). As a result, older people need to consume larger amounts of protein to preserve and enhance muscle mass than young adults and this is reflected in age-related recommendations for dietary protein intake (82, 83). Whereas recommendations for dietary protein intake in non-elderly adults usually recommend approximately 0.8 g/kg/day, the European PROT-AGE group, for example, recommends 1.0–1.2 g/kg dietary protein for

healthy older adults, 1.2-1.5 g/kg for older adults with chronic illness. This equates to about 30–45 g protein per serving, whereas lower doses < 20 g are sufficient for younger adults (84). In order to meet these guidelines, older people are often recommended to consume high protein foods, plus or minus protein or protein-enriched supplements. Further detail about the type of protein, amount and timing along and possible adverse effects of protein supplements are detailed in **chapter 2, page 58-67.**

Because dairy products are a good source of protein, increasing the intake of milk and dairy products is often recommended as a way to increase protein intake (85, 86).

1.2.2 Whey protein

Our group has been interested in the nutritional management of under-nutrition and sarcopaenia, particularly the role of whey protein.

Whey protein is obtained from milk, as a by-product of cheese making process in which it is separated from casein. It accounts for 20% of the total milk protein. As compared to casein it is considered a “fast protein” as it is more soluble in stomach resulting in rapid digestion. It is also considered a “complete” protein with a high biological value of 104 (proportion of protein retained in the body for growth and/or maintenance and expressed in percent of nitrogen absorbed) and net protein utilisation of 92 (ratio of amino acid mass converted to proteins to the mass of amino acids supplied) (87, 88). It contains bioactive peptides with β-lactoglobulin (45–57%) present in high amounts, whereas α-lactalbumin (15–25%), immunoglobulin (10–15%), glycomacropeptide (10–15%), bovine serum albumin (10%), lactoferrin

(~1%) and lactoperoxidase (< 1%) accounts for smaller amounts (4, 15, 16) (Table 1).

Whey protein drinks in doses of 30–70 g slow gastric emptying in a dose-responsive manner (42, 44, 50), and probably slow it more in older than in young adults. Whey ingestion has effects on a number of gastrointestinal factors controlling appetite and food intake, particularly mediated by the release of satiety-inducing hormone e.g. concentrations of cholecystokinin (CCK), insulin, glucagon, gastric inhibitory peptide (GIP), glucagon-like peptide-1 (GLP-1), peptide tyrosine-tyrosine (PYY), and amino acids increase, while glucose does not change and ghrelin concentrations decrease (89). Food intake is reduced via the release of gut hormones such as CCK and GLP-1 in response to amino acid and peptide release by consumption of whey protein (89). Ingestion of nutrients results in CCK and GLP-1 release from small bowel that slows gastric emptying and reduces food intake, typically seen in older people causing satiety (29, 90). Bioactive peptides present in whey or formed during digestion are the main stimulators of GIP secretion which increase more after oral whey ingestion in older than in young adults (11, 12, 14, 91). Whey suppresses circulating concentrations of the appetite-stimulating hormone ghrelin, and increases glucagon concentrations in older and younger adults (90).

An important factor influencing the satiating effect of different proteins is their amino-acid composition and effects on circulating amino acid concentrations after ingestion (89).

Table 1: Essential amino acid content of whey protein

Amino acid	Amount
Leucine	8.6
Threonine	5.4
Methionine	1.8
Phenylalaninie	2.5
Histadine	1.4
Lysine	7.1
Valine	3.5
Isoleucine	3.8
Sum of essential amino acid	34.1

Values presented are in g per 100 g raw material

Whey has a relatively high content of the branched chain amino acids leucine, isoleucine and valine and these amino acids, particularly leucine are more potent than other amino acids in stimulating muscle protein synthesis (92, 93). In older people, muscle protein synthesis was increased with 42% content of leucine and not with a mixture having leucine content of 26% (94). Consequently whey protein is considered to be an effective anabolic protein. It is commercially available in varying forms, including as a whey protein concentrate, whey protein isolate, and whey protein hydrolysate (86, 95, 96). These are increasingly used as supplements in an attempt to preserve or increase muscle function.

Previous studies by our group and others have indicated that oral ingestion of whey is capable of suppressing appetite and food intake in older, as in young adults, but

that this effect is less potent in older adults, with substantially higher doses of whey necessary to suppress food intake in older people. In one study when intraduodenal infusions of 8 g, 24 g and 48 g of whey protein were administered to older and young men, the older men had suppression of *ad libitum* energy intake only with the highest dose, whereas the younger men suppressed with lower doses (9). This finding has been supported by the results of other studies (27). Our group has conducted a number of studies using 30 g and 70 g of whey protein, 30 g approximating the minimum amount required in older people to stimulate muscle protein synthesis (see 1.2.1.1 above) (63, 83, 97). Compared to ingestion of an energy-free control drink oral administration of 30 g of whey protein suppressed energy intake less in older (1 ± 5%) than compared to younger men (15 ± 2%; $P = 0.008$) (14, 15, 98) Appetite decreased in young, while it increased in older men (desire to eat, young vs control, a decrease of 647 ± 910 mm vs an increase of 1027 ± 458 mm, $P = 0.024$; prospective food consumption, young vs control, a decrease of 1616 ± 706 mm vs an increase of 119 ± 370 mm, $P = 0.021$) (15). This reduced suppressive effect of whey on feeding behaviour in older people is likely to be a good thing in most situations, as whey ingestion is therefore less likely to lead to appetite suppression and weight loss when whey supplements are used in an attempt to enhance muscle function in older people.

The optimum timing of ingestion of whey supplements in older people is uncertain. If maximisation of protein intake without suppression of appetite and food intake overall, and whey suppresses appetite, it might be best to give the supplements between meals, so as not to reduce food intake at main meals. If, on the other hand, as suggested by the above studies, appropriate doses of whey have little or no appetite suppressant effect in older people, the timing of ingestion may be less important. Also, by ingesting whey with a main meal, which contains other protein in “usual”

foods, it may be possible to achieve optimum single dose protein intake for muscle benefit (30 g or higher) with a lower dose of whey that would be needed if ingested between meals. The duration of effect of whey on appetite and feeding behaviour in older people have also not been well defined.

1.2.3 Obesity in older people

Our group's previous studies have focused on the effects of whey on non-obese older people. Given the adverse effects of obesity and its rising prevalence, including in older people, we have now examined the effect of whey on feeding behaviour in obese, older people.

Increasing rates of obesity are a global health problem (99-101). Although weight loss is more common than weight gain in older people (see 1.2.1), obesity rates are rising in Australia, including in older people, mainly because of the increasing number of people achieving old age already overweight (102, 103). This is a key health issue for older Australians as it is associated with an increased risk of developing long term, chronic degenerative disorders including T2D, osteoarthritis, cardiovascular disorders, hypertension, certain types of cancers and increased rates of overall poor health and disability (104, 105). For example, accumulation of adipose tissue, particularly when sufficient to cause obesity, plays a critical role in causing T2D by causing insulin resistance and other mechanisms (106). Over recent decades the increasing prevalence of obesity has been closely associated with a marked rise in T2D, and this frequent association has been termed diabesity (107). In 2017-2018, 78% of Australians between 68-74 years were overweight (BMI 25-29.9 kg/m^2) or obese (BMI > 30 kg/m^2) as compared to 65% aged 18-64 years (103).

It is expected that by 2030, 51% of the world's population will be obese by BMI criteria (108). Around 50–90% of people with T2D have a BMI > 25 kg/m^2, with even higher rates reported in older people with T2D (109).

Due to various hormonal and other changes, not always well understood, ageing is associated with a substantial accumulation of body fat (110) and redistribution of fat from peripheral and subcutaneous sites to central and other sites, resulting in visceral and ectopic (hepatic and intramuscular) accumulation of adipose tissue. This is reflected in increased waist circumference and abdominal obesity in older adults (111). According to the World Health Organization (WHO), obesity contributes substantially to the development of 44% cases of T2D (see also 1.3), 23% of heart diseases and 7-41% of all cancers (112).

The combination of age-related muscle loss (see 1.2.1) and fat gain can result in older people developing sarcopaenic obesity. Sarcopaenia has been defined as a skeletal muscle mass two standard deviations below the sex-specific reference for a young, healthy population (49) while obesity is usually defined as a BMI greater than 30 kg/m^2 (113-115). Sarcopaenic obesity is the combination of both and is associated with greater adverse effects than either obesity or sarcopaenia alone including metabolic diseases, disability, increased institutionalization, inflammation and all risk mortality (116-118).

Lifestyle interventions (diet and exercise) are the first line of treatment for obesity followed by pharmaceutical interventions and/or bariatric surgery (119, 120). Increasing physical activity promotes weight loss (121). However, exercise-induced weight loss is often not dramatic and marked increases in exercise may not be

possible in older people with mobility limitations due to sarcopaenia, arthritis and other medical conditions (53). Energy restricted diets, preferably rich in fibre and micronutrients, along with exercise may be beneficial. When body weight is lost deliberately in response to energy restricted diets, lean tissue, including skeletal muscle, is lost along with adipose tissue (122). Given their age-related reduced muscle mass, older people are therefore at increased risk of developing adverse effects from reduced energy intake, whether it be involuntary or deliberate as part of a weight loss diet. Protein supplementation has been shown to have a role in preventing and treating loss of muscle in older people (3, 8, 123). It has been hypothesized that that there is a reduction in the risk of frailty with higher protein intake by increase in muscle anabolism, and improvement in muscle quality (124).

While protein is the most satiating macronutrient in young adults and may aid weight loss, the effect of proteins, including whey, to suppress appetite and reduce food intake is reduced in older, non-obese, people (see 1.2.2). The interacting effects of ageing and obesity on appetite and feeding behaviour are not well understood.

1.2.4 Questions about effect of whey on nutritional factors addressed in this thesis

1. Duration of the effect of whey on appetite and feeding beyond the next meal in healthy younger and older men (chapter 3)
2. Effect of whey on appetite and feeding behaviour in obese, younger and older men participants (chapter 4)

1.3 Effect of whey protein ingestion on glucose metabolism

1.3.1 Type 2 diabetes mellitus (T2D)

1.3.1.1 Type 2 diabetes mellitus: Demographics and impact

The prevalence of T2D in most countries has increased over recent decades as their populations have become older and heavier. T2D accounts for about 85% of all diabetes and is the most rapidly growing chronic disease in Australia (125). According to the International Diabetes Federation, in 2015 the number of people with diabetes worldwide was 415 million, and this is expected to rise to 642 million by 2040 (126). Older people with T2D are at increased risk of cognitive and functional impairment, depression, falls and other diabetes related comorbidities (127, 128). A number of factors contribute to the increasing incidence (and hence prevalence) of T2D with increasing age. These include declining insulin secretion, increased insulin resistance, hormonal impairment, inflammatory and genetic factors, increased oxidative stress and increased use of medication promoting hyperglycaemia (e.g. glucocorticoids). Fasting and postprandial hyperglycaemia is a characteristic feature of T2D (129).

1.3.1.2 Management of T2D: Focus on dietary measures

Due to the complexity of factors associated with poor glycaemic control in T2D, achieving optimal levels of glycaemic control is challenging (130). Lifestyle measures are the first line treatment and should be continued even after medications are started, if needed.

Medications include a variety of oral agents, usually used first, then injected

medications in the form of GLP-1 receptor agonists and/or insulin. Oral metformin is the most widely used first line medication and works by several mechanisms including a reduction in hepatic glucose production; sulphonylureas (for example gliclazide, glipizide) increase insulin secretion; DPP-IV inhibitors (linagliptin, saxagliptin, sitagliptin) and GLP-1 receptor agonists (exenatide, liraglutide, lixisenatide, dulaglutide, semaglutide) increase insulin secretion and reduces glucagon secretion; sodium-glucose cotransporter-2 (SGLT-2) inhibitors (dapagliflozin, empagliflozin) inhibit renal glucose reabsorption, whereas insulin (short acting, immediate-acting and long acting) increases glucose disposal and reduces hepatic glucose production (131).

Lifestyle measures, particularly modifications to diet and increased physical exercise remain important treatment for people with T2D. Recommendations for the dietary management of T2D vary between recommending bodies, but generally include an emphasis on energy intake reduction to promote weight loss in overweight people with T2D, and reduction in the intake of unprocessed, high glycaemic index carbohydrates. They contain little mention of dietary protein intake (132).

The multiple determinants of postprandial glycaemia include pre-prandial blood glucose concentrations, the rate of gastric emptying, meal composition, carbohydrate absorption in the small intestine, insulin and glucagon secretion, incretin release and hepatic and peripheral glucose disposal (133). Protein ingestion alone usually has little effect on blood glucose concentrations, due to counteracting stimulatory effects on release of both insulin and glucagon (134, 135). However, when combined with carbohydrates, protein ingestion has been shown to reduce blood glucose levels significantly compared to ingestion of the carbohydrates alone, in younger and

middle-aged adults (86, 95, 136). Ingestion of protein appears to mainly stimulate insulin secretion by direct effects on the pancreas (137, 138). Oral protein ingestion also stimulates the gastrointestinal release of the incretin hormones glucagon-like peptide 1 and gastric inhibitory protein, which in turn stimulate insulin release and inhibit glucagon secretion. GLP-1 in particular stimulates the beta cell function, augment insulin release and regulates gastric emptying rate particularly via the vagal afferents that send signals to the brain. In addition, the release of gut peptides such as CCK and PYY is also stimulated by ingestion of whey protein, with the effect of delaying gastric emptying and regulating the gastrointestinal transit of food. Carbohydrates stimulate insulin secretion via sodium-glucose cotransporter-1 (SGLT-1) and intestinal "sweet taste" receptors (139). Variations in the rate of gastric emptying have an impact on postprandial glycaemia in people with and without T2D. Ageing is associated with modest slowing of gastric emptying thereby lower postprandial glucose excursions, while abnormally delayed gastric emptying may be present in people long standing diabetes, particularly if poorly controlled. In contrast, gastric emptying may be more rapid in people with newly developed and well-controlled. The rate of gastric emptying influences postprandial blood glucose concentrations (see 1.5.1). Due to the ability of insulin to cross the blood brain barrier, increased brain insulin concentrations of insulin after protein ingestion may also play a part in its appetite suppressant effects (140) and possibly reduce hepatic glucose production via the brain-liver axis (141). As explained later co-ingestion of proteins such as whey with carbohydrates can result in a more than additive effect on the increase in circulating insulin concentrations (see chapter 5 and 6). This may be because some amino acids act as substrates in the Krebs cycle to create ATP, which acts directly on the beta cells to increase their insulin secretory response to glucose (142).

Previous studies in young and middle-aged adults with and without T2D have shown that co-ingestion of protein with carbohydrate reduces the postprandial increase in blood glucose after carbohydrate ingestion alone with marked protein-induced increases in circulating insulin concentrations (143, 144). Nuttall reported a significant rise in insulin concentration with co-ingestion of 50 g protein with 50 g of carbohydrate as compared to glucose or protein alone (247 ± 33 μU • h/ml vs 97 ± 35, 83 ± 19 μU • h/ml) and the plasma glucose area above the baseline following a glucose meal was reduced 34% when protein was given with the glucose in 9 men with T2D with a mean age of 61 years (68). Similar results have been reported by other studies. However, those studies largely used casein or beef protein (145, 146), rather than whey, our protein of interest. Frid et al. (136) studied the effect on post prandial glucose and insulin concentrations of adding 27.6 g of whey protein to high glycaemic index meals at breakfast and lunch in non-elderly participants with T2D aged 27–69 years and found a whey induced increase in the insulin responses at breakfast and lunch of 31% and 57% respectively, with an associated whey-induced reduction in blood glucose concentrations.

Ma et al. (147) gave 55 g of whey protein in meal or as a preload along with carbohydrate to 8 participants with diet-controlled T2D with a mean age of 58 years and showed a substantial reduction in postprandial glycaemia. The incremental areas under the curve (iAUC) for insulin, GLP-1, GIP, and CCK were significantly greater and for glucose significantly lower when whey was co-administered. Because of this effect to reduce blood glucose concentrations ingestion of whey with or just before a meal has been proposed as a possible strategy in the management of T2D (147-149). Therefore, it is not known if the above effects of whey on postprandial glycaemia in people with and without T2D persist into old age, particularly over 70

years - the age group with the highest prevalence of diabetes – or if 30 g of whey protein (a muscle protecting dose) has this effect. This led our group conduct studies to determine the effect of combining 30 g whey protein with carbohydrate in men > 65 years with and without T2D.

1.3.2 Questions about effect of whey on glucose metabolism addressed in this thesis

1. Impact of ageing on the effects of whey on glucose metabolism in healthy younger and older men (chapter 5)
2. Effect of whey on glucose metabolism in older men with and without T2D (chapter 6).

1.4 Effects of whey on blood pressure and pulse rate in older people

1.4.1 Physiological cardiovascular responses to eating

Ingestion of food has many physiological effects. The cardiovascular and metabolic responses to feeding are driven both peripherally and centrally (150). Meal ingestion results in blood flow redistribution to the gut, splanchnic blood pooling and a rise in mesenteric blood flow. This diversion of blood from the systemic circulation to aid nutrient absorption and digestion results in a blood pressure (BP) fall unless there is an adequate compensatory increase in pulse rate and hence cardiac output (151). Usually these compensatory mechanisms work well enough to prevent excessive post-prandial BP falls in most young people (152-154). However, these compensatory responses to food ingestion are less effective in older people who experience greater food-induced BP decreases than younger adults (155).

1.4.2 Postprandial hypotension

An excessive fall in BP after a meal has been termed postprandial hypotension (PPH) and defined as a decrease in SBP of > 20 mm Hg within 2 hr of a meal (156). The effectiveness of cardiovascular compensatory responses to food intake declines with advancing age and PPH is not uncommon in older people (157-159). The prevalence of PPH has been reported to be 24% to 38% in community-dwelling elderly and residential facilities (160, 161), 20% to 91% in hospitalized geriatric patients (162, 163), 40% in people with T2D (164, 165), and 40% to 100% in people with Parkinson disease (166). While the majority of people with PPH are probably asymptomatic (166), when severe, PPH can lead to nausea, light headedness, weakness, dizziness, falls, fractures, syncope, and even cerebrovascular events (strokes), thus increasing morbidity and mortality rates in older people (158, 160, 167, 168).

The pathophysiology of PPH is multifactorial. Causative factors, which vary between individuals, include baroreceptor dysfunction, gastric distension, variations in the rate at which nutrients are delivered from the stomach to the small intestine, splanchnic blood flow and release of vasodilatory hormones, and altered neural and hormonal mechanisms. After meal ingestion, secretion of neurotensin and insulin may cause excessive vasodilation, particularly of vessels supplying the gastrointestinal tract thus lowering cardiac output (169-171). In healthy young adults, excessive BP decreases after food ingestion are prevented by increased vasoconstriction of blood vessels in the leg and other compensatory mechanisms which act to increase cardiac output mainly by increasing heart rate (172). On average these compensatory mechanisms become less effective with increasing age

(172).

Other factors affecting postprandial BP responses include meal composition (173-175) and temperature, the timing of food intake and the posture of the participant after food ingestion (155). Smaller meals are associated with less diversion of blood to gut, thereby requiring less cardiovascular compensation. In patients with chronic autonomic failure, consumption of 3 large meals has been associated with lower postprandial BP as compared to 6 smaller meals distributed throughout the day SBP; lying, sitting and standing; large vs. small; 131 vs. 151 mm Hg, 109 vs. 124 mm Hg, 89 vs. 103 mm Hg, $P = 0.005$; DBP: 76 vs. 90 mm Hg, $P = 0.02$, 66 vs. 78 mm Hg, $P = 0.07$ and 50 vs. 66 mm Hg, $P = 0.06$ (176). The magnitude of postprandial BP changes varies with the nutrient composition of the food eaten (173, 174). Carbohydrates, particularly, glucose and sucrose, lower BP more than the other macronutrients (168, 177). Carbohydrate rich meals cause greater activation of the sympathetic nervous system and decrease in peripheral resistance than protein rich meals (178). Nevertheless, in older participants all macronutrients appear to have the capacity to decrease BP when given orally or intraduodenally (179-181).

Only a limited number of studies have been performed, with somewhat conflicting results, on the influence of fat and protein ingestion on postprandial BP and these have produced inconsistent results (173, 174, 182-186). Observational studies indicate that increased dietary protein intake may be associated with reductions in BP (187-190). Milk protein has been associated with reduced BP (191). A number of clinical trials have been carried out to evaluate the effect of milk proteins from whole foods and supplements on BP (192-195). A reduction of 3.33 mm Hg in SBP and 1.08 mm Hg in DBP has been observed with milk proteins (196). A randomized double blind controlled trial was conducted to determine the effect of dietary proteins

on BP (PROPRES) and investigate whether a moderately increased protein intake (+10% of energy) compared with iso-calorically increased maltodextrin intake for 4 weeks lowered BP in 99 overweight individuals aged 20–70 years with grade 1 hypertension (SBP of 130–159 mm Hg and/or DBP of 85–99 mm Hg). Participants were supplemented for 4 weeks with 3x 20 g protein per day (20% pea, 20% soy, 30% egg and 30% milk-protein isolate) or control (with 3 × 20 g maltodextrin/d). Office SBP and DBP were 4.9 ± 1.7 mm Hg and 2.7 ± 1.3 mm Hg (both $P = 0.05$) lower in the protein group and daytime SBP was 4.6 ± 1.7 mm Hg lower in the protein group ($P = 0.006$), with no daytime DBP difference between groups ($P = 0.37$). While this result suggests a BP lowering effect of protein no differences in postprandial BP responses between high-protein and high-carbohydrate meals have been found it other studies (181, 197).

The specific effects of whey protein on BP have been studied, but not in elderly people. It has been proposed that bioactive peptides in whey, particularly lactokinins are involved in inhibition of renin-angiotensin aldosterone system by binding with the zinc fingers on the angiotension converting enzymes (ACE) molecule resulting in the inability of ACE to further cleave angiotensin I into angiotensin II, thus lowering BP (198, 199). Results of studies with whey in non-elderly adults are inconsistent (200) but mostly indicate that it does lower post-prandial BP. Fekete et al. showed a significant reduction in SBP and DBP with 28 g of whey protein/day as compared to 28 g of calcium caseinate/day or 27 g of maltodextrin/day for 2 days in 38 participants for 8 weeks (191). Fluegel et al. also found that whey beverage consumption for 6 weeks significantly decreased SBP and DBP in adults with elevated BP (201). In a randomised controlled trial, a significant decrease in SBP was observed with a diet containing whey protein (40% carbohydrate: 15% mixed

protein: 15% whey protein: 30% fat) as compared to mixed protein (40% carbohydrate: 30% protein: 30% fat) or control (55% carbohydrate: 15% protein: 30% fat) (65) in 18 healthy adults. However, Lee indicated that the regular consumption of a milk drink containing whey peptides for 12 weeks has no effect on BP in mildly hypertensive individuals (202). Given this, it is possible that the BP changes reported in our studies in a single day treatment would not persist and might even be reversed with longer treatment. It is not clear whether whey exerts hypotensive effect in older men and if so if those effects are dose-dependent.

Various treatments have been proposed for people experiencing adverse effects of PPH. These include medications such as alpha glucosidase inhibitors (acarbose) which delay the digestion of carbohydrates, and non-pharmacological/lifestyle measures such as postprandial exercise, consumption of small frequent meals, and measures to slow the gastric emptying of nutrients into the small intestine such as increased water drinking to increase gastric distension (176, 203-208). A better understanding of the effects of ingestion of proteins including whey, when ingested alone and with other macronutrients, on postprandial BP, is likely to help decisions about the dietary treatment of PPH when present.

The effect of whey protein, administered alone and with other macronutrients, on postprandial BP in older people has not been clarified. The relative effects on BP of whey when administered alone and in combination with other macronutrients are also unclear. If oral ingestion of proteins, including whey has the ability to lower postprandial BP in older people, who are more at risk than younger people of PPH and its adverse consequences, the use of whey supplements for muscle-preservation or other reasons, by older people may be associated with adverse excessive BP

lowering effects. Therefore, we conducted studies to see the effect of 0 g, 30 g and 70 g whey protein alone or mixed with other macronutrients on SBP, DBP and HR in younger and older men.

1.4.3 Questions about effect of whey on blood pressure and pulse rate addressed in this thesis

1. The impact of ageing on BP responses to whey protein (0 vs 70 g in older vs younger men, study in chapter 7).
2. Having found that 70 g decreases BP in older men (chapter 7), does a lower, probably more "physiological dose" (30 g) have the same effect (study in chapter 8).
3. The effect of whey, ingested alone and together with other macronutrients on BP and pulse rate in older men (chapter 9).

1.5 Gastric emptying

1.5.1 Control of gastric emptying

Gastric emptying involves a coordinated set of activities of the proximal (fundus and gastric body) and distal stomach (pylorus and antrum) and duodenum. There is a relaxation of proximal stomach as meal intake is commenced, as it is a reservoir for solid foods. The distal stomach then grinds the solid foods to small particles by contractions thereby creating flow across pylorus (70, 209). The gastric emptying rate is regulated by the interstitial cells of Cajal as it is involved in antrum contractions and neuromuscular transmissions (210). Gastric emptying rate is influenced not only by the physical characteristics of the meal such as whether it is solid, semi-solid, liquid, viscosity, volume but also the chemical composition such

as nutrient and energy content, osmolarity and pH (110).

In this thesis, gastric emptying was measured in the studies described in chapters 3, 4 5, 6, and 7. These measures were obtained because the rate of gastric emptying has been shown in previous studies by our group and others to be associated with the outcomes of interest in the studies in this thesis.

Variations in the rate of gastric emptying are known to be associated with

1. Appetite and feeding behaviour (e.g. hunger, fullness, satiety) – Ingestion of nutrients triggers a series of mechanisms both intragastric (gastric distension) and small intestinal (changes in antropyloroduodenal motility and the release of appetite-regulating hormones) that regulates appetite and feeding behaviour (165). When ingested, nutrients interact in the small intestine resulting in generation of neural and humoral signals which controls gastric emptying (211-213). Secreted by stomach, ghrelin stimulates appetite and energy intake. Small intestine secretes PYY CCK and GLP-1 that suppresses food intake. The delayed proximal gastric distention to a meal observed in the elderly might contribute to early satiation (91). Ageing is associated with reduced proximal gastric distension, and greater distension antral area, resulting in slightly slower gastric emptying, that favours reductions in energy intake (25, 214, 215).

2. Blood glucose concentrations after nutrient ingestion – The rate of gastric emptying is a critical determinant of postprandial blood glucose concentrations (216, 217). In healthy individuals the stomach empties at the rate of 1-4 kcal/min after meal ingestion. Rapid gastric emptying is associated with a greater, more rapid increase in post-prandial blood glucose concentrations. Ageing is associated with modest slowing of gastric emptying, while gastric emptying may

be more rapid in people with well-controlled T2D and delayed gastric emptying may be present in long-standing, poorly controlled T2D (149, 215, 218-223). In people with poorly controlled and long-standing diabetes (HbA1c > 8.5% and > 10 years), gastric emptying of solid meal is delayed in 30-50% of participants (220, 224-226).

3. Greater post prandial BP decreases have been associated with more rapid gastric emptying (227, 228). The fall in BP after a meal is greater when the rate of gastric emptying is faster in both healthy older participants (13, 14, 166, 229) and people with T2D (163, 227).

4. Ageing – ageing is associated with modest slowing of gastric emptying of both solid and liquid meals (230) and this slowing may potentially contribute to reduce food intake in older people (214). With ageing, there is a loss of interstitial cells of Cajal and enteric neurons. Slowing of gastric emptying is due to increased pyloric pressure waves and reduction in duodenal pressure wave (210). Various other factors such as higher fasting and postprandial CCK and GLP-1 concentrations and higher satiating effect of intraduodenal glucose infusion are responsible for altered nutrient response with ageing (25, 26, 28, 231, 232). Perceptions of gastric distension are diminished in the healthy elderly, indicating a reduction in visceral sensitivity (91).

1.5.2 Measurement of gastric emptying rate

The gastric emptying rate can be measured by a number of methods and is often expressed as the time taken for 50% of the ingested meal to leave the stomach (218).

Methods used include scintigraphy, breath tests, ultrasound, paracetamol absorption, radiology, magnetic resonance imaging (MRI), computerized tomography (CT) scan, manometry and two-dimensional (2D) or three-dimensional (3D) ultrasonography (70, 165, 233). Scintigraphy is usually considered the "gold standard" and allows measurement of the gastric residue of both solid and liquid food using dual isotopes. Due to the need for complex equipment including a gamma camera and radiation exposure (70, 230) other methods, validated against scintigraphy are often used.

In the studies described in this thesis, a non-invasive method of measuring gastric emptying with a stable isotope breath test, ^{13}C octanoic acid was used. This technique has been validated against and has been shown to be of comparable accuracy to scintigraphy in the measurement of gastric emptying of both solid and liquid meals in people without (234) and with T2D (235). In the validation paper by Ghoos et al., a test meal with egg yolk labelled with ^{14}C octanoic acid was given to participants as scrambled eggs. It was also labelled with another marker 99mTc-albumin colloid. Simultaneous measurements of scintigraphy and breath test were performed on 36 participants. Breath sampling was performed over 4 h at 15-minute intervals. Two curves were obtained for each participant: the scintigraphic and the %dose/h curves. A strong correlation between coefficient of gastric emptying and the scintigraphic half-emptying time ($r = -0.88$); breath test half-emptying time and the scintigraphic half-emptying time ($r = 0.89$); and between the scintigraphy and breath test the lag phases ($r = 0.92$) was obtained.

It is possible to replace ^{14}C with ^{13}C in the octanoic acid breath test with results of similar accuracy (234). Studies in adults participants have demonstrated a close correlation between ^{13}C octanoic acid breath test and gastric emptying scintigraphy

(r = 0.74) (236-238). This method has been used in previous published studies by our group and others (217, 239-241). Essentially the Carbon-13-isotope is emptied from the stomach and oxidized to $^{13}CO_2$ after transport to the liver via portal vein. The participant ingests a ^{13}C labelled standardized drink/meal. End-tidal breath samples are collected at different time intervals and labelled carbon dioxide content is measured using isotope ratio mass spectrometry in each breath sample. T_{50} (t1/2) of ^{13}C excretion is further determined using the Wagner-Nelson method (242).

The following chapter provides further insights into the rational use of whey protein supplements, particularly in older people, with details extending to include the evidence for benefits of nutritional supplements, potential adverse effects, and relevance of gastrointestinal mechanisms along with the role of gut hormones (chapter 2).

CHAPTER 2: RATIONAL USE OF PROTEIN SUPPLEMENTS IN THE ELDERLY- RELEVANCE OF GASTROINTESTINAL MECHANISMS

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i) the candidate's stated contribution to the publication is accurate (as detailed above);

ii) permission is granted for the candidate in include the publication in the thesis;and

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2.1 Abstract

Protein supplements are increasingly used by older people to maintain nutrition and prevent or treat loss of muscle function. Daily protein requirements in older people are in the range of 1.2 gm/kg/day or higher. Many older adults do not consume this much protein and are likely to benefit from more. Protein supplements are probably best taken twice daily, if possible soon after exercise, in doses that achieve protein intakes of 30 gm or more per episode. It is probably not important to give these supplements between meals, as we have shown no suppressive effects of 30 gm whey drinks, and little if any suppression of 70 gm given to older participants at varying time intervals from meals. Many gastrointestinal mechanisms controlling food intake change with age but their contributions to changes in responses to protein are not yet well understood. There may be benefits in giving the supplement with rather than between meals, to achieve protein intakes above the effective anabolic threshold with lower supplement doses, and have favourable effects on food-induced blood glucose increases in older people with, or at risk of developing type 2 diabetes mellitus; combined protein and glucose drinks lower blood glucose compared to glucose alone in older people.

2.2 Introduction

This review will focus on appetite, feeding and gastrointestinal responses to protein ingestion in older people. As a background, the high rates of under-nutrition, sarcopenia and probably also sub-optimal dietary protein intake in older people will be outlined, in support of the logical use of protein or protein-rich supplements by older people. The type, amount and timing of such supplements will be covered and possible side effects of these supplements. Results of studies with whey protein by our group will be used to illustrate a number of gastrointestinal and cardiovascular responses to protein ingestion and to support some preliminary recommendations about beneficial use of protein supplements by older people. A number of issues remain unresolved.

2.2.1 Ageing, weight loss and undernutrition

Healthy ageing is associated with a physiological reduction in appetite and food intake, the so-called "anorexia of aging" (243); people over 80 years consume about 30% less energy per day than those in their 20s (71). Consequently, after about 65 years in developed countries body weight tends to decrease (71). This age-related weight loss, particularly when substantial and involuntary, has been associated with increased mortality (71). Adverse factors prevalent in older people can be superimposed on the age-related changes to cause pathological under-nutrition, with even greater associated increases in morbidity and mortality (32). Rates of under-nutrition increase with age and loss of independence; it is present in up to 45% of community dwelling elderly people, 50-80% in hospital, and 80-100% in residential facilities (32). The major markers of existing or pending under-nutrition in older

people are low body weight (particularly Body Mass Index < 22 kg/m²); loss of weight, particularly when involuntary and > 5%; substantial loss of muscle mass which can lead to sarcopenia (see below); reduced muscle strength and function; reduced appetite and food intake; and frailty (32, 71).

2.2.2 Ageing and sarcopenia

The weight lost with increased ageing is disproportionately made up of lean tissue, particularly skeletal muscle, but also bone mass. In contrast, fat mass increases with ageing, tending to mask the extent of lean tissue loss (71). After approximately age 30 years about 5% of lean muscle mass is lost per decade (71). The loss of lean tissue, when large, can result in sarcopenia, an excessive and damaging loss of muscle. Sarcopenia is prevalent in older people, present in up to 50% of people over 80 years (244). Sarcopenia has functional adverse consequences, with increased morbidity due to falls, fractures, infections and other conditions, and increased mortality (245).

2.3 Prevention and management of under-nutrition and sarcopenia in older people

A greater appreciation of the high rates and significant adverse effects of undernutrition and sarcopenia, which often co-exist in older people, has led to attempts to prevent, and treat these conditions. Both exercise and nutritional measures have been shown to have benefits, particular when combined (246, 247).

2.3.1 Nutritional measures

Nutritional measures often start with encouragement and assistance to consume greater quantities of usual foods to maintain body weight, the so-called "Food First" approach (248). These measures may include providing meals at home, supervising food intake and/or having the person eat with others in aged care settings (to counteract reduced appetite and reduced spontaneous food intake), increasing the nutrient and energy density of the food, adding flavour boosters and fortifying the food with additional fats, protein and carbohydrates (248).

2.3.2 Nutritional supplements

While these "Food First" approaches may be sufficient, they may be difficult to implement due to cost of foods or time/staffing/convenience constraints, and not always successful. Consequently, nutritional supplements are increasingly recommended for, and used by older people, both as a means of increasing total energy intake (when harmful weight loss is a concern) and to increase protein intake. Mixed macronutrient supplements are used for the former, supplements of pure protein or very high in protein more specifically for the latter indication.

The use of such supplements offers the opportunity to more closely tailor the timing and composition of added protein intake to that what is likely to be most beneficial. It is often recommended that older people take nutritional supplement drinks between and well separated from regular meals so as not to reduce energy intake at those meals; maintenance of weight or even weight gain being a desirable outcome in the many older people with, or at risk of, under-nutrition.

Consequently specific nutritional supplements, usually commercial preparations, are used widely by older people. When the aim is to maintain body weight and general nutrition, rather than specifically enhance skeletal muscle and function, these usually take the form of a mixture of macronutrients in a drink containing 1-1.5 kcal/ml of protein, fat and carbohydrates. Frequently used preparations contain about 9-15gm protein (15-17% of total energy) in a 200-240 ml serve.

2.3.3 Protein nutritional supplements

When the aim is to preferentially prevent and/or reverse ageing-associated muscle loss and sarcopenia the use of such mixed macronutrient nutrient supplements may not be sufficient and pure protein or high protein supplements may be used.

2.4 Dietary protein requirements in older people

Recommended dietary protein intakes for adults of all ages, based on nitrogen balance studies, are 0.8 gm/kg body weight in most countries (249), with no adjustments for age or gender. There have been increasing calls, however, for the minimum recommended daily protein intake in older people to be higher than that (97, 250). One reason is concern that the traditional nitrogen balance study methods used to determine appropriate protein requirements in adults of any age underestimate true requirements, with requirements as determined by the newer indicator amino acid oxidation (IAAO) methods up to 25-75% higher (249). The minimal protein intake for healthy younger adults determined by IAAO is approximately 1.0-1.2 gm/kg/day (249).

Secondly, older people are likely to need higher dietary protein intakes than younger adults for the maintenance of good health, for a number of reasons. These include an age-related reduction in muscle anabolic response to ingested protein; while digestion and absorption of dietary protein is not apparently affected by ageing, there is evidence for an age-related reduction in the anabolic muscle response to ingested protein – anabolic resistance (see (250). This is due to both a redistribution of ingested proteins away from the muscle to splanchnic tissues and a reduced anabolic effect on muscle of the amino acids that do reach the muscle. In addition, catabolic conditions associated with increased muscle breakdown and needs for dietary protein intake, become more prevalent with increasing age. These include chronic diseases such as obstructive airways disease, heart failure, renal failure, malignancies, inflammatory forms of arthritis and polymyalgia rheumatica, and acute conditions such as infections and cardiovascular/cerebrovascular events.

Although not conclusive, there is some evidence from balance studies that older people do have higher dietary protein requirements than young adults. One meta-analysis was reported to show approximately 6% higher protein intakes needed to maintain nitrogen balance in people 60 year and older compared to those younger (251), and another approximately 26% higher intakes needed in those over 55 years compared to those younger (252), although neither difference achieved statistical significance due to small participant numbers. Using the indicator amino acid oxidation method mentioned above to study small numbers (12 or less per group) of participants protein requirements have been reported as 1.24 gm/kg/day in men over 65 years (253), and 1.15-1.29 gm/kg/day in women over 65 years (254).

2.4.1 Do men and women have different dietary protein needs?

Women, on average consume less protein than men, in absolute terms due to lower body weight, and possibly also less compared to body weight. Women over 70 years in the 2003-4 US NHANES study consumed about 10% less protein per kg body weight than men of the same age, with 50% of these older women reporting daily protein intakes ≤ 0.9 gm/kg ideal body weight/day, compared to only 25% of older men (255). These reduced intakes in women are probably in line with reduced needs. Results of several meta-analyses of nitrogen balance studies indicate that adult women need approximately 10% less dietary protein per kg body weight than men to maintain nitrogen balance (251, 252), probably due to lower muscle mass relative to body weight. Nevertheless, there were few older participants in the studies examined in those meta-analyses, and not all studies show lower requirements in women (256). While protein needs/kg body weight of older women may be lower than those of older men, therefore, the difference does not appear to be great. In the interests of simplicity and the absence of evidence that higher protein intakes, if achievable, do much harm (see below) it seems reasonable to maintain gender-neutral protein intake recommendations for older people at this time.

2.4.2 Summary recommendations for dietary protein intake in older people

2.4.2.1 Total Daily Protein Intake

For reasons outlined above, recent recommendations for total daily protein intake in healthy older people are usually in the range of 1-1.5 gm/kg per day. The European PROT-AGE study group for example, has recommended 1.0-1.2 gm/kg dietary protein for healthy older adults (97). Others have suggested that even higher intakes

(> 1.2 gm/kg/day) are needed for maintenance of muscle mass and function (250). Even higher intakes than these are likely to be needed at times of catabolic stress due to acute or chronic illness, with the PROT-AGE group recommending 1.2-1.5g/kg/day for those with acute or chronic illness, and up to 2.0 gm/kg/day for those with severe illness or injury or with marked malnutrition (97).

2.4.3 How many older people need supplements to reach the recommended protein intake?

It is unclear how many older adults consume less than the recommended protein intake (either current RDA or the newer recommendations), but probably a substantial proportion. The US NHANES study of 2003-4 reported dietary protein intake substantially lower in healthy older than young adults; in people over 70 years mean total protein intake was 64.7 gm with a mean intake of 1.0 gm/kg ideal body weight, 27% and 23% respectively below reported intakes in adults 19-30 years (255). Over 75% of people over 70 years reported intakes of less than 1.2 gm/kg ideal body weight (255). Similarly, in the Quebec NuAge study the mean protein intake of older participants was very close to 1 gm/kg/day (256).

2.4.4 Possible adverse effects of protein supplements

Some concerns have been raised about increasing the dietary protein intakes of older people over current recommended levels. These include:

2.4.4.1 Renal effects

Renal function declines with increasing age (257). High protein intakes can increase renal filtration and accelerate the progression of established renal disease; restricted protein intakes of 0.3-0.8 gm/kg/day have been shown to delay the progression of established renal disease and are often prescribed for this indication. The adverse effects of high dietary protein intakes on impaired renal function appear to be increased in those with diabetes mellitus, hypertension or obesity. It is possible, therefore, that increasing the dietary protein intake of older people, whose renal function has already undergone age-related declines, to levels of 1.2 gm/kg/day or higher may induce renal impairment or accelerate the progression of pre-existing renal disease. While this is possible, it is not established that older people with relatively good renal function and without significant risk factors are at such risk. Evidence does not support a relationship between increased dietary protein content and a decline in renal function (258) and high protein diets undertaken for up to 2 years have not been shown to impair renal function in otherwise healthy people (259). Advanced age alone does not, therefore, appear to be a reason to avoid increased dietary protein intake when indicated, although caution should be exercised in those with pre-existing renal impairment or risk factors for renal function deterioration.

2.4.4.2 Bone effects

Previous suggestions that increased dietary protein intake may have adverse effects on bone health by increasing bone mineral loss and increasing the risk of fractures, have not been supported by more recent studies (249, 260), which have, if anything,

shown beneficial effects of dietary protein on bone health.

2.4.4.3 Post-prandial hypotension

Ingestion of nutrients leads to redirection of blood flow from other organs to the splanchnic circulation to aid digestion. As a result blood pressure can drop. This is largely prevented in young, healthy adults by an increase in heart rate and other compensatory mechanisms, but in older people, these are less effective. As a result, older people have greater food-induced BP decreases than younger adults (155). In some this can be excessive and lead to symptoms of dizziness and in some cases falls and cardiovascular events (261). The excessive drop in BP after food ingestion has been termed post-prandial hypotension and defined as a decrease in systolic BP of 20 mm Hg or more within 2 hours of food ingestion (262). Ingestion of all three macronutrients, alone or together causes post-prandial BP falls in older people. Available evidence indicates that carbohydrate and protein have equivalent BP lowering effects, although the BP decreases occur sooner after carbohydrate than protein and fat (263). We have recently reported substantial systolic BP decreases after ingestion of a 70 gm whey protein drink by healthy, older men, with 58% having a decrease of 20 mg Hg systolic or more within three hours of protein ingestion, with maximum decreases occurring between two and three hours after the drink (264). In contrast, maximum decreases after carbohydrate alone, or mixed drinks, appear to occur earlier. Seventy grams of whey is a high dose, higher than is generally recommended or likely to be ingested by older people (see below), and the hypotensive effects of protein and other macronutrients do seem to be at least partly dose-responsive (265). Nevertheless high-energy nutrient supplement drinks decrease BP in older people and the hypotensive effects of pure or high protein drinks

may be quite prolonged and possibly greater in those already on antihypertensive medications. Appropriate advice and precautions are indicated after such drinks, particularly in at-risk individuals.

2.4.5 Evidence for benefits of nutritional supplements in older people

A detailed review of this matter is beyond the scope of this review. Study methods and results are variable. Not all studies show benefits (256). Nevertheless, in our view, a number of conclusions can be drawn; the use of such supplements is generally safe (see above) and relatively easy to implement. Few, if any adverse effects of such supplements have been reported; use of these supplements by older people improves nutritional intakes and is associated with weight gain and an increase in lean body mass in many cases, and in some cases improvements in muscle function, hospitalisation rates and even death rates (266). The greatest benefit of taking nutritional supplements are obtained by the most undernourished older people (266).

For these reasons, and because many older people appear to have a suboptimal dietary protein intake, protein or protein- rich supplements are increasingly recommended to older people.

In summary, there is evidence that:

1. Older people have higher dietary protein needs than young adults, particularly if they have lost weight and/or are undernourished, are sarcopenic, or have acute or chronic medical conditions which contribute to muscle catabolism. These requirements are in the range of 1.2 gm/kg/day or higher.

2. Many, (probably the majority of those > 70 years) older people do not consume these minimum protein requirements. It can be difficult for older people to increase their dietary protein intake by increasing their intake of usual foods due to anorexia and the cost of high protein foods.
3. Protein supplements increase muscle mass and strength, and may also reduce morbidity and mortality, particularly in undernourished and/or sarcopenic older people.

2.4.6 Does the type of protein or amino acid in the supplement matter?

Yes, it probably does. Not all ingested proteins are the same, particularly in terms of their anabolic effects on skeletal muscle (see (249) for review). Available evidence indicates that branched chain amino acids, particularly leucine, are the most effective amino acids in stimulating muscle protein synthesis (MPS). Consistent with this, in one longitudinal study older people (> 65 years) with dietary leucine intakes in the upper quartile had preservation of lean body mass over 6 years whereas those with intakes in the lowest quartile had loss of lean body mass (267). The extent of MPS is proportional to the peak leucine plasma concentration after protein ingestion (268).

Animal derived dietary proteins appear to be stronger in stimulating MPS than plant-based proteins and milk-based proteins in particular are a good source of leucine containing proteins (249). Whey protein, obtained from milk in the cheese making process, is high in leucine, about 10-15%, and more rapidly absorbed than casein, factors which may contribute to its greater stimulatory effect than casein on MPS in older men (7). Maximum stimulatory effects on MPS probably occur after ingestion of 2-2.5 gm leucine in young and middle aged adults, which little additional effect

of higher intakes. Ingestion of 20gm whey protein concentrate at one time is probably sufficient to optimise MPS in young and middle aged adults (269). In contrast, because of anabolic resistance, older adults appear to need 30-35 gm or more of whey to achieve similar anabolic effects (270).

The effects of protein supplements to increase muscle mass and strength and to have functional benefits, appear greatest in those most at risk ie malnourished, sarcopenic, or at risk of sarcopenia. Several interventional studies have demonstrated that combined dietary supplements of whey protein and leucine increase muscle mass and strength and improve function in older sarcopenic adults see (249). A recent meta-analysis reported increased lean body mass without an increase in strength in sarcopenic older adults taking 2-7.8 gm per day of leucine supplements/day (271). It is difficult to separate the effects of leucine supplements from those of other amino acids or proteins such as whey when they are given in combination. It may be, particularly in the case of already leucine rich whey protein supplements, that they have benefits in addition to those of leucine, and that when a sufficient amount is ingested, further leucine fortification has little further benefit.

2.4.7 How much protein should there be in the supplement?

Owing to age-induced "anabolic resistance" older people probably require 30-45 gm protein per serve to stimulate muscle protein synthesis after that meal, whereas lower doses ($\leq$ 20 gm) are sufficient in young adults (see above and (84)). The consumption of 1-2 daily meals with protein content of 30 to 45 g may be an important strategy for increasing and/or maintaining lean body mass and muscle strength with ageing (84). There is evidence that older men and women with more-evenly mealtime

distributed protein intakes have higher muscle strength irrespective of their total protein intake (256). It seems reasonable, therefore, that if protein supplements are being used for their effects on muscle, they be used in a way that provides at least 1.2 gm/kg/protein per day (food plus supplement) with at least two protein intake episodes/day (food plus supplement) containing at least 30 gm protein each. As the post protein BP drop, which could be potentially harmful, appears to be dose-responsive, it is also likely that smaller, twice daily or even more frequent, doses of protein supplement will have more beneficial effects on BP than once daily larger doses.

2.4.8 Timing of protein intake relative to exercise

Both protein ingestion and resistance exercise independently stimulate muscle protein synthesis in older people, although the response to both is blunted in the elderly (272, 273). These two stimuli have synergistic anabolic effects on MPS, particularly when the protein is ingested soon after the resistance exercise (274). While ingestion of approximately 20 gm of a high-quality protein in sufficient to maximize skeletal muscle protein synthesis rates during recovery from resistance-type exercise in younger adults, doses up to 40 gm or possibly even higher, are needed in older adults (272, 273).

2.5 Gastrointestinal responses to protein ingestion: Effects of ageing

Appetite and food intake in free living humans are dependent on a complex interplay of environmental factors and central and peripheral physical mechanisms. The latter mechanisms include intra-gastric and small intestinal sensory and motor functions

and their interactions (71). Their study has been a focus of our group. We have used whey protein drinks and intra-duodenal infusions of whey protein to investigate peripheral responses to protein in young and older adults and have identified a number of age-related differences between these age groups in gastrointestinal responses to protein ingestion. The doses of oral whey used have mainly been 30 gm and/or 70 gm, which is of significance as 30gm appears to approximate the amount required in older people to stimulate muscle protein synthesis (see above), while higher doses are used by some older people.

Older people have reduced appetite compared to young adults and consume less food. There is no change, however, with ageing in the preference for particular macronutrients i.e. the percentage of total energy ingested as protein does not seem to change with increasing age (275).

2.5.1 Effect of ageing on appetite and feeding responses to whey protein

There is evidence that protein is the most satiating of the macronutrients in young adults (276), although this effect may be less in women than men (17). Our studies have demonstrated that healthy ageing is associated with a marked and significant reduction in the satiating effects of whey protein, administered by both intra-duodenal (9) and oral routes (15, 277).

2.5.1.1 Intra-duodenal whey

Healthy ageing is associated with a marked reduction in the suppressive effect of whey protein administered directly into the duodenum, on appetite and subsequent

food intake. Sixty minute intra-duodenal infusions of 8 gm (0.5 kcal/min), 24 gm (1.5 kcal/min) and 48 gm (3 kcal/min) of whey had a dose responsive suppressive effect on subsequent ad libitum food intake in young men, whereas older men experienced suppression of food intake only after the 48gm infusion (~33% vs 17% suppression by 48 gm whey in young vs older, $P < 0.05$) (9). Baseline hunger ratings were lower in the older than young men and were suppressed less by the protein infusions in the older than young men, consistent with the reduced effects of intra-duodenal fat and carbohydrate infusions on appetite in older people (27).

2.5.1.2 Oral whey

We have reported that healthy, non-obese, young men but not women experience significant suppression of hunger ratings and ad libitum food intake three hours after 30 gm and 70 gm whey protein drinks (12, 15, 17). In contrast, and consistent with their reduced responses to intra-duodenal whey, healthy, non-obese, men and women people over 65 years, experience little reduction in hunger and no suppression of ad libitum food intake three hours after either 30gm or 70gm whey drinks (12, 14-16, 278).

This age-related reduction in the satiating effects of whey drinks is observed in our studies largely irrespective of the timing of the whey drink relative to later ad libitum food intake at test meals. Thirty gram whey protein drinks do not suppress appetite ratings or subsequent ad libitum food intake in healthy older people immediately after the drink, or 35 minutes, 1 hour, 2 hours, 3 hours, 265 minutes and 510 minutes after the drink (14, 15, 98, 277, 278). In only one study have we detected any reduction in subsequent food intake by older people after a whey drink (277). In that

study older men received a 30 gm or 70 gm whey drink then ate at libitum at breakfast (30 minutes later), lunch (265 min later) and dinner (510 min later). There was no reduction in food intake compared to the control day at any of the three meals on the 30 gm day. On the 70gm day there was no effect on breakfast intake, but a 15% reduction in lunch energy intake ($P < 0.05$ vs control), followed by a compensatory 7% increase at dinner.

When intake from whey drinks plus subsequent food intake is calculated, absent (usually) or only minor suppression of subsequent food intake by whey drinks has resulted in consistent findings of increases in total energy intake and even greater proportional increases in protein intake after whey drinks in our short-term studies of older people. For example, in the study above where whey drinks were taken before breakfast and men studied for the rest of the day (277), there were non-significant 4% and 3% increases in total energy intake on the 30 gm and 70 gm days respectively compared to the control day. Total daily protein intake was increased significantly by the whey drinks in a dose-responsive manner , with increases almost equal to the protein content of the whey drinks (+31 gm on the 30 gm whey drink day, + 62 gm on the 70 gm whey drink day, $P < 0.001$ vs control (277).

These findings suggest that it should be possible to give enough extra protein to older people to preserve or increase muscle mass and function without suppressing energy intake and promoting weight loss, particularly if they are encouraged to continue their usual non-supplement food (energy) intake.

2.5.2 What is the best timing of the protein supplement use by older people?

Because older men can increase their protein intake in a single episode into the range of 30-40 gm - enough to maximize the protein's anabolic effects on muscle without the timing of that supplement's ingestion making much if any difference to subsequent appetite and food intake, these supplement can probably be ingested as a between-meal supplement, close to, or even with meals. Effects in women are likely to be similar (14), but require further study. The effects of more frequent protein doses across the day also need to be determined.

Indeed, if the supplement is given with a protein-containing meal, instead of between meals, less supplementary protein is needed to reach the 30-40 gm anabolic threshold described above. Older men in the NuAge study for example had a mean daily protein intake of 1 gm/kg (mean of 18 gm at breakfast and 23 gm at lunch and dinner) (256). Their protein intake could be increased to 1.33 gm/kg/day with a total protein intake of 35 gm per meal two meals a day by adding as little as 12 gm protein with each of lunch and dinner. A pragmatic measure, to allow for those with below average dietary protein intakes, would be take 20 gm of a protein supplement with the two meals each day already containing the most protein. This dose is unlikely to have many if any side effects for most older people.

2.5.3 Effect of ageing on gastric function and emptying

Healthy ageing is associated with changes in gastric function. These include reduced perceptions of proximal gastric distension and delayed gastric accommodation (91), probable changes in the intra-gastric distribution of food after its ingestion, greater

stimulation of phasic pyloric pressure waves by intra-duodenal lipid (46), and slowing of gastric emptying. It is unclear, however, how much, if at all, these changes contribute to the age-related reduction in the satiating effect of ingested protein and responses to protein supplements in older people. It is possible, however, that they may contribute to age-related reduced appetite and food intake.

2.5.3.1 Intra-gastric food distribution and antral area

After food is ingested, it is distributed throughout the stomach, with varying amounts in the proximal versus distal stomach (antrum). Antral distension appears to be a greater determinant of satiety and satiation than proximal gastric distension; the more distal the intra-gastric distribution of food, the greater the antral distension, the greater the sensations of fullness, the less the hunger, and the lower the subsequent food intake (25). Owing to impaired receptive relaxation of the gastric fundus (91) and other factors, food is distributed more distally in older people, as indicated by them having larger antral areas than young adults after ingestion of the same mixed macro-nutrient loads (25). As a result, they probably experience greater fullness and lower hunger ratings. While the more distal movement of food after its ingestion thus probably contributes to reduced hunger and increased fullness in older people, it is unclear how these changes could contribute to the reduced suppression of appetite ratings and food intake after oral ingestion of protein, alone or combined with other nutrients.

2.5.3.2 Gastric emptying

The oral ingestion of nutrients, irrespective of their type, slows gastric emptying.

Gastric emptying of non-nutrient drinks is slightly slower in older than in young adults (12, 14-16, 25, 27, 91, 98, 214, 277, 278). Whey protein drinks in doses of 30–70 gm slow gastric emptying in a dose-responsive manner (14, 15, 17), and probably slow it more in older than in young adults; the stomachs of healthy older men emptied whey into the duodenum at approximately 0.8 kcal/min compared with 1.0 kcal for young adult men ($P < 0.05$) in one study (15). Gastric emptying of whey protein drinks is faster in young, non-obese men than women (17), but this sex difference is no longer present in healthy people over 65 years (14). Gastric emptying of whey drinks occurs at a similar rate in obese young and older men (278), with neither age group having suppression of appetite or food intake 3 hours after ingestion of 30 gm whey—a suppressive dose in young, non-obese men (9, 15, 17). The findings suggest that obesity may blunt the effects of whey on both food intake and the slowing of gastric emptying.

Delayed gastric emptying results in more food in the stomach at any given time after food ingestion than would otherwise be the case. This increases gastric distension and, depending on the distribution of the food within the stomach, might be expected to produce greater feelings of fullness and reduced subsequent food intake. Consistent with this, the intentional creation of gastric distension and fullness by implanting gastric balloons has had some success in the treatment of obesity (279). Greater gastric distension after eating, due to slower gastric emptying, in older than in young adults, might thus be expected to cause greater post-eating suppression of appetite and food intake in older than in young adults. Existing evidence does not suggest, however, that this is so. Perceptions of gastric distension are less in older than young adults (91), and healthy older people have fullness ratings (unlike hunger ratings) 3 h after a whey drink, at the start of an ad libitum meal, that do not relate

significantly to energy intake at that meal (15). Furthermore, as outlined above, older people have less, not more, suppression of appetite and food intake by whey drinks than younger adults, despite slower gastric emptying and presumably greater gastric distension at any given time after the drinks. Our groups' studies have largely involved administering the ad libitum test meal 3 hours after the whey test drink, in order to allow the full effect of gastric mechanisms to be studied. The stomach is empty or almost empty by then after both 30 gm and 70 gm whey protein drinks (15). While it is possible that, if the meal had been given earlier, greater suppressive effects on intake due to greater gastric distension would have been present, this is not supported by our finding of a lack of suppression by 30 gm whey of food intake immediately after the drink and hourly up to 3 hours after the drink (98).

2.5.3.3 Small intestinal satiety mechanisms

As ageing has similar qualitative effects on appetite and food intake after intra-duodenal protein (whey) to those of oral whey (see above), and the age-related changes in gastric mechanisms do not appear to explain the reduced suppression of appetite and food intake by protein in older people, it seems likely that the reduced age-related changes in response to protein are mediated mainly by reductions in the satiating effects of the protein after it enters the small intestine. Nevertheless, slower gastric emptying in older people may modify these post-gastric effects by delaying their onset and prolonging their duration. Sixty minute duodenal infusions of 0.5 kcal/min and 1.5 kcal whey/minute do not suppress subsequent food intake in healthy older men, but 3 kcal/min infusions do, albeit less in older than in younger men (9). As healthy older men empty whey drinks from the stomach at a rate of approximately 0.8 kcal/min (15), it is likely that, even if a very high protein load is taken in (drink

form, for most older people, their age-related slowing of gastric emptying and markedly reduced effect of whey once it enters the duodenum combine to result in no suppression of appetite or subsequent energy intake. In contrast, duodenal infusions of whey in doses of 0.5, 1.5, and 3 kcal/in have dose-responsive suppressive effects on energy intake in young men, who empty whey drinks from the stomach faster than older men (~1 kcal/min) (15). Together, these findings explain the suppressive effects of both 30 gm and 70 gm whey drinks on subsequent energy intake in young, but not older adults (15).

2.5.4 Selected hormones

Ingestion of protein, either orally or by infusion directly into the duodenum, results in changes in circulating concentrations of a number of hormones with definite or possible effects on appetite and subsequent food intake. Among them, concentrations of cholecystokinin (CCK), insulin, glucagon, gastric inhibitory peptide (GIP), glucagon-like peptide-1 (GLP-1), peptide tyrosine-tyrosine (PYY), and amino acids increase, while glucose does not change and ghrelin concentrations decrease (12-14, 16, 17).

A number of these small intestinal responses to protein ingestion differ between healthy older and younger adults and these age-related changes in turn possibly affect the responses to protein or protein containing supplements in older people.

2.5.4.1 Cholecystokinin (CCK)

CCK is released by the small bowel after nutrient ingestion and acts to slow gastric

emptying and reduce food intake. Circulating CCK concentrations increase less after oral protein ingestion in young women than in young men, which may be one reason for the lower satiating effect of whey protein in young women than men (17). Circulating CCK concentrations are higher in healthy fasting older than young adults (29, 90), and older adults retain their sensitivity to the satiating effects of exogenous CCK (231). Increased CCK activity may thus be a cause of the anorexia of ageing and reduced hunger pre meals observed in older adults. Increases in circulating CCK concentrations are at least as great (53), if not greater (58), after whey drinks in healthy older than young adult men and women. The cause of this possibly greater rise is not known; it may relate to slower transition of whey through the small bowel in older people and, therefore, more prolonged contact with the CCK releasing cells. Ageing-related increases in CCK secretion and action are unlikely, however, to contribute to the reduced suppression of appetite and food intake by whey protein observed in older people compared with young adults. The opposite might be expected. The exact role of CCK in mediating age-related responses to protein supplements remains to be determined.

2.5.4.2 Glucagon-like peptide 1 (GLP-1)

GLP-1 is released by the small bowel and colon in response to food ingestion. Like CCK, it slows gastric emptying and has satiating effects. Fasting GLP-1 concentrations are higher in healthy older than young ageing (29, 90), which may thus contribute to lower basal (fasting) hunger in older people. Circulating GLP-1 concentrations appear to increase to a similar extent after whey drinks in young and older adults (13, 214, 279), which does not support a role for the lesser suppression of food intake by whey protein in older adults.

2.5.4.3 Gastric inhibitory peptide (GIP)

Along with GLP-1, GIP is an incretin that plays roles in the control of glucagon, insulin, and blood glucose concentrations. It is not clear what role GIP plays in the control of appetite and feeding, but it may have some effect to stimulate food intake (280). Circulating fasting GIP concentrations are not affected by normal ageing, but GIP concentrations increase more after oral whey ingestion in older than in young adults (12, 14, 16, 25, 27, 91, 98, 214, 277, 278). This greater increase might act to reduce the whey-induced suppression of food intake that would otherwise occur. It might also act to limit blood glucose concentration increases in older people after protein is co-ingested with other nutrients.

2.5.4.4 Insulin and glucose

Insulin plays a key role in glucose homeostasis, while its role in appetite and feeding control is less clear. Oral ingestion of protein, including whey, stimulates insulin secretion in a dose-responsive manner (90). While oral ingestion of protein on its own has little, if any, effect on blood glucose concentrations, its co-ingestion with glucose by non-elderly adults with type 2 diabetes results in significantly smaller increases in blood glucose concentrations than ingestion of the same amount of glucose on its own (68). The stimulation of insulin secretion by whey drinks is not apparently affected by ageing, and remains robust (90). We have recently found that co-ingestion of 30 gm whey protein with 30 gm glucose in drink form significantly reduces the increase in blood glucose concentrations compared with ingestion of 30 gm glucose alone (peak glucose 7.4 vs. 9.0 mmol/L, $P < 0.01$) in men over 65 years

(265), and are now extending these studies to older people with type 2 diabetes. These findings suggest that moderately high whey protein intake together with carbohydrate might improve postprandial glycaemia in older people, particularly those with diabetes, and provide an additional benefit of taking a protein supplement with, rather than between, meals.

2.5.4.5 Glucagon

Circulating concentrations of glucagon are not affected by ageing (13, 214, 279). Whey protein drinks act to increase glucagon concentrations, to a similar degree in older and young adults (90).

2.5.4.6 Ghrelin

Ghrelin is an orexigenic hormone secreted by the enteroendocrine cells of the gastrointestinal tract, particularly the stomach. Circulating concentrations are highest in the fasting state and decrease after food ingestion. Fasting circulating concentrations may be slightly lower in older than in young adults, and thus contribute to the anorexia of ageing (26, 281, 282). Ghrelin concentrations are suppressed to a similar degree after whey drinks in healthy older and young adults (90), so it is not clear what role, if any, ghrelin plays in mediating age-related reductions in feeding responses to protein supplements.

2.6 Future directions

Future studies should focus on women as well as men to determine whether our

findings in older men also apply to women. They should also determine whether the results of our short-term studies with protein supplements, such as post-supplement blood pressure drops and failure to suppress appetite and food intake, are replicated in studies of longer term protein supplement use. Further studies, both short- and longer-term, of the effect of protein when co-ingested with carbohydrates on glucose metabolism in older people with and without diabetes mellitus would also be of interest.

2.7 Conclusions

Nutritional supplements, including pure-protein or protein-enriched drinks, are increasingly used by older people to maintain body weight and nutrition, and specifically to prevent or treat loss of muscle function, sarcopenia, and frailty. Daily protein requirements in older people appear to be higher than those of young adults—in the range of 1.2 gm/kg/day or more. Many older adults do not consume this much protein in their usual diet and are likely to benefit from an increase.

Protein supplements appear relatively safe, although care should be taken in those with or at risk of renal impairment, or prone to post-nutrient hypotension. Protein supplements (alone or in a mixed macronutrient supplement) in doses sufficient to reach the above daily intake are likely to be beneficial, perhaps best taken twice daily, if possible soon after resistance exercise, in doses that achieve protein intakes of 30 gm or more per episode. Our study results suggest that it is probably not important to give these supplements between meals, because they have little, if any, suppressive effect on appetite and later food intake in older people, owing to age-related changes in gastrointestinal and other mechanisms that are as yet poorly understood. Adding

protein supplements to usual food intake is very unlikely to reduce energy intake and instead is likely to increase overall energy and protein intake, particularly if encouragement is given to continue usual food intake. There may even be benefits in giving the supplement with meals, to achieve protein intakes above the effective anabolic threshold with lower supplement doses, and have favorable effects on food-induced blood glucose increases in older people with, or at risk of, developing type 2 diabetes mellitus.

Further studies are indicated to determine the following:

1. The acute effects of whey and other proteins, when co-ingested with other macronutrients, with and between meals, on appetite and food intake in older people.
2. The longer term effects of protein ingestion, alone and combined with other macronutrients, on appetite, food intake, and glucose homoeostasis, in older people; i.e., whether effects observed in acute studies persist with longer-term administration.

A better understanding of the mechanisms underlying the reduced suppression of appetite and food intake by protein and other macronutrients in older compared with younger adults, whether gastro-intestinal, central, or both, can be used to develop ways of improving nutrition in at-risk older individuals. Although our studies with whey have identified a number of age-related changes in gastro-intestinal responses to protein ingestion, it is not yet clear exactly how they act to mediate age-related changes in appetite and feeding.

2.8 Overall aims and hypotheses

In order to address the questions raised in sections 1.2.4, 1.3.2 and 1.4.3, the overall aims and hypotheses of the thesis are that:

• Suppression of energy intake by whey protein when compared to control will be less in healthy older than younger adults, resulting in an increase in cumulative energy and protein intake in older men (chapter 3).

• The acute suppression of energy intake by whey protein, will be less in older men with obesity as compared to younger men with obesity (chapter 4).

• Insulin concentrations following both glucose and protein drinks would increase and, to determine whether the effect on insulin of the combination drink is synergistic or additive, we compared the effect of combined whey protein and glucose on the rise in plasma insulin concentrations with that of the sum of the effects of glucose and whey protein alone on insulin concentrations using a paired t-test (chapter 5).

• Insulin concentrations following both glucose and protein drinks would increase, and to determine if the effect on insulin of the combination drink is synergistic or additive, we compared the effect of combined whey protein and glucose (GP drink) on the rise in plasma insulin concentrations to that of the sum of the effects of glucose and whey protein alone (G drink + P drink) on insulin concentrations, using a paired t test (chapter 6).

• Older, when compared to younger, men will exhibit a greater fall in blood pressure, and a smaller increase in heart rate, following whey protein ingestion compared to control (chapter 7).

• The decrease in blood pressure in response to ingestion of 30g whey protein will be less compared to 70g whey protein in healthy older men (chapter 8).

• The hypotensive effects of whey protein containing drinks will be dependent on the energy, rather than protein, content of the drink and persist for greater than 2 h (chapter 9).

CHAPTER 3: WHEY PROTEIN DRINK INGESTION BEFORE BREAKFAST SUPPRRESSED ENERGY INTAKE AT BREAKFAST AND LUNCH, BUT NOT DURING DINNER, AND WAS LESS SUPPRESSED IN HEALTHY OLDER THAN YOUNGER MEN

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Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i) the candidate's stated contribution to the publication is accurate (as detailedabove);

ii) permission is granted for the candidate in include the publication in the thesis;and

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3.1 Abstract

Ageing is associated with changes in feeding behavior. We have reported that there is suppression of energy intake three hours after whey protein drink ingestion in young, but not older, men. This study aimed to determine these effects over a time period of 9 h. Fifteen younger (27 ± 1 years, 25.8 ± 0.7 kg/^{m2}) and 15 older (75 ± 2 years, 26.6 ± 0.8 kg/^{m2}) healthy men were studied on three occasions on which they received, in a randomized order, a 30 g/120 kcal, 70 g/280 kcal whey-protein, or control (~2 kcal) drink. Ad-libitum energy intake (sum of breakfast, lunch, and dinner) was suppressed in a protein load responsive fashion (P = 0.001). Suppression was minimal at breakfast, substantial at lunch (~16%, P = 0.001), no longer present by dinner, and was less in older than younger men (-3 ± 4% vs. 8 ± 4%, P = 0.027). Cumulative protein intake was increased in the younger and older men (+20% and +42%, P < 0.001). Visual analogue scale ratings of fullness were higher and desire to eat and prospective food consumption were lower after protein vs. control, and these effects were smaller in older vs. younger men (interaction effect P < 0.05). These findings support the use of whey-protein drink supplements in older people who aim to increase their protein intake without decreasing their overall energy intake.

3.2 Introduction

The number of older people with malnutrition, both under- and over-nutrition, is rising (283). Healthy ageing is associated with a reduction in appetite and food intake, including protein intake, which predisposes older people to loss of body weight and in particular, skeletal muscle mass (10, 71). The latter is associated with a decrease in function and quality of life (284). The causes of the reduction in food intake during healthy ageing are likely to be heterogeneous, including changes in gastrointestinal mechanisms induced by nutrient intake, such as slowing of gastric emptying (70, 285).

A common strategy to increase energy intake and body weight in undernourished older people is the use of > 25–30 g whey protein-enriched supplements (286), which may result in preserved or even increased muscle mass and strength (286, 287). We reported that in healthy older adults, when compared to younger adults, the acute suppression (up to 3 h following ingestion) of energy intake by protein administered orally or infused directly into the duodenum is less, resulting in an increase of overall energy and protein intake in the older adults (9, 15, 98). In healthy, younger adults, protein is considered to be the most satiating macronutrient and protein-rich supplements and diets are often recommended as a weight loss strategy in obese, younger individuals. There is a lack of definitive evidence on their efficacy (288, 289), especially in older adults.

In this study, we aimed to characterize the effect of ageing on the suppression of food intake at breakfast, lunch, and dinner over a time period of 9 h by a pre-breakfast whey protein load (30 g and 70 g) compared to a control drink in healthy younger

and older men. We hypothesized that suppression of energy intake by whey protein when compared to control would be less in healthy older than younger adults, resulting in an increase in cumulative energy and protein intake in the older men.

3.3 Materials and methods

3.3.1 Participants

The study included 15 healthy younger men (mean ± standard error of the mean (SEM) age: 27 ± 1 years; body weight: 76.1 ± 2.0 kg; height: 1.73 ± 0.02 m; body mass index (BMI): 25.8 ± 0.7 kg/^{m2}) and 15 healthy older men (75 ± 2 years; 80.7 ± 2.9 kg; 1.75± 0.01 m; 26.6 ± 0.8 kg/^{m2}). Body weight and BMI of the younger and older men did not differ significantly ($P > 0.05$). Participants were recruited by online advertisement and by flyers placed on notice boards at the University of Adelaide, Adelaide, Australia.

Exclusion criteria included smoking; alcohol intake of > 2 standard drinks on > 5 days per week; being vegetarian; intake of any illicit substance; use of prescribed or non-prescribed medications that may affect appetite, body weight, gastrointestinal function, or energy metabolism; food allergy(s); diabetes mellitus (fasting glucose concentration > 6.9 mmol/L); epilepsy; gallbladder, pancreatic, cardiovascular, or respiratory diseases; significant gastrointestinal symptoms, disease, or surgery; any other illness deemed significant by the investigator; and an inability to comprehend the study protocol. Inclusion criteria included being weight stable (< 5% fluctuation in their body weight) at study entry, as assessed by their self-reported weight in the preceding 3 months, and maintenance of usual physical activity level.

All participants gave written informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Ethics Committee of The Royal Adelaide Hospital (HREC/18/CALHN/132) and registered under trial registration number ACTRN12618000881235.

3.3.2 Protocol

Each participant was studied on three occasions, separated by ~3–10 days. On each occasion, they received, in a randomized order (using the method of randomly permuted blocks; www.randomization.com), a single drink of either flavoured water (control; ~2 kcal), 30 g whey protein (120 kcal), or 70 g whey protein (280 kcal). The drinks were equivolaemic (~450 mL) and contained different quantities of food-grade unflavoured whey protein isolate (Bulk Nutrients, Tasmania, Australia) dissolved in varying amounts of distilled water, sodium chloride, and low-calorie lime cordial (Bickford's "diet lime" cordial) (15).

Volunteers arrived at the laboratory at ~8.00 a.m. after fasting for ~12 h overnight and refraining from strenuous exercise and alcohol for 24 h. The participants were provided with a standard meal the night before each study day (beef lasagne, McCain Foods Pty Ltd., Wendouree, VIC, Australia ~591 kcal). Participants were told that we were assessing perceptions of appetite around the 3 meals, but not that we measured their food/energy intake.

At baseline (t = -5 min), perceptions of appetite were assessed by visual analogue scales (VAS) and the antral area of the stomach (cm^2) was measured with a LogiqTM

e-ultrasound machine (GE Healthcare Technologies, Sydney, NSW, Australia). Subsequently, the drink was administered at t = -2 min (~8.30 a.m.) and was served in an opaque cup to ensure that the volunteers were blinded. Participants were asked to ingest the drink within 2 min. Following consumption of the drink (t = 0 min), palatability of the drink and perceptions of appetite were assessed by VAS. The antral area of the stomach was measured at several time points between the drink and breakfast (t = 0, 5, 20, 35 min) and not thereafter. Energy intake was measured at breakfast (t = 35–65 min; ~9 a.m.), lunch (t = 275–305 min; ~1 p.m.), and dinner (t = 515–545 min; ~5 p.m.). Breakfast and lunch consisted of a cold buffet-style meal (Table 3.1) and dinner consisted of a warm meal and a small variation of buffet items (Table 3.2). Participants were instructed to consume food until they were comfortably full. Before and after consumption of the meals, perceptions of appetite, in terms of hunger, fullness, desire to eat, and prospective food consumption, were assessed (t = 0, 5, 20, 35, 65, 80, 95, 275, 305, 320, 335, 515, 545, 560, 575 min). Participants were not permitted to consume any food or drink between ingesting the study drink and the end of the study day, except at the breakfast, lunch, and dinner meals provided during the study day. Water intake in between meals was allowed, but not within 30 min before their next meal.

Table 3.1: Composition of the cold buffet-style breakfast and lunch meal

Food items	**Amount served (g)**	**Energy Content (kcal)**	**Protein (g)**	**Carbohydrate (g)**	**Fat (g)**
Wholemeal bread, 4 slices ∗	125	308	13.8	54.8	4.9
White bread, 4 slices ∗	125	304	11.1	61.4	2.7
Cheese, sliced †	85	346	22.6	0.9	29.2
Ham, sliced ‡	100	95	17.1	3.5	1.8
Chicken, sliced §	100	104	19.4	3.7	1.7
Margarine ‖	20	108	0.0	0.0	12.4
Mayonnaise ¶	20	137	0.4	0.7	15.2
Tomato, sliced	100	13	1.0	2.0	0.1
Cucumber, sliced	100	11	0.5	2.0	0.1
Lettuce	100	5	0.9	0.4	0.0
Apple	170	89	0.5	2.0	0.1
Banana	190	166	3.3	39.0	0.2
Fruit salad **	140	81	0.4	17.7	1.3
Strawberry yogurt ††	175	162	9.1	25.0	3.4
Chocolate custard ‡‡	100	105	3.3	16.9	3.1
Milky Way §§	12	52	0.3	9.0	1.9
Orange juice, unsweetened‖‖	300	117	1.9	22.6	2.7
Iced coffee ¶¶	375	254	12.4	38.3	6.6
Water	600	0	0.0	0.0	0.0
Total		*2,457*	*19%*	*49%*	*32%*

∗ Sunblest, Tiptop, George Weston Foods Ltd, Enfield, NSW, Australia. † Coon Tasty Cheese slices, Australian Cooperative Foods Ltd, Sydney Olympic Park, NSW, Australia. ‡ KR

Castlemaine boneless leg ham, George Weston Foods Ltd, Enfield, NSW, Australia. § Inghams chicken breast, Inghams Enterprises Pty Ltd, Burton, SA, Australia. || Vita-Lite canola, Peerless Holdings Pty Ltd, Braybook, VIC, Australia. ¶ MasterFoods, Mars Food Australia, Berkeley Vale, NSW, Australia. ∗∗ Goulburn Valley, SPC, Ardmona Operations Ltd, Shepparton, VIC, Australia. †† Yoplait, LD&D Foods Pty Ltd, Docklands, VIC, Australia. ‡‡ Yogo, LD&D Foods Pty Ltd, Docklands, VIC, Australia. §§ Mars Chocolate Australia, Wendouree, VIC, Australia. |||| Golden Circle Orange juice, Golden Circle Limited, QLD, Australia ¶¶ Farmers Union, LD&D Foods Pty Ltd, Docklands, VIC, Australia.

Table 3.2 Composition of the dinner meal

Food items	Amount served (g)	Energy Content (kcal)	Protein (g)	Carbohydrate (g)	Fat (g)
Pasta with Meatballs ¤	500	720	27.7	78.4	35.0
Whole meal bread, 4 slices ∗	125	308	14.0	55.5	4.9
Margarine ||	20	108	0.0	0.0	12.5
Philadelphia cream cheese º	68	175	3.8	2.1	17.3
Apple	170	89	0.5	2.0	0.1
Banana	190	166	3.3	39.5	0.2
Fruit salad **	140	81	0.4	17.9	1.4
Strawberry yogurt ††	175	162	9.2	25.3	3.4
Chocolate custard ‡‡	100	105	3.3	17.1	3.1
Muesli bar Ɔ	35	185	5.6	12.5	13.1
Orange juice, unsweetened ||||	300	117	1.9	22.9	2.7
Water	600	0	0.0	0.0	0.0
Total		*2,216*	*13%*	*49%*	*38%*

¤ Man Size Spaghetti and Meatballs, McCain Foods Pty Ltd, Wendouree, VIC, Australia. ∗ Sunblest, Tiptop, George Weston Foods Ltd, Enfield, NSW, Australia. || Vita-Lite canola, Peerless Holdings Pty Ltd, Braybook, VIC, Australia. º Philadelphia Spreadable Cream Cheese snack tubs, Consumer Advisory Service, Melbourne, VIC, Australia. ∗∗ Goulburn Valley, SPC, Ardmona Operations Ltd, Shepparton, VIC, Australia. †† Yoplait, LD&D Foods Pty Ltd, Docklands, VIC, Australia. ‡‡ Yogo, LD&D Foods Pty Ltd, Docklands, VIC, Australia.

3.3.3 Measurements

The primary outcome of the study was ad libitum energy intake at the buffet-style meal and secondary outcomes include antral area and appetite.

3.3.3.1 Energy intake

To quantify the amount eaten, the weights of the food items were recorded before and after they was offered to the participants (15). Energy intake and macronutrient composition was calculated using commercially available software (Foodworks 3.01, Xyris Software, Highgate Hill, QLD, Australia). Absolute (kcal) and percentage suppression of energy intake (expressed as % of energy intake of the control day) by protein were calculated.

3.3.3.2 Antral area

Gastric emptying (gastric retention) was determined by measuring the antral area of the stomach. The circumference of the antral area was measured with a LogiqTM e-ultrasound machine (GE Healthcare Technologies, Sydney, NSW, Australia) by using a 3.5 C broad spectrum 2.5–4 MHz convex linear array transducer. Antral area (cm^2) was determined with the use of a caliper and calculation program built into the ultrasound machine. Volunteers were seated on a chair and were asked to be still during the measurement. The transducer was positioned vertically to obtain a parasagittal image of the antrum, with the superior mesenteric vein and the abdominal aorta in a longitudinal section. If gastric contractions were observed, the acquisition was paused until the contraction wave had passed. To calculate meal

retention in the whole stomach, the fasting antral area (measured at baseline) was subtracted from subsequent measurements performed after ingestion of the drinks (290). Gastric retention was then calculated at a given time point as:

$$\text{Retention (\%)} = [AA(t) - AA(f)]/[AA(max) - AA(f)] \times 100,$$

where AA(t) = antral area measured at a given time point, AA(f) = fasting antral area, and AA(max) = maximum antral area recorded after drink ingestion (15).

3.3.3.3 Perceptions of appetite and palatability

Perceptions of appetite in terms of hunger, fullness, desire to eat, and prospective consumption were assessed by use of a VAS questionnaire (291). The questionnaire consisted of 100 mm horizontal lines, where 0 represented that the sensation was "not felt at all" and 100 represented that the sensation was "felt the greatest." Volunteers placed a vertical mark on each horizontal line to signify the strength of each sensation at the specified time points. Baseline fasting ratings were calculated as the mean of the three study days. Total area under the curve (AUC) was calculated over 0–180 min (15). Palatability of the drink was assessed by ratings of pleasantness, intenseness, full of taste, sweetness, saltiness, sour, bitterness, umami, and creaminess immediately after drink intake; palatability of the meal was assessed by like of taste, like of aftertaste, and enjoyability of the meal by use of a VAS questionnaire.

3.4 Data and statistical analysis

Statistical analyses were performed using SPSS software (version 24; IBM, Armonk, NY, USA). Power calculations were performed for the primary outcome of energy intake using measures of variance obtained from previous data (standard deviation (SD) of 181 kcal) (15) to detect a minimum difference in suppression of energy intake by the treatment condition compared with the control of 251 kcal between younger and older participants. Age and protein load main effects and the age by protein load interaction on outcomes were determined by using two-way repeated-measures analysis of variance (ANOVA). Residuals from all models were checked for normality and constant variance and all assumptions were found to be met. When significant treatment and/or interaction effects were present, Bonferroni corrected post hoc tests were performed to determine which specific drink conditions were different between age groups. Statistical significance was accepted at $P < 0.05$. All data are presented as means ± SEMs.

3.5 Results

The study protocol was well tolerated by all participants.

3.5.1 Energy intake

Energy intake after the drink (sum of breakfast, lunch, and dinner; Figure 3.1) was suppressed by whey protein compared to control (protein load main effect on energy intake $P = 0.012$), driven by the suppression of the 70 g whey protein drink (young: -251 ± 117 kcal, -8 ± 4%; older: -184 ± 96 kcal, -5 ± 4%; post-hoc test $P = 0.023$),

which was greater (P = 0.027) when compared with the 30 g protein drink (young: -88 ± 108 kcal, -3 ± 4%; older: -5 ± 99 kcal, 0 ± 4%; Table 3.3). Suppression of energy intake by the 70 g whey protein compared to control (protein load main effect, P = 0.007) was greatest at lunch (young: -181 ± 83 kcal, -17 ± 8%; older: -154 ± 49 kcal, -15 ± 5%; P = 0.001; Figure 3.2). Protein intake of the drink, before breakfast, did not affect ad libitum energy intake at dinner in either age group. Suppression of energy intake (sum of breakfast, lunch, and dinner) by whey protein was less in healthy older men: -94 ± 82 kcal when compared to younger men -169 ± 100 kcal (there was a main effect of age on suppression of energy intake by protein compared to control P = 0.027).

Cumulative energy intake (sum of energy in test drink, breakfast, lunch, and dinner) was not significantly different between study days and age groups (young: control: 2929 ± 131 kcal, 30 g whey protein: 2961 ± 161 kcal and 70 g whey protein: 2958 ± 163 kcal; older: 2878 ± 165 kcal, 2993 ± 122 kcal and 2974 ± 148 kcal, all P > 0.05).

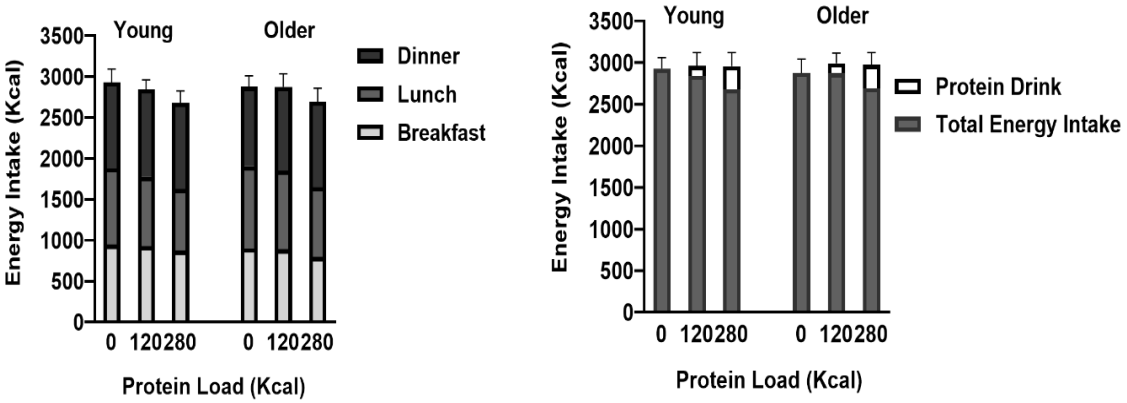

Figure 3.1: Energy intake at breakfast, lunch, and dinner following whey protein ingestion in healthy young and older men. Mean (± SEM) ad libitum energy intake (kcal; left) at breakfast (light grey bars), lunch (dark grey bars), and dinner (black bars) following drink ingestion containing flavored water (control, ~2 kcal) or whey protein

(30 g/120 kcal or 70 g/280 kcal) and cumulative energy intake (kcal; right; sum total energy intake at breakfast, lunch, and dinner combined (dark grey bars) and protein drink (white bars)) in young (left; n = 15) and older (right; n = 15) men. Age and protein load main effects and interaction effects were determined by repeated measures ANOVA. * The 70 g protein drink suppressed energy intake (sum of breakfast, lunch, and dinner) compared with the control (protein load effect P = 0.012, post-hoc P = 0.023).

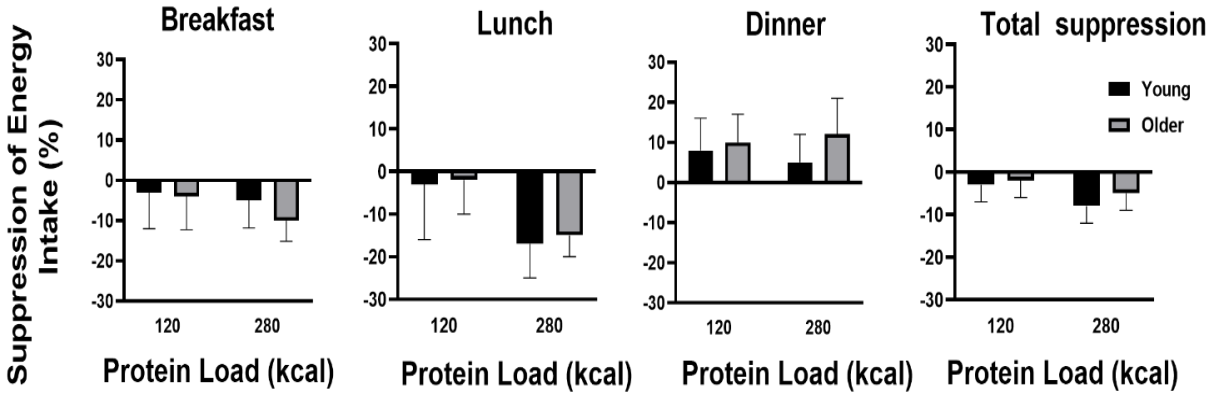

Figure 3.2: Suppression of energy intake by whey protein at breakfast, lunch and dinner and total suppression of energy intake in healthy young and older men. Mean (± SEM) suppression of energy intake (kcal) at breakfast lunch and dinner), and total intakes (sum breakfast, lunch and dinner) following drink ingestion containing flavoured water (control, ~2kcal) or whey protein (30 g/120kcal or 70 g/280kcal) in young (grey shading; n = 15) and older (black shading; n = 15) men. Age and protein load effects and interaction effects were determined by using repeated-measures ANOVA. *Energy intake was suppressed by protein compared to control (protein-load effect P = 0.012). Suppression of energy intake by 70g protein compared to control (P=0.007) was particularly occurring during lunch (P = 0.001). Suppression of energy intake (sum of breakfast, lunch and dinner) by protein was less in healthy older when compared to younger men (age effect P = 0.027).

Table 3.3: Energy intake at, and macronutrient composition of, breakfast, lunch and dinner following whey protein ingestion in healthy younger and older men

	Young (n=15)				Older (n=15)			
	Breakfast	**Lunch**	**Dinner**	**Total**	**Breakfast**	**Lunch**	**Dinner**	**Total**
Control drink								
Energy intake (kcal)	947 ± 64	933 ± 74	1049 ± 68	2929 ± 131	896 ± 74	1007 ± 62	975 ± 79	2878 ± 165
Fat (energy %)	34 ± 1	34 ± 2	36 ± 2		29 ± 2	33 ± 6	39 ± 2	
Carbohydrate (energy %)	43 ± 2	43 ± 2	50 ± 2		51 ± 2	46 ± 2	47 ± 2	
Protein (energy %)	23 ± 1	23 ± 1	14 ± 1		20 ± 1	21 ± 1	14 ± 1	
30 g (120 kcal) protein drink								
Energy intake (kcal)	925 ± 67	848 ±89	1068 ±48	2841 ± 161	888 ± 60	962 ± 84	1023 ± 66	2873 ± 122
Fat (energy %)	34 ± 2	30 ± 3	38 ± 2		30 ± 1	34 ± 1	38 ± 2	
Carbohydrate (energy %)	43 ± 2	48 ± 4	47 ± 1		51 ± 3	46 ± 2	48 ± 1	

Protein (energy %)	23 ± 1	22 ± 2	15 ± 0		19 ± 1	20 ± 1	14 ± 1	
70g (280kcal) protein drink								
Energy intake (kcal)	874 ± 70	752 ± 85*	1052 ± 56	2678 ± 163	794 ± 72	853 ± 69*	1047 ± 82	2694 ± 148
Fat (energy %)	34 ± 1	27 ± 2	48 ± 2		30 ± 2	32 ± 1	38 ± 2	
Carbohydrate (energy %)	43 ± 2	54 ± 3	47 ± 1		51 ± 4	46 ± 2	48 ± 1	
Protein (energy %)	23 ± 1	19 ± 2	15 ± 0		19 ± 1	22 ± 1	14 ± 0	

Mean (± SEM) ad libitum energy intake (kcal) at, and macronutrient composition (energy percentage) of, breakfast, lunch and dinner, following drink ingestion containing flavoured water (control, ~2 kcal) or whey protein (30 g/120 kcal or 70 g/280 kcal) in young (left; n = 15) and older (right; n = 15) men. Main age and protein-load effects and interaction effects were determined by using repeated-measures ANOVA. *Energy intake was suppressed by protein compared to control (protein-load effect $P = 0.012$). Suppression of energy intake by 70 g protein compared to control ($P = 0.007$) was particularly occurring during lunch ($P = 0.001$).

3.5.2 Protein intake

1. The sum of breakfast, lunch, and dinner protein intake after the test drinks decreased after the 70 g (P = 0.023), but not 30 g, whey protein drink when compared to the control day (protein load main effect P = 0.009, main effect of age P = 0.71, interaction effect P = 0.54).
2. Cumulative protein intake (sum of protein in the drink plus protein intake at the meals) was increased in a protein load responsive fashion (young: control: 143 ± 10 g, 30 g whey protein: +17%, 167 ± 9 g and 70 g whey protein: +36%, 195 ± 9 g; older: control: 133 ± 10 g, 30 g whey protein: +23%, 164 ± 10 g and 70 g whey protein: +47%, 195 ± 9 g; P < 0.001) comparably in the healthy younger and older men (main effect of age P = 0.71, interaction effect of age x protein load P = 0.54; Figure 3.3).

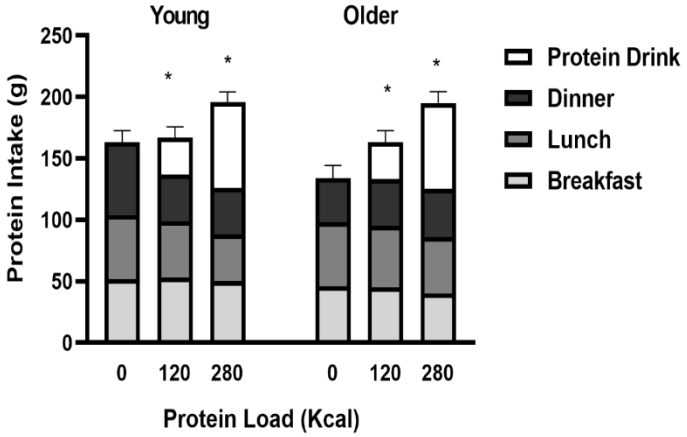

Figure 3.3: Mean (± SEM) protein intake (g) at breakfast (light grey bars), lunch (dark grey bars), and dinner (black bars) following drink ingestion containing flavored water (control, ~2 kcal) or whey protein (30 g/120 kcal or 70 g/280 kcal; white bars) in young (left; n = 15) and older (right; n = 15) men. Age and protein load main effects and interaction effects were determined by using repeated-measures ANOVA. * Cumulative protein intake (sum of protein drink plus protein

intake at meals) was increased in a protein load responsive fashion comparably in the healthy young and older men (main effect of age $P = 0.71$, protein load main effect $P < 0.001$, interaction effect $P = 0.54$).

3.5.3 Gastric emptying

Antral areas following overnight fasting (control: 3.4 ± 0.8 cm^2; 30 g whey protein: 2.8 ± 0.7 cm^2; 70 g whey protein: 2.9 ± 0.8 cm^2; protein load main effect $P = 0.21$) and immediately after drink consumption (control: 15.6 ± 0.8 cm^2; 30 g whey protein: 16.2 ± 0.8 cm^2; 70 g whey protein: 16.4 ± 0.8 cm^2; protein load main effect $P = 0.76$) were comparable between the study days for both the age groups. Gastric retention was greater after both protein drinks compared to control (main effect of age $P = 0.27$, protein load main effect $P < 0.001$, interaction effect $P = 0.091$; Figure 3.4).

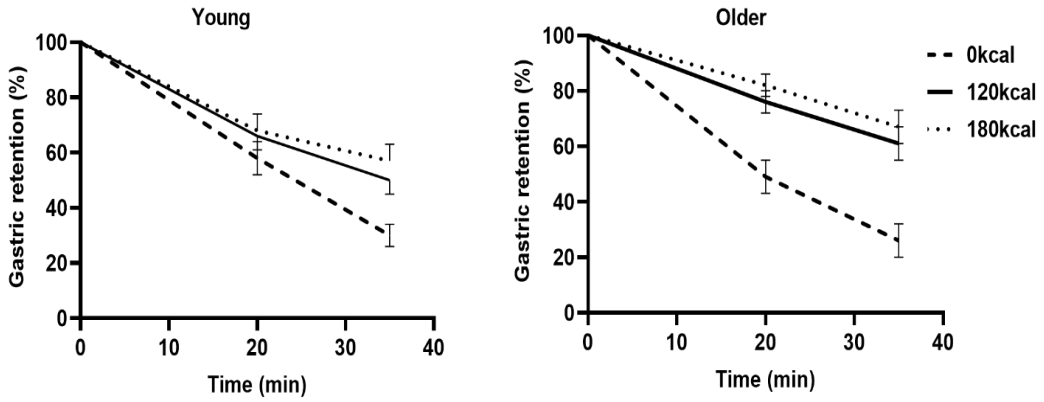

Figure 3.4: Mean (± SEM) Gastric Retention (%) of drinks containing flavored water (control, ~2 kcal) or whey protein (30 g/120 kcal or 70 g/280 kcal; open bars) in young (left; n = 15) and older (right; n = 15) men. Age and protein load main effects and interaction effects were determined by using repeated-measures ANOVA. *Gastric Retention, calculated based on the antral areas, were larger after both protein drinks compared to control (main effect of age $P = 0.27$, protein main effect

$P < 0.001$, interaction effect $P = 0.091$).

3.5.4 Appetite

Baseline perceptions of appetite in terms of hunger (young: 61 ± 8 mm; older: 59 ± 9 mm), fullness (13 ± 4 mm; 5 ± 2 mm), desire to eat (61 ± 7 mm; 52 ± 8 mm), and prospective food consumption (67 ± 5 mm; 55 ± 6 mm) were not significantly different between study days and age groups after overnight fasting (all $P > 0.05$). Protein drink ingestion affected fullness (protein main effect $P < 0.001$), desire to eat ($P < 0.001$), and prospective food consumption ($P = 0.002$; Figure 3.5) in a protein load related fashion; fullness was higher (AUC, both $P < 0.001$) and desire to eat (AUC, $P = 0.035$ and $P = 0.009$) and prospective food consumption (immediately before lunch, $P = 0.025$, $P = 0.006$) were lower after the 70 g whey protein drink compared to control and the 30 g protein drink. Older compared to younger men had a lesser desire to eat (main effect of age $P = 0.028$) but also less fullness (main effect of age $P = 0.003$, interaction effect of age x protein load $P < 0.001$) throughout the day (Figure 3.5).

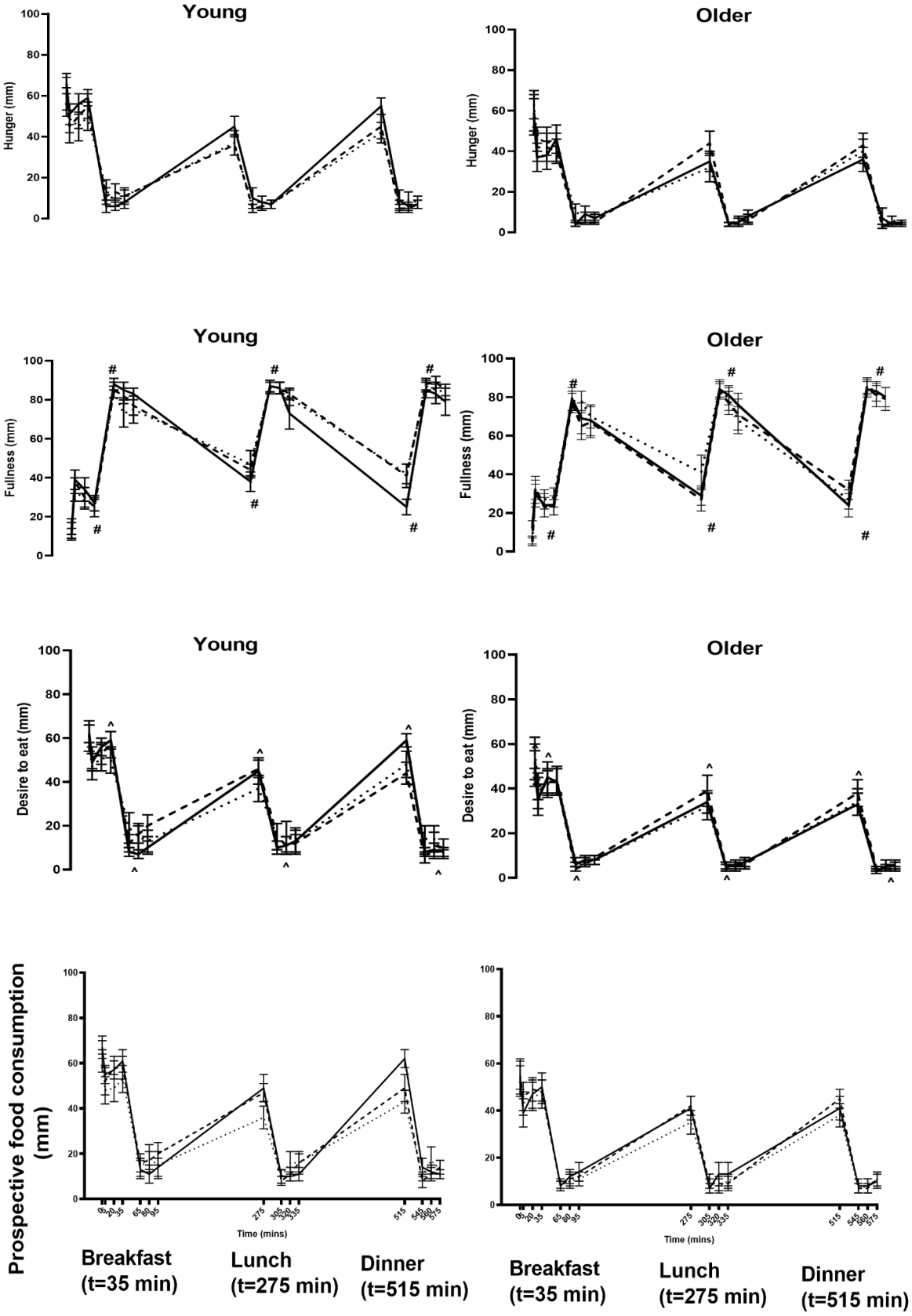

Figure 3.5: Mean (± SEM) visual analogue scores (VAS; 0–545 min) of hunger (A, B), fullness (C, D), desire to eat (E, F), and prospective food consumption (G,H)

following overnight fasting (t = −5) and after drink ingestion (t = 0, 5, 20, 35, 65, 80, 95, 275, 305, 320, 335, 515, 545, 560, 575 min) containing flavored water (control, ~2 kcal) or whey protein (30 g/120 kcal or 70 g/280 kcal; open bars) and immediately before and after breakfast (B), lunch (L), and dinner (D) in young (left; n = 15) and older (right; n = 15) men. Age and protein load main effects and interaction effects were determined using repeated-measures ANOVA. Protein affected #fullness (protein load main effect $P < 0.001$), ^desire to eat ($P < 0.001$), and *prospective food consumption ($P = 0.002$) in a protein load related fashion. Older compared to younger men had lower desire to eat (main effect of age $P = 0.028$) and fullness ($P = 0.003$, interaction effect $P < 0.001$).

3.5.5 Palatability of drinks and meals

The 70 g whey protein drink was perceived to be creamier when compared to the flavored control drink ($P = 0.016$). Ratings of pleasantness, intenseness, full of taste, sweetness, saltiness, sour, bitterness, umami, and creaminess of the drinks were not significantly different (main effect of protein $P > 0.05$). The healthy younger men rated the drinks as more bitter than the older men (young: 19 ± 4mm; older: 26 ± 3 mm, main effect of age $P = 0.037$). All other palatability ratings of the drinks were comparable between the age groups: pleasant (young: 47 ± 5 mm; older: 44 ± 4 mm), intense (51 ± 4 mm; 55 ± 3 mm), fullness (59 ± 4 mm; 59 ± 3 mm), sweet (53 ± 3 mm; 48 ± 3 mm), salty (31 ± 6 mm; 37 ± 4 mm), sour (34 ± 6 mm; 39 ± 4 mm), umami (34 ± 5 mm; 35 ± 3 mm), refreshing (40 ± 6 mm; 41 ± 4 mm), creaminess (27 ± 5 mm; 31 ± 3 mm, main effect of age all $P > 0.05$). Palatability of the meals, assessed as ratings of taste, aftertaste, and enjoyability, were comparable between study days and age groups (control, 30 g, 70 g protein: young: taste: 73 ± 5 mm, 75

± 5 mm, 72 ± 6 mm, after taste: 73 ± 5 mm, 72 ± 5 mm, 73 ± 5 mm, enjoyable: 73 ± 5 mm, 75 ± 5 mm, 74 ± 5 mm; older: taste: 72 ± 4 mm, 74 ± 3 mm, 72 ± 4 mm, after taste: 71 ± 3 mm, 71 ± 3 mm, 72 ± 4 mm, enjoyable: 73 ± 4 mm, 76 ± 3 mm, 73 ± 4 mm; main effects of age, protein load main effects and interaction effects all $P > 0.05$).

3.6 Discussion

This study compared the acute effects of ingestion of whey protein drinks containing 30 g and 70 g to those of a flavored control drink consumed 35 min before breakfast on ad libitum energy intake at breakfast, lunch, and dinner, perceptions of appetite throughout the day, and gastric emptying (antral area) in healthy younger and older men. Energy intake (sum of breakfast, lunch, and dinner) was suppressed in a protein load-responsive fashion at breakfast and in particular, at lunch, but not at dinner. Suppression of combined energy intake at breakfast, lunch, and dinner by the protein drink was less in healthy older (−3%) when compared to younger (−7%) men. Cumulative protein intake (sum of protein drink plus protein intake at the meals) was increased in a protein load responsive fashion (+20% and +42%) in the healthy younger and older men. Gastric emptying of the protein drinks in the 35 min before breakfast was slower than that of the control. Fullness was higher and desire to eat and prospective food consumption lower after protein intake when compared with the control in a protein load related fashion. Older compared to younger men had a lower desire to eat but also lower fullness throughout the day, suggesting that older people experience lower sensitivity of the appetite-suppressing effects of a protein drink and may have a decreased perception of gastric distension as seen in our previous study (12, 91).

Overall, suppression of energy intake by protein was less in healthy older than younger men in this study, confirming the results of our previous studies (14-16, 18, 25), e.g., in a study with a comparable design, suppression of energy intake by oral whey protein ingestion was ~−15% in healthy young compared to ~−1% in older men. In the present study, energy intake (sum of breakfast, lunch, and dinner) was suppressed most by the 70 g whey protein load compared to control (~7%) and at lunch, 4 h 35 min after the drink (~−20% in young and ~−15% in older men). In contrast, there was no suppression of energy intake by pre-breakfast protein at dinner time, 8 h 35 min after the drink, in either age group (~+7% compared to control dinner). We reported previously that in healthy older people, the timing of a 30 g whey protein drink (3 h, 2 h, 1 h, and immediately before the buffet-style meal) does not affect subsequent energy intake in older people. The effect of the whey protein ingestion on energy intake throughout the day may be associated with the slightly slower gastric emptying, reported by us and others in previous studies measuring gastric emptying for a period of 3 h in healthy older, when compared with younger, people (14-16, 18). Gastric emptying may be associated with postprandial satiety by affecting plasma gut hormone concentrations (90) in healthy younger adults (91, 165, 290, 292).

The cumulative energy intake (sum of drink, breakfast, lunch, and dinner) was comparable between study days while cumulative protein intake was elevated during the protein conditions in both age groups. Cumulative energy intake on the protein days compared to control was slightly higher in older (+4%) than younger men (−1%), as was reported in our previous studies determining ad libitum energy intake 3 h after oral whey protein ingestion (15) and following 1 h whey protein infusions directly into the small intestine (9). The insignificant effect of the whey drink on

cumulative daily energy intake in this study may indicate that the ingestion of a single daily dose of whey protein, in doses up to 70 g, is unlikely to be a successful weight loss strategy to achieve a negative energy balance, without taking the effects on energy expenditure and muscle anabolism into account. Even if whey protein was given more than once a day, we have no evidence that this would have resulted in a greater cumulative energy deficit, particularly in older adults. The energy content of the protein drink would have equalled or outweighed suppression of energy intake produced by the protein drink. Given our finding with one protein drink before breakfast, it is likely that suppression of cumulative energy intake with multiple drinks would have been even less (293). The participants in this study were not aware, however, that we were interested in or measuring their ad libitum meal energy intake throughout the day in response to the different drinks. Young adults using protein supplements to lose weight may have different responses to those in this study. Cumulative protein intake was significantly increased by the 30 g and 70 g whey protein loads, particularly in the older men (young: +17% and +36% and older: +23% and +47%), reaching meaningful amounts sufficient to result in postprandial muscle anabolism in older adults (123, 287)—the 70 g whey protein drink increased protein intake by 62 g, or ~0.8 g/kg body weight, in the older men.

A limitation of the study was that we only studied men. This was to enable comparisons with the results of our previous studies conducted in men which clearly showed the effect of protein load. As men generally show greater variations in appetite and food intake in response to energy manipulation than women (17, 72), the effects of the protein drinks may be different in women and it would be appropriate to perform further studies including women. The healthy older participants were well nourished, unrestrained eaters, had an active lifestyle, and

comparable energy intake on the control day to the younger men. It has been reported numerous times that healthy ageing is associated with reduced food intake (25, 30) and hunger (25, 26, 214) and a blunting of the regulation of food intake (72, 294) as suggested by the findings of this study, i.e., less suppression of energy intake by protein. The suppressive effect of whey protein in younger adults may be affected by having dietary restraints or actively trying to lose body weight (295, 296). Furthermore, the overall suppressive effect of protein supplements may be influenced by protein supplement intake before each meal of the day. The significant increase in cumulative protein intake and slight increase in cumulative energy intake in the older men suggests that whey protein can be given at breakfast, and possibly also at other meals, without decreasing overall daily energy intake, which would benefit malnourished, frail, older people—further studies are warranted. Another possible limitation was that the study was limited to 9 h after drink ingestion. As the effect of the pre breakfast drink on energy and protein intake had worn off by dinner, however, it seems unlikely that it would have had any effect after that.

3.7 Conclusions

Energy intake was suppressed by whey protein drinks in a protein load-responsive fashion at breakfast and particularly, at lunch, but not at dinner, and suppression of energy intake by protein was less in healthy older than younger men. Cumulative protein intake was increased in a protein load responsive fashion. These findings support the use of whey-protein drink supplements in healthy older patients who aim to increase their protein intake without decreasing their overall energy intake.

CHAPTER 4: ACUTE EFFECTS OF WHEY PROTEIN ON ENERGY INTAKE, APPETITE AND GASTRIC EMPTYING IN YOUNGER AND OLDER, OBESE MEN

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Contribution	Contributed to the overall design of the manuscript, literature review, data analysis and interpretation, drafting and revision of the manuscript.		
Overall percentage	75%		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	October 2022

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By signing the Statement of Authorship, each author certifies that:

i) the candidate's stated contribution to the publication is accurate (as detailedabove);

ii) permission is granted for the candidate in include the publication in the thesis;and

iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution	Conception and design of the study, data interpretation, statistical analysis, drafting of the manuscript and overall responsibility for the study.		
Signature		Date	October 2022

4.1 Abstract

Background: Obesity is becoming more prevalent in older people. A management strategy in obese, young adults is to increase dietary protein relative to other macronutrients. It is not clear if this is effective in obese, older individuals. Obesity may be associated with diminished sensitivity to nutrients. We have reported that a 30-g whey protein drink slows gastric emptying more, and suppresses energy intake less, in older, than younger, non-obese men. The aim of this study was to determine the effect of a 30 g whey protein drink on energy intake, gastric emptying (GE) and glycaemia in obese, older and younger men.

Methods: In randomized, double-blind order, 10 younger (age: 27 ± 2 years; BMI: 36 ± 2 kg/m²), and 10 older (72 ± 1 years; 33 ± 1 kg/m²), obese men were studied twice. After an overnight fast, participants ingested a test drink containing 30 g whey protein (120 kcal) or control (2 kcal). Postprandial gastric emptying (antral area, 2D Ultrasound) and blood glucose concentrations were measured for 180 min. At t = 180 min participants were given a buffet meal and ad libitum energy intake was assessed.

Results: Older participants ate non-significantly less (~20%) that the younger participants (effect of age, $P = 0.16$). Whey protein had no effect on subsequent energy intake (kcal) compared to control in either the younger (decrease 3 ± 8%) or older (decrease 2 ± 8%) obese men (age effect $P > 0.05$, protein effect $P = 0.46$, age × protein interaction effect $P = 0.84$). Whey protein slowed gastric emptying, to a similar degree in both age groups (50% emptying time: control vs. protein young men: 255 ± 5 min vs. 40 ± 7 min; older men: 16 ± 5 min vs. 50 ± 8 min; protein effect $P = 0.001$, age effect $P = 0.93$, age × protein interaction effect $P = 0.13$).

Conclusions: Our data suggest that obesity may blunt/abolish the age-related effect

of whey protein on suppression of energy intake.

4.2 Introduction

While the number of older people is increasing worldwide (297, 298), it is not as well appreciated that obesity rates are rising across the age range. Approximately one-third of people over 65 years are obese BMI > 30 kg/m^2) in Australia (299) and other developed countries (US, UK etc.) (300, 301) and this proportion is increasing. Although the BMI range associated with lowest morbidity and mortality increases with age (302), a BMI higher than 30 kg/m^2 in older people is still associated with adverse health outcomes, including diabetes, hypertension and heart disease. Obesity is therefore a serious problem in older people (303-306) and intentional weight loss is often recommended for obese, older adults (307).

Ageing is also associated with changes in feeding behaviour, gut function, and body composition, which impact on life expectancy and quality (9, 10, 13, 14, 16, 90). Skeletal muscle mass decreases, while fat mass increases (308). Insufficient protein intake in obese older individuals exacerbates muscle loss (309). This muscle loss, which may lead to sarcopaenia, is associated with functional impairment, increased rates of falls, increased nursing home admissions, and other adverse outcomes (111, 306, 310-315).

Protein supplements, often rich in whey protein, are commonly part of weight-loss strategies, based on the rationale that protein is more satiating than the other macronutrients (276, 316-318), although that effect may be modest, particularly when the protein is consumed as part of typical, ad libitum eating situations. Whey protein is high in essential amino acids which are rapidly digested, resulting in postprandial amino acid availability, stimulating muscle protein accretion more

effectively than casein and casein hydrolysate in older men (7, 319). We have recently shown that the acute administration of 30 g (120 kcal) and 70 g (280 kcal) oral whey-protein loads suppressed subsequent energy intake by 12–17% in young people without suppression in healthy older men (15). The whey-induced suppression of appetite and energy intake in young people may reflect an increase in pyloric and reduction in antral and duodenal motility, factors important in the regulation of gastric emptying (18, 320). Obesity appears to have minor, inconsistent, effects on gastric emptying (321- 323), whereas gastric emptying is modestly slower in older, than younger, adults (15). The potential influence of gastric emptying and energy/ protein load of an ingested meal on subsequent voluntary food intake is complex and likely to depend on the time of subsequent eating, the degree of gastric distension and initiation of small intestinal mechanisms to stimulate satiety after food leaves the stomach (25, 322, 324).

Information about the effects of whey protein on energy intake in obese, older people is limited. We have reported that the acute suppression of energy intake by whey protein, administered either intraduodenally (9), or orally (15), is less in healthy, non-obese, older than younger men, suggesting that the use of protein supplements to promote weight loss may not be as affective in older as younger adults (15). However, ad libitum energy intake responses to oral whey protein have not been evaluated in older, obese, adults. These may have implications for the use of high protein dietary strategies to manage obesity and maintain muscle mass in older people.

The primary aim of this study was to determine the effects of a 30 g whey protein drink on energy intake in obese older and younger men.

4.3 Materials and methods

Ten younger (Mean ± standard error of mean (SEM): age: 27 ± 2 years; body weight: 112 ± 8 kg; height: 1.75 ± 2.81 m; BMI: 36 ± 2 kg/m^2) and ten older (age: 72 ± 1 years; body weight: 103 ± 4 kg; height: 1.76 ± 2.56 m; BMI: 33 ± 1 kg/m^2) obese men were recruited by advertisement. Body weight and BMI of the younger and older participants did not differ significantly ($P > 0.05$).

On the basis of our previous study in lean participants (9), we determined that ten participants/group would be sufficient to detect a difference in the suppression of energy intake by 30 g whey protein of 395 kcal, with standard deviations (SDs) of 316 kcal (younger participants) and 180 kcal (older participants) (9), and in 50% gastric emptying time (T50 min) of 80 min with (SD's) of 38 min (younger participants) and 63 min (older participants) (214, 215, 325), between younger and older participants, with $\alpha = 0.05$ and power of 80%.

Participants were excluded on the basis of smoking, alcohol abuse, diabetes (HbA1C > 6%), significant gastrointestinal surgery, gastrointestinal symptoms (pain, reflux, diarrhoea, or constipation), and the use of medications known to affect energy intake, appetite, or gastrointestinal motor function. For older people, additional exclusion criteria were impaired cognitive function (score < 25 on Mini Mental State (326)) and depression (score ≥ 11 on the Geriatric Depression Questionnaire (327)).

The Royal Adelaide Hospital Human Research Ethics Committee approved the protocol which was conducted in accordance with the Declaration of Helsinki. The study was registered with the Australian New Zealand Clinical Trial Registry

(www.anzctr.org.au, registration number ANZCTR12616001216404). All participants provided written informed consent prior to their study inclusion.

4.3.1 Protocol

Participants were studied twice, separated by ≥ 3 days, to determine the effects of a whey protein drink (30 g/120 kcal) and a control drink (~0 g whey protein/~2 kcal) on energy intake, in randomized order (1 block with balanced permutations; www.randomization.com), double-blind, and cross-over design.

The protein drink (~450 mL) was prepared by dissolving whey protein (Bulk Nutrients, Tasmania, Australia) in demineralized water and diet lime cordial (Bickford's Australia, South Australia (SA), Australia) to achieve the desired load i.e. 30 g whey (volume of the powder: 19 mL) in 335 mL distilled water and 85 mL cordial (2.5 kcal/100 mL). The 'control' drink contained 0 g protein, 360 mL water, and 90 mL cordial. Sodium chloride, 0.3 g and 1.2 g, was added to the whey and control drinks so that the osmolarity (88 mOsm/L) was matched. To ensure even mixing drinks were stirred continuously at low speed on a stirring plate. The volumes of the drinks differed slightly (control: 450 mL; 30 g protein: 439 mL). Drinks were prepared by a research officer not involved in data analysis and served in a covered cup, so that both investigators and participants were blinded to the treatment.

Participants were provided with a standardized meal [beef lasagne (McCain Foods Pty Ltd, Wendouree, Victoria (VIC), Australia), ~591 kcal] to consume on the night before each study day at ~1900 h. They were instructed to fast overnight ~12 h from solids and liquids except water, and to refrain from strenuous physical activity until

they attended the laboratory at ~0830 h.

On arrival, participants were seated in a chair, where they remained throughout the study day, and an intravenous cannula was inserted. Measurements of antral area and perceptions of appetite and gastrointestinal symptoms were performed immediately before (during fasting; 0 min), immediately after ingestion of the drink (5 min), and then at 15 min intervals until 180 min. Participants consumed the drink within 2 min. Antral area was measured by 2-dimensional (2D) ultrasonography (15). Perceptions of appetite and gastrointestinal symptoms were assessed using visual analogue scales (VAS) and blood samples were collected to measure blood glucose. At 180 min, participants were given a cold, buffet-style meal, as described (15), in excess of what they were expected to consume (total energy content of 2,457 kcal; 19% protein, 50% carbohydrates, 31% fat) and instructed to eat freely for up to 30 min until comfortably full (180–210 min) (15).

4.3.2 Measurements

4.3.2.1 Energy intake

The amount eaten (g) was quantified by weighing the meal before and after consumption. Energy intake (kcal) and proportions of intake of protein, carbohydrate, and fat were calculated using commercially available software (Foodworks; 3.01, Xyris Software, Highgate Hill, Queensland, Australia). Energy intake was calculated both as intake at the buffet meal and as the cumulative energy intake (sum of energy intake at the buffet meal and from the preload drink). Absolute (kcal) and percentage suppression/change (energy intake as % of control day intake) of energy intake at the buffet meal by 30 g protein compared with control were

calculated (16).

4.3.2.2 Gastric emptying

Gastric emptying was measured at baseline, after overnight fasting (t = 0min), immediately after drink consumption and then every 15min to 180min. Gastric emptying rates were calculated from ultrasound measurements of gastric antral area (cm^2), as previously described (25) (292). Measurements were performed with a LogiqTM ultrasound machine (GE Healthcare Technologies, Sydney, New South Wales, Australia) using a 3.5 C broad spectrum 2.5–4MHz convex linear array transducer. The transducer was positioned vertically to obtain a para-sagittal image of the antrum with the superior mesenteric vein and the abdominal aorta in a longitudinal section. Measurements were performed at the end of inspiration. To calculate meal retention in the stomach, fasting antral area (measured at baseline) was subtracted from subsequent measurements performed after ingestion of the drinks (290). Gastric retention was calculated as

Retention (%) = [AA(t)-AA(f)] / [AA(max)-AA(f)] * 100

where AA(t) = antral area measured at a given time, AA (f) = fasting antral area, and AA(max) = maximum antral area recorded after drink ingestion (290). When ultrasound images lacked sufficient clarity, data were imputed by linear interpolation. The time at which 50% of the preload drink had emptied from the stomach (time to halving of post-drink maximum antral area: T_{50}; min) was calculated for both conditions.

4.3.2.3 Perceptions of appetite and gastrointestinal symptoms

Perceptions of hunger, desire to eat, prospective consumption, fullness, nausea, and bloating were rated using a 100mm visual analogue scale (VAS) questionnaire at t = 0, 5, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, and 210 min as previously described (291). Area under the curve (AUC) was calculated for appetite ratings (0–180 min) using the trapezoidal rule.

4.3.2.4 Blood glucose

Blood glucose concentrations (mmol/L) were determined by the glucose oxidase method using a portable glucometer (Optium Xceed, Abbott Laboratories, Australia). Area under the curve (AUC) was calculated for blood glucose 0–180 min using the trapezoidal rule.

4.4 Data and statistical analysis

Statistical analyses were performed using SPSS software (version 25; IBM, Armonk, NY, USA). Effects of age and treatment and their interaction effect were determined using a repeated measures mixed-effects model. An unstructured covariance structure was used to account for the repeated treatments by subject. Suppression of energy intake by protein and outcomes of the control condition were compared between age groups with a paired t-test. Statistical significance was accepted at $P < 0.05$. All data are presented as mean values ± SEM.

4.5 Results

The study protocol was well tolerated by all participants.

4.5.1 Energy intake

Older participants consumed ~20% less energy after the drinks than younger participants, although this difference was not statistically significant (P = 0.16). There was no difference in the amount of energy consumed after the protein drink compared to control, in either younger (control 1173 ± 130 kcal vs. protein 1114 ± 124 kcal) or older (control 926 ± 99 kcal vs. protein 892 ± 127 kcal) men (age effect P = 0.16, protein effect P = 0.46, age × protein interaction effect P = 0.84; Figure 4.1). There was no suppression of energy intake by whey protein in either age group (younger −3.0 ± 7.7% vs. older −2.3 ± 8.4%, P = 0.95).

Furthermore, cumulative energy intake (intake at buffet meal plus test drink) during the protein day did not differ with age or treatment (age effect P = 0.16, protein effect P = 0.25, age × protein interaction effect P = 0.84).

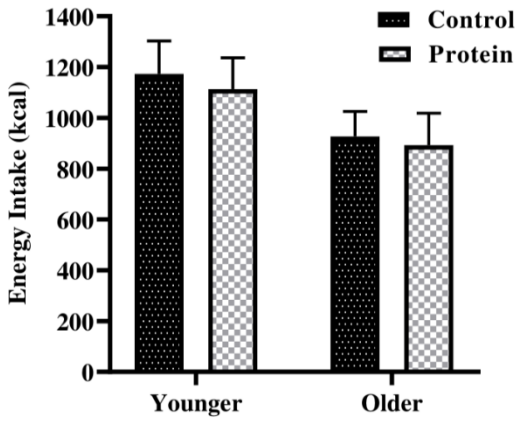


Figure 4.1: Mean ± standard error of mean (SEM) energy intake at the buffet meal

(kcal) in younger (n = 10) and older (n =10) men after drinks containing water (control-black) and 30 g whey protein (black border). Main age and protein effects and age × protein interaction effect were determined by using mixed model analysis. The protein drink did not suppress subsequent energy intake at the buffet meal compared with control. The main effects of age ($P = 0.16$) and protein ($P = 0.46$) and the age × protein interaction effect ($P = 0.84$) for energy intake were not significant.

4.5.2 Gastric emptying

In one older participant the quality of antral images was insufficient to determine gastric emptying on both days, so data for this participant was excluded from analysis. There was no difference in baseline (after overnight fasting) antral areas between age groups or study treatments (young men control vs. protein: 3.20 ± 0.37 cm^2 vs. 2.98 ± 0.31 cm^2; older men control vs. protein: 2.68 ± 0.29 cm^2 vs. 3.19 ± 0.29 cm^2; age effect $P = 0.64$, protein effect $P = 0.61$, age × protein interaction effect $P = 0.23$).

The protein drink emptied more slowly than control in both groups (T_{50} young men control vs. protein: 25 ± 5 min vs. 40 ± 7 min; older men control vs. protein: 16 ± 5 min vs. 50 ± 8 min; protein effect $P = 0.001$, age effect $P = 0.93$, age × protein interaction effect $P = 0.13$; Figure 4.2). Gastric emptying of the control drink was not significantly different between both age groups ($P = 0.21$).

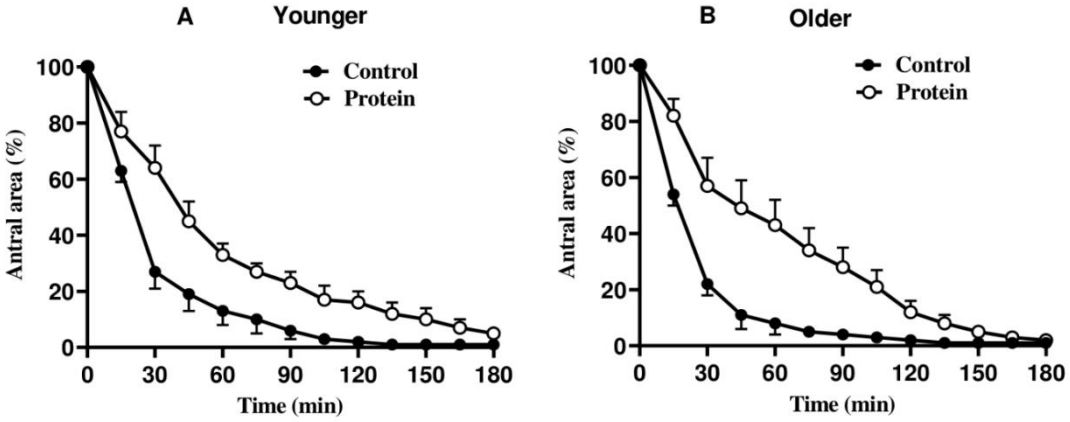


Figure 4.2: Mean ± standard error of mean (SEM) antral area (%) in younger (n = 10) and older (n = 10) men after drinks containing water (control) and 30 g whey protein. Main age and protein effects and age x protein interaction effect were determined by using mixed effects analysis. Gastric emptying (antral area) of the protein drink was slower than control to a similar degree in both the age groups. The main effect of protein for 50% gastric emptying time (T50 min; P = 0.001) was significant; the age (P = 0.93) and the age × protein interaction (P = 0.13) effects were not significant.

4.5.3 Blood glucose concentrations

Fasting blood glucose concentrations were higher in older (control 6.1 ± 0.2 mmol/L, protein 6.2 ± 0.2 mmol/L) than younger (control 5.4 ± 0.1 mmol/L, protein 5.4 ± 0.2 mmol/L) men (age effect *P* = 0.003, protein effect *P* = 0.76, age × protein interaction effect *P* = 0.58) and throughout both study days ($AUC_{0\text{-}180}$ min, young men control: 1003 ± 17 mmol/L, protein: 981 ± 21 mmol/L; older men control: 1112 ± 28 mmol/L, protein: 1108 ± 37, age effect *P* = 0.005). There was no effect of protein on blood glucose concentrations in either age group (protein effect *P* = 0.42, age × protein interaction effect *P* = 0.54; Figure 4.3).

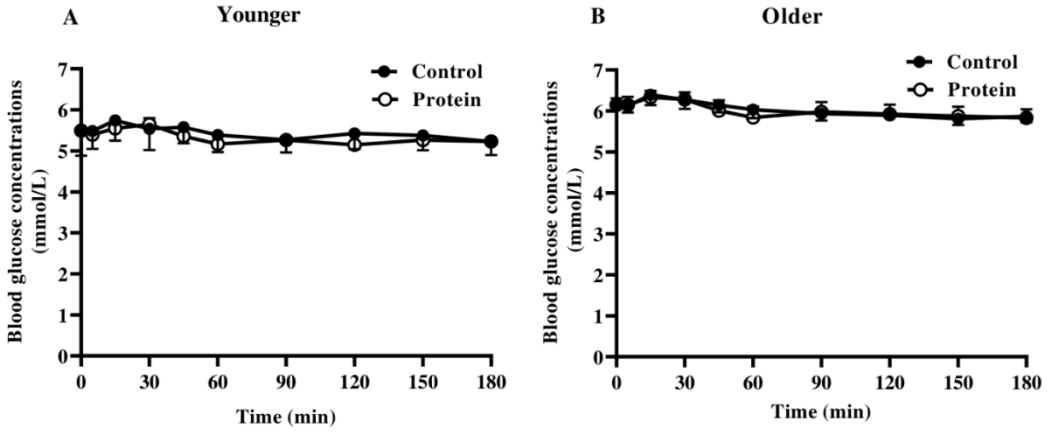


Figure 4.3: Mean ± standard error of mean (SEM) blood glucose concentrations (mmol/L) in younger (n = 10) and older (n= 10) men after drinks containing water (control) and 30 g whey protein. Main age and protein effects and age × protein interaction effect were determined by using mixed effects analysis. There was no effect of protein on the blood glucose concentrations in either age group. The main effect of age (P = 0.005) was significant; the protein (P = 0.42) and age × protein interaction (P = 0.54) effects were not significant.

4.5.4 Perceptions of appetite and gastrointestinal symptoms

4.5.4.1 Baseline

Baseline ratings of hunger [younger men control (YC) 53 ± 9 mm, younger men protein (YP) 39 ± 9 mm; older men control (OC): 37 ± 9 mm, older men protein (OP) 44 ± 6 mm], fullness (YC 19 ± 5 mm, YP 20 ± 6 mm; OC 16 ± 6 mm, OP 9 ± 5 mm), nausea (YC 14 ± 6 mm, YP 8 ± 4 mm; OC 4 ± 1 mm, OP 5 ± 1 mm) and bloating (YC 8 ± 3 mm, YP 9 ± 5 mm; OC 7 ± 4 mm, OP 5 ± 1.9 mm) were not different between younger and older men or between control and protein days (all main effects $P > 0.05$).

There were significant but modest age × protein interaction effects for ratings of prospective consumption and desire to eat at baseline. Pairwise comparisons for prospective food consumption (age × protein interaction effect $P = 0.001$) showed that in the young, scores were higher on the control day than protein day, whereas in theolder group, scores were higher on the protein day than control day (YC 62 ± 8 mm, YP 51 ± 8 mm, P = 0.040 vs. OC 41 ± 8 mm, OP 58 ± 8 mm, $P = 0.004$).

For desire to eat (age × protein interaction effect $P = 0.020$), there was no difference between treatments in either age group ($P > 0.05$). Scores were higher in the younger than the older group on the control day ($P = 0.040$), but not on the protein day (YC 52 ± 8 mm, YP 39 ± 7mm vs. OC 30 ± 7 mm, OP 39 ± 7 mm, $P = 0.98$).

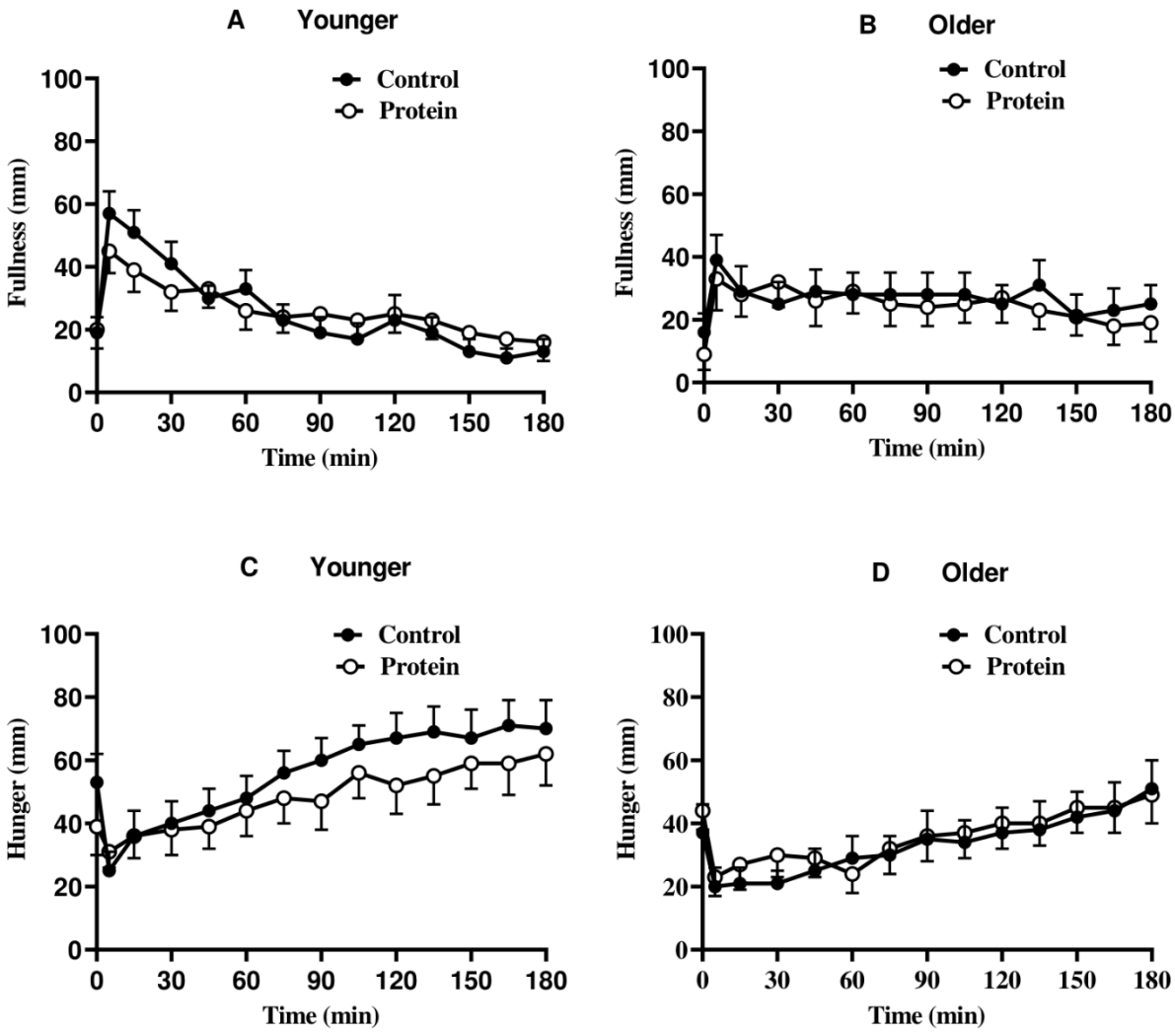
4.5.4.2 After study drink

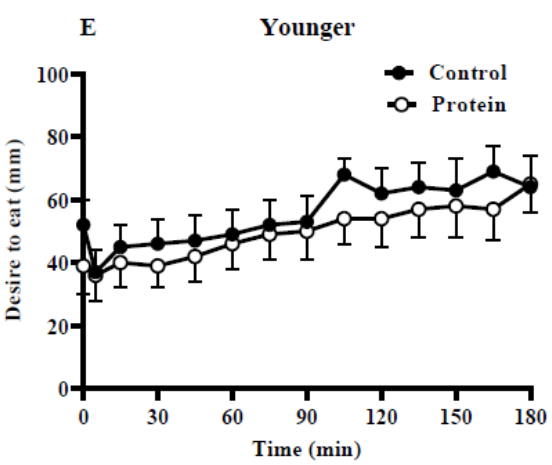
Prospective food consumption

The main age and protein effects for ratings of desire to eat (total AUC; YC 10,019 ± 1174 mm.min, YP 8,925 ± 1156 mm.min vs. OC 6,003 ± 1173 mm.min, OP 6,763 ± 1156 mm.min; age effect $P = 0.07$, protein effect $P = 0.71$), hunger (YC 10,041 ± 1180 mm.min, YP 8,676 ± 1289 mm.min vs. OC 5,873 ± 1180 mm.min, OP 6,324 ± 1289 mm.min; age effect $P = 0.07$, protein effect $P = 0.35$), fullness (YC 4,705 ± 897 mm.min, YP 4,703 ± 959 mm.min vs. OC 4,884 ± 897 mm.min, OP 4,703 ± 959 mm.min; age effect $P = 1.0$, protein effect $P = 0.57$), prospective food consumption (YC 10,279 ± 1198 mm.min, YP 9,572 ± 1143 mm.min vs. OC 7,341 ± 1198 mm.min, OP 7,607 ± 1143 mm.min; age effect $P = 0.15$, protein effect $P = 0.57$), bloating (YC 3,151 ± 875 mm.min, YP 2,666 ± 653 mm.min vs. OC 1,973 ± 875 mm.min, OP 1,495 ± 653 mm.min; age effect $P = 0.28$, protein effect $P = 0.11$) were

not significant. In younger participants nausea ratings were higher after the test drink than older participants (YC 3,127 ± 741 mm.min, YP 2,892 ± 827 mm.min vs. OC 771 ± 741 mm.min, OP 708 ± 827 mm.min; age effect $P = 0.04$). The effect of protein was not significant ($P = 0.74$).

The age × protein interaction effect for ratings of hunger, fullness, prospective food consumption, nausea, and bloating were not significant ($P > 0.05$), although there was a strong trend for desire to eat to be less in the older participants (age × protein interaction effect $P = 0.051$; Figure 4.4).

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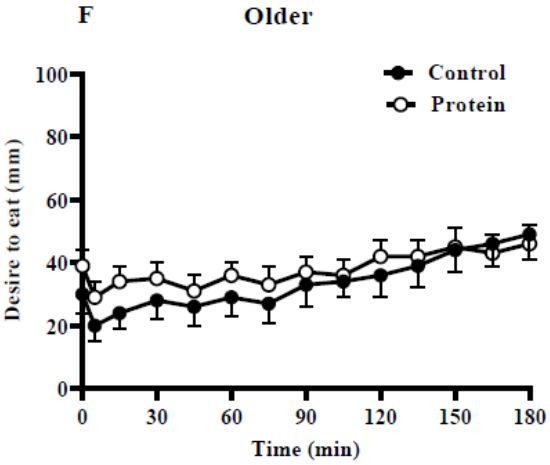
Control
Protein
Desire to eat (mm)
Time (min)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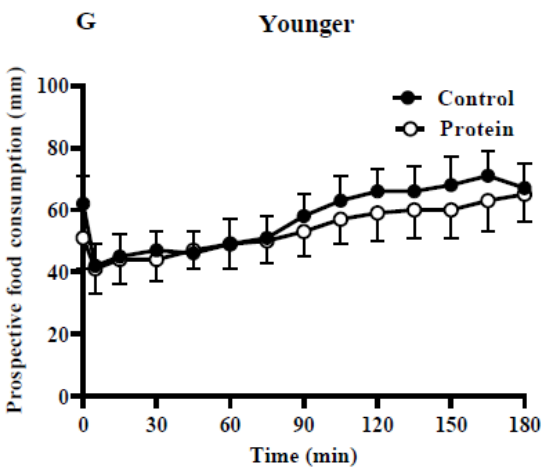
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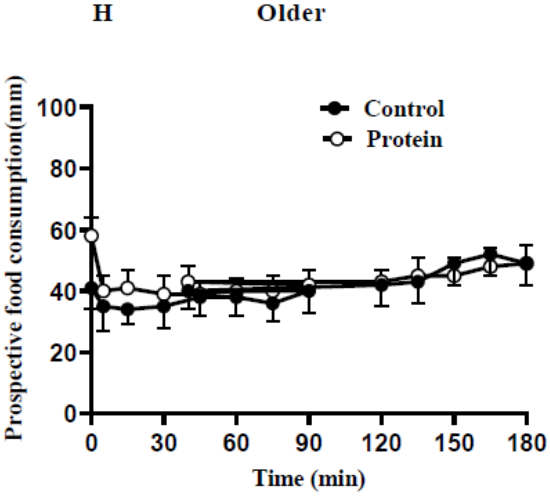
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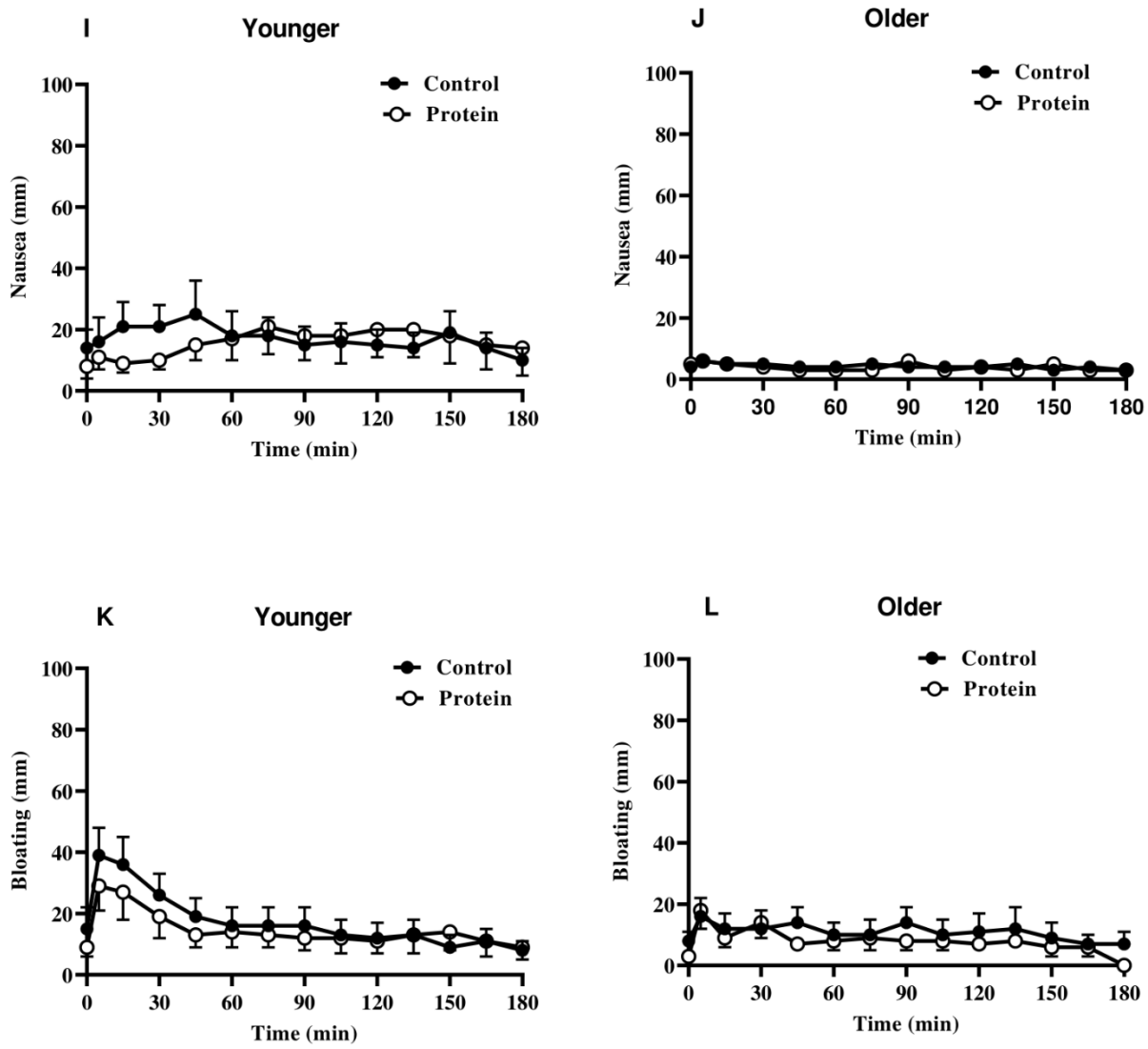

Figure 4.4 Perceptions of appetite and gastrointestinal symptoms. Mean ± standard error of mean (SEM) visual analogue scores (VAS; mm; 0−180min) of fullness (A, B), hunger C, D), desire to eat (E, F), prospective food consumption (G, H), nausea (I, J) and bloating (K, L) in younger (n = 10) and older (n =10) men immediately before and after drinks containing water (control) and 30 g whey protein. Time (0−180min) effects were determined by using mixed effects analysis. Ratings of fullness, hunger, nausea, and bloating did not change from baseline during all study days in both age groups ($P > 0.05$). Scores for prospective food consumption were significantly higher in the young on the control day ($P = 0.040$), whereas in the older

group, scores were significantly higher on the protein day (protein effect $P = 0.004$; age x protein interaction effect $P = 0.001$). For desire to eat prospective consumption was significantly higher in the younger than older group on the control day (age effect $P = 0.040$), but not on the protein day (age effect $P = 0.98$; age × protein interaction effect $P = 0.020$).

4.6 Discussion

The major findings of this study are:

1. There was no suppression of ad libitum energy intake after 30 g whey protein ingestion in either older or young, obese men. This is in contrast to our previous studies in lean, non-obese men, where greater (and significant) suppression of energy intake was observed in younger than older men, after both a 30 g whey protein drink (1% vs. 15% suppression) (15) and intraduodenal whey infusions (1% vs. 19% suppression) (9).
2. The whey protein drink slowed gastric emptying, to a comparable degree in both age groups.
3. There was no effect of the protein drink on hunger, desire to eat, fullness, satiety and bloating.

Numerous studies, including our own, have shown that ingestion of protein, by mouth and directly into the stomach or small intestine, acutely suppresses appetite and ad libitum energy intake in young, non-obese adults. Evidence that protein is the most satiating of the macronutrients in young adults (276, 316), has led to the development of high protein (usually energy-restricted) diets and their recommendation to young, overweight people trying to lose weight. Increased

protein intake in these diets is often at the expense of reduced carbohydrate intake (328-330). Many people, however, fail to achieve substantial, long-term weight loss on such diets. One possible explanation for this is that protein is not as satiating in obese as in non-obese individuals. In the present study we examined the impact of obesity on the satiating effects of protein ingestion (15). In our previous study of young non-obese men (mean BMI 23 kg/m^2) we showed that a 30 g whey protein drink significantly suppressed ad libitum energy intake at a test meal 3 h later by 17% compared to control day intake. That suppression was associated with reduced appetite ratings. In contrast, in the present study, using the same whey dose and study protocol, the protein drink suppressed neither energy intake nor appetite ratings in young obese men.

These results contrast with those of Brennan et al. (331), who reported that high protein meals suppress energy intake in both lean and obese younger men 3 h after an energy preload. While the energy preloads in that study were larger than we employed (213 kcal vs. 120 kcal), and administered as a fat, protein and carbohydrate mixture, we have reported previously that pure whey drinks of 280 kcal and mixed macronutrient drink of 504 kcal protein (280 kcal), carbohydrate (112 kcal) and fat (112 kcal) drinks also did not suppress voluntary energy intake at 3 h in non-obese older men (16). The preloads in Brennan et al. were solid food (a meat and pasta dish), compared to a whey drink in our study. There is evidence that drinks are less satiating than solid foods of the same nutrient composition (331). Mourao et al., for example, reported greater ad libitum energy intake after drinks than solid food of comparable energy content (332). These results are consistent with the possibility of a reduced satiating effect of liquid vs. solid protein. This would support the use of increased oral protein in solid rather than liquid form when the intention is to promote

weight loss. Nevertheless, the whey protein drink did suppress appetite and food intake in young, non-obese men in our previous study, an effect not present in the obese young men in this study. These results suggest that obesity may blunt the satiating effects of protein, at least whey protein, and that these effects of obesity may be similar to those of physiological ageing; both healthy ageing and obesity have been associated with a loss of suppression of subsequent food intake by a whey drink.

The mechanism(s) by which obesity blunts the satiating effects of whey protein are not known. Blood glucose concentrations were predictably higher in the older than younger men, but not affected by protein ingestion, as in our previous study of non-obese men (15), suggesting that glucose was not involved.

Gastric emptying was slowed by ingestion of the whey protein in both younger and older men with no difference between them. Despite this, subsequent food intake was suppressed by whey in neither age group. This suggests, at least under these study conditions, that gastric distension due to retention of the protein load in the stomach and delayed entry of test drink into the small intestine, did not affect appetite and food intake. To allow a comprehensive assessment of gastric emptying the test meal was started 180 min after the study drink, by which time the stomach was almost completely empty. It is possible that if the test meal was given earlier, when the difference between the study days in how much drink remained in the stomach was greater, there may have been reduced food intake on the whey drink day and an association between food intake and gastric emptying. Nevertheless, further evidence for the lack of such an association is provided by the results of our previous study in non-obese men (15), in which gastric emptying was markedly slowed by the whey drink in both older and young men, with almost complete emptying of the drink from

the stomach before buffet meal ingestion at 180 min. In that study, there was a reduction in ad libitum energy intake in the non-obese young men, suggesting that slowing of gastric emptying by protein ingestion is not involved in the satiating effect (or lack of) of the protein drinks under these study conditions.

Increasing age is associated with a physiological reduction in hunger and food intake, the so-called "anorexia of ageing" (214, 325). Consistent with that reduction, the older participants consumed about 20% less energy in this study than the younger participants, although that reduction was not statistically significant. On average, body weight decreases after about age 60–70 years. Much of the weight lost is lean tissue, including skeletal muscle and bone (243, 333). Muscle loss can be marked, particularly when pathological processes are superimposed, and can lead to sarcopaenia and frailty. One way to prevent this skeletal muscle loss, and thus preserve function and quality of life, is with dietary protein supplements, that can help maintain lean body mass and improve health (334). In contrast, when body weight is deliberately lost with energy-reduced diets, lean tissue is lost as well as fat. This may have adverse effects in older people with baseline low muscle mass. The results of this and our previous acute studies suggest that protein supplementation as whey protein drinks can be given to older people as an aid to skeletal muscle preservation and even augmentation, without risk of appetite suppression and weight loss. Conversely, such protein drinks are likely to have little effect to promote weight loss in overweight, older people trying to lose weight, and are likely to be even less effective than in younger adults. Longer-term studies in both obese and non-obese older adults evaluating the effects on appetite and food intake of increased protein intake in solid foods would be of interest.

Limitations of this study include the relatively modest participant numbers and the use of only one dose of whey protein. We did not assess the participants' perceptions of taste and/or pleasantness of the drinks. Gastric emptying rate was measured indirectly by 2D Ultrasound. 3D ultrasonography, which we have used previously (15, 230, 335) is probably more accurate in non-obese people, but was not used here as the results are less reliable in obese individuals due to tissue layers. Scintigraphy is the 'gold standard' technique for measurement of gastric emptying but was not available for this study. We studied only men, as they appear to have the greatest ability to regulate energy intake in response to energy manipulation (320). The results do not necessarily apply to women.

In summary a 30 g whey protein drink did not suppress appetite or energy intake in obese younger or older men. We speculate that obesity might mimic the effects of ageing to inhibit responses to protein. The use of liquid whey supplementation is unlikely to be helpful as a weight loss strategy in men of any age. Liquid whey protein supplements, the use of which may be of benefit for the preservation of function in older people, are unlikely to suppress food intake in older people of any weight.

CHAPTER 5: COMPARATIVE EFECTS OF CO-INGESTING WHEY PROTEIN AND GLUCOSE ALONE AND COMBINED ON BLOOD GLUCOSE, PLASMA INSULIN AND GLUCAGON CONCENTRATIONS IN YOUNGER AND OLDER MEN

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Candidate	Avneet Oberoi		
Contribution	Conducted the clinical trial, contributed to the overall design of the manuscript, literature review, data analysis and interpretation, drafting and revision of the manuscript.		
Overall percentage	75%		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	October 2022

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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i) the candidate's stated contribution to the publication is accurate (as detailedabove);

ii) permission is granted for the candidate in include the publication in the thesis;and

iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution	Conception and design of the study, data interpretation, statistical analysis, drafting of the manuscript and overall responsibility for the study.		
Signature		Date	October 2022

5.1 Abstract

The ingestion of dietary protein with, or before, carbohydrate may be a useful strategy to reduce postprandial hyperglycemia, but its effect in older people, who have an increased predisposition for type 2 diabetes, has not been clarified. Blood glucose, plasma insulin and glucagon concentrations were measured for 180 min following a drink containing either glucose (120 kcal), whey-protein (120 kcal), whey-protein plus glucose (240 kcal) or control (~2 kcal) in healthy younger (n = 10, 29 ± 2 years; 26.1 ± 0.4 kg/m^2) and older men (n = 10, 78 ± 2 years; 27.3 ± 1.4 kg/m^2). Mixed model analysis was used. In both age groups the co-ingestion of protein with glucose (i) markedly reduced the increase in blood glucose concentrations following glucose ingestion alone (P < 0.001) and (ii) had a synergistic effect on the increase in insulin concentrations (P = 0.002). Peak insulin concentrations after protein were unaffected by ageing, whereas insulin levels after glucose were lower in older than younger men (P < 0.05) and peak insulin concentrations were higher after glucose than protein in younger (P < 0.001) but not older men. Glucagon concentrations were unaffected by age. We conclude that the ability of whey-protein to reduce carbohydrate-induced postprandial hyperglycemia is retained in older men and that protein supplementation may be a useful strategy in the prevention and management of type 2 diabetes in older people.

5.2 Introduction

Type 2 diabetes mellitus is a major and increasing problem worldwide (336-338). The prevalence of diabetes increases with age; in Australia, from less than 0.3% of those under age 35 years to 14–16% in those 65 years or more (339). Postprandial hyperglycemia is a major determinant of overall glycemic control—when HbA1C is < 8% postprandial glycemic excursions are the dominant determinant of HbA1C (340-343)—and an independent risk factor for cardiovascular disease (344, 345). While ingestion of protein on its own has little effect on blood glucose concentrations (90, 346), co-ingestion of dietary protein with other macronutrients may influence postprandial blood glucose concentrations when combined with carbohydrate. In non-elderly adults with and without type 2 diabetes, the oral ingestion of proteins or their component amino acids substantially reduces the increase in postprandial blood glucose concentrations when compared with carbohydrate ingestion alone (69, 147, 347-350), with different proteins and amino acids varying in their ability to have this effect (44, 346, 351, 352). Whey is a protein with potent glucose-lowering action (44, 86, 138, 350, 351, 353, 354) and there has been increasing interest in the possible benefits of whey protein in lowering blood glucose concentrations when ingested with a meal or as a preload (86, 147).

Human ageing is characteristically associated with a loss of skeletal muscle mass and function; when severe this leads to sarcopenia and other adverse outcomes (63). Increasing protein ingestion can preserve and increase muscle mass and function in older people and protein supplements have been shown to reduce morbidity and mortality in the elderly (63). Whey protein is ideal for this anabolic effect as it is rich in the branched chain amino acids, particularly leucine (249). Due to age-related

anabolic resistance, more protein per serve is required to stimulate muscle protein synthesis in older than younger adults (63) and a minimum of 25–30 g protein per meal has been recommended for older people (97).

Ageing is associated with changes in glucose metabolism, including impaired glucose tolerance and reduced insulin secretion (355-357). There may also be changes in the responses to dietary protein, including differing effects of both oral (12, 15, 90) and intraduodenal (9) whey protein administration on appetite and gut hormone release. Whey protein, when consumed orally alone, slows gastric emptying load dependently in healthy young men (18) and suppresses appetite less in healthy older men than younger men (9). It is possible, therefore, that the capacity of whey protein to attenuate the rise in carbohydrate-induced "postprandial" blood glucose concentrations may differ between older people and young adults. Previous studies examining the effects of protein ingestion on blood glucose concentrations after carbohydrate ingestion have been conducted in young- and middle-aged adults (44, 86, 138, 350, 351, 353, 354). We conducted this study to determine whether the comparative effects of whey protein/carbohydrate co-ingestion on postprandial blood glucose concentrations persists into old age. We used a whey protein dose of 30g because of its tolerability in older people and likely beneficial effects on skeletal muscle.

5.3 Materials and methods

5.3.1 Participants

This was a randomized double-blind cross-over study comprising 10 healthy younger men (age range 18–35 years, mean ± SEM age: 29 ± 2 years; body weight: 84 ± 4

kg; height: 1.75 ± 0.02 m; body mass index (BMI): 26.1 ± 0.4 kg/m^2) and 10 healthy older men (68–87 years, 78 ± 2 years; 82 ± 2 kg; 1.76 ± 0.02 m; 27.3 ± 1.4 kg/m^2). The body weight and the BMI of the younger and the older men did not differ significantly ($P > 0.05$).

The participants were recruited by online advertisement and by flyers placed on notice boards at the University of Adelaide, Australia.

The exclusion criteria included smoking, alcohol intake of > 2 standard drinks on > 5 days per week, being vegetarian, intake of any illicit substance, use of prescribed or non-prescribed medications which may affect appetite, body weight, gastrointestinal function or energy metabolism, food allergy(s), known diabetes mellitus (or fasting blood glucose concentration > 6.9 mmol/L), epilepsy, gallbladder, pancreatic, known cardiovascular or respiratory disease, significant gastrointestinal symptoms, disease or surgery, any other illness deemed significant by the investigator and inability to comprehend the study protocol. Inclusion criteria included being weight stable (< 5% fluctuation in weight) at study entry, as assessed by self-reported weight in the preceding 3 months, and willingness to maintain usual physical activity level throughout the study.

The Royal Adelaide Hospital Human Research Ethics Committee approved the protocol, which was conducted in accordance with the Declaration of Helsinki. The study was registered with the Australian New Zealand Clinical Trial Registry (www.anzctr.org.au (2 June 2022), registration number ACTRN12619000420145). All participants provided written informed consent prior to their study inclusion.

5.3.2 Protocol

Each participant was studied on four occasions, separated by ~7–10 days. On each occasion, they received, in randomized order (using the method of randomly permuted blocks; www.randomization.com), a drink of either 30 g glucose (G, 120 kcal) (glucose monohydrate, Sigma-Aldrich, St. Louis, MI, USA), 30 g whey protein (P, 120 kcal) (Bulk Nutrients, Tasmania, Australia), 30 g whey protein plus 30 g glucose (GP, 240 kcal) or flavored water (control, C; ~2 kcal). The effects on blood glucose, plasma insulin, glucagon, gastric emptying, energy intake and perceptions of appetite were evaluated. The drinks were equivolemic (~250 mL) and were prepared by a research assistant who was not involved in the analysis of the study results. They were flavored with varying amounts of distilled water, sodium chloride and light lime cordial (Bickford's Australia Pty Ltd., Adelaide, SA, Australia) and 100 mg [^{13}C] sodium acetate to match for taste and served in a covered cup to achieve blinding. To ensure that all ingredients were dissolved evenly throughout, and to minimize the layer of foam on top of the solution, the drinks were stirred continuously at low speed on a stirring plate. Both the investigators conducting the study and the participants were blinded to the drink composition.

Participants were told to consume the same meal on the night before each study day at around 1900 h. They were instructed to fast overnight from solids and liquids and to refrain from strenuous physical activity and alcohol for 24 h prior to their attendance at the laboratory at the Clinical Research Facility, Adelaide Health and Medical Sciences Building, the University of Adelaide at 08:30 am. Upon arrival, participants were seated in a chair, and a cannula was inserted in an antecubital vein for blood sampling. A heated pad was used so that the samples were arterialized.

Once baseline measurements were taken (blood samples, breath sample and appetite ratings), participants were instructed to consume the test drink within 2 min. Gastric emptying (% intragastric retention) of the drink was measured with a ^{13}C-sodium acetate breath test t = 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 90, 105, 120, 135, 150, 165 and 180 min. Perceptions of appetite were assessed with validated visual analog scales and blood samples were collected into ice chilled EDTA-coated tubes for the measurement of blood glucose, plasma insulin and plasma glucagon concentrations at t = 0, 15, 30, 45, 60, 90, 120, 150 and 180 min. No inhibitors were added (33). At 180 min, each participant was presented with a standardized cold buffet-style meal, in excess of what they were expected to consume (total energy content of 2457 kcal: 19% protein, 50% carbohydrates, 31% fat) (36), in a room by themselves to limit external distractions, and were allowed to eat freely for 30 min (180–210 min) until comfortably full.

5.3.3 Measurements

5.3.3.1 Blood glucose and plasma insulin and glucagon concentrations

Blood glucose concentrations (mmol/L) were quantified by the glucose oxidase method using a glucose analyser (YSI 2900 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH, USA). Intra- and inter-assay coefficient of variations (CVs) were ≤ 2%. Plasma was obtained by centrifugation for 15 min, at 3200 rpm at 4 °C and samples were stored at −80 °C for further analysis of insulin and glucagon concentrations. Total plasma insulin (milliunits per liter) was measured by enzyme-linked immunosorbent assay (ELISA) immunoassay (10-1113; Mercodia, Uppsala, Sweden). The sensitivity of the assay was 1.0 mU/L and the coefficient of variation was 2.9% within and 11.6% between assays (358). Plasma glucagon concentrations

were measured by ELISA immunoassay (10-1271-01, Mercodia, Uppsala, Sweden). The lower limit of quantification (LLOQ) was 1.5 pmol/L and the detection limit was 0.75 pmol/L. The coefficient of variation was 9.3% within assays and 7.5% between assays.

5.3.3.2 Gastric emptying

As the stomach empties the test drink containing [^{13}C] sodium acetate, the substrate is rapidly absorbed in the proximal small intestine and metabolized in the liver. $^{13}CO_2$ is produced, which can then be measured in the breath, thus reflecting gastric emptying of nutrients (359). The participants were asked to exhale through a mouthpiece to collect an end-expiratory breath sample into a 100 mL foil bag at certain time intervals. The breath samples were collected and the quantity of $^{13}CO_2$ (disintegrations per min) was measured by a non-dispersive infrared spectrometer (FANci2, Fischer ANalysen Instrumente, Leipzig, Germany) (360). The gastric half-emptying time (T_{50}) and the intragastric retention were calculated using the Wagner–Nelson method (361). This method has been shown to be of comparable accuracy to scintigraphy in the measurement of the gastric emptying of both solid and liquid meals (242, 362).

5.3.3.3 Energy intake

The energy consumed at the buffet meal (g) was quantified by weighing the food before and after consumption. The energy intake was calculated as absolute (kcal) at the buffet meal using commercially available software (Foodworks version 8; Xyris Software Pty Ltd, Highgate Hill, QLD, Australia (15).

5.3.3.4 Perceptions of appetite

The perceptions of hunger and fullness were rated using a 100 mm visual analog scale questionnaire immediately before the test drinks and at t = 15, 30, 45, 60, 90, 120, 150 and 180 min as described (355).

5.4 Data and statistical analysis

Statistical analyses were performed using SPSS software (version 25; IBM, Armonk, NY, USA). Sample size was based on the statistical power functions of the between-group contrasts of older versus younger with overall P = 0.05, statistical power of 0.8 and anticipated drop-out rate of ~10%, and significance levels adjusted to account for the 4 comparisons (control, whey protein, glucose, whey protein plus glucose). Calculations were performed for the primary outcome of area under the curve (AUC) of blood glucose concentrations, assuming a within-participant SD of 0.5 mmol/L, and a between-participants SD of 1.4 mmol/L (14, 15, 90) to detect a difference between groups of 1.5 mmol/L and a difference between treatments of 0.4 mmol/L.

The net incremental area under the curve (Net iAUC0–180/min), peak, time to peak and time to return to baseline was calculated for blood glucose, insulin and glucagon. The Net $iAUC_{0\text{-}180/min}$ was calculated from baseline using the trapezoidal rule and then divided by time (min) to present a weighted average value over the time interval.

The main effects of age and drink condition, and their interaction effects, on blood glucose, plasma insulin and glucagon concentrations were determined using a mixed-effects model with the drink condition as the within-participant factor and age as the

between- participant factor, including baseline values at each treatment visit as a covariate. Post hoc comparisons, which were adjusted for multiple comparisons with Bonferroni correction, were performed when there were significant drink-condition or interaction effects. Peak concentrations, time to peak and time to return to baseline were determined for blood glucose, plasma insulin and glucagon concentrations in those drink conditions associated with changes from baseline using a repeated measures ANOVA test (G and GP for glucose; G, P and GP for insulin and glucagon). When the time to return to baseline was between 2 blood sampling time points, an interpolated value was estimated assuming a linear relationship between the 2 time points. If a blood glucose (n = 5 of 80 study days) or plasma insulin (n = 8) or glucagon concentration (n = 20) did not return to baseline by 180 min, the time to return to baseline was calculated using a linear extrapolation from the values at 150–180 min. We hypothesized that insulin concentrations following both G and P drinks would increase and, to determine whether the effect on insulin of the combination drink is synergistic or additive, we compared the effect of combined whey protein and glucose (GP drink) on the rise in plasma insulin concentrations with that of the sum of the effects of glucose and whey protein alone (G drink + P drink) on insulin concentrations using a paired t-test. The inhibition of the increase in blood glucose concentrations following glucose ingestion by whey protein when compared with glucose ingestion alone was calculated as the difference between the Net iAUC of G and GP as a percentage of the Net iAUC of G. The increase in plasma insulin concentrations following GP when compared with G ingestion alone was calculated as the difference between the Net iAUC of GP and G as a percentage of the Net iAUC of G. The increase in plasma glucagon concentrations following P when compared with G ingestion alone was calculated as the difference between the Net iAUC of P and G as a percentage of the Net iAUC of G. Statistical significance

was accepted at $P < 0.05$. Data are presented as mean values ± SEM.

5.5 Results

The study protocol was well tolerated by all participants and no adverse effects were reported.

5.5.1 Blood glucose

Baseline glucose concentrations did not differ between age groups ($P = 0.68$) or study days ($P = 0.24$, Table 5.1).

5.5.1.1 Interaction effects

The time to peak glucose concentrations were longer after G in older than younger men ($P = 0.03$, Table 5.1). The age by drink interaction effect was non-significant for all other outcomes.

5.5.1.2 Drink-condition effects

Blood glucose concentrations increased from baseline following glucose ingestion, alone (G) and when combined with whey protein (GP) (both $P < 0.05$), to peak mean concentrations of 6.3–8.1 mmol/L (Table 5.1) but did not change following the control (C) or protein (P) drinks.

Co-ingestion of whey protein with glucose reduced the peak and the Net iAUC blood

glucose concentrations when compared with glucose ingestion alone (both $P < 0.001$), in both older and younger men, without any effect of age on this inhibitory effect ($P > 0.05$). Peak blood glucose concentrations occurred earlier after GP than G ($P < 0.001$) and returned to baseline at a comparable time after GP and G ($P = 0.83$, Figure 5.1).

The inhibition of the increase in blood glucose concentrations following glucose ingestion by whey protein, when compared with glucose ingestion alone, was 44 ± 38% over 3 h ($P < 0.001$; 50 ± 13% in the first hour $P < 0.001$, 66 ± 33% in the second hour $P = 0.001$ and −1 ± 23% in the third hour $P = 0.21$).

5.5.1.3 Age effects

Peak glucose concentrations after GP and G drinks were non-significantly higher in older than younger men (Table 5.1). Peak glucose concentrations occurred later after GP and G in the older than the younger men ($P = 0.007$) and took longer to return to baseline in the older men ($P = 0.03$).

There was no effect of age on the inhibitory effect of whey protein (GP) on the rise in blood glucose compared with after glucose alone (G). The inhibition of the rise in blood glucose concentrations over 3 h was 62 ± 27% in the older men and 32 ± 92% in the younger men ($P = 0.75$) and 41 ± 6% in the older men and 53 ± 8% in the younger men in the first hour after drink ingestion ($P = 0.46$).

Table 5.1: Blood glucose, plasma insulin and plasma glucagon concentrations following control drink (C), 30g whey protein drink (P), 30g glucose drink (G) and 30g whey protein plus 30g glucose drink (GP) ingestion

	Younger men				**Older men**				P value age effect	P value drink-condition effect	P value interaction effect
	C	**P**	**G**	**GP**	**C**	**P**	**G**	**GP**			
Blood glucose (mmol/L)											
Fasted	4.3±0.1	4.3±0.2	4.2±0.2	4.5±0.1	4.5±0.1	4.2±0.2	4.4±0.2	4.5±0.1	0.68	0.24	0.23
Peak	4.7 ±0.1	4.8 ±0.1	7.8±0.3	6.3±0.3	4.9 ±0.1	4.7 ±0.1	8.1±0.3	6.9±0.3	0.27	<0.001	0.54
Time to peak (min)	50±13	68±17	34±3[1]	30±3	26±13	75±17	51±3[1]	36±3	0.007	<0.001	0.031
Return to baseline (min)	88±26	41±13	106±15	91±18	60±22	125±23	132±15	153±18	0.03	0.83	0.23
Net $iAUC_{0\text{-}60min}$	-0.1±0.1	0.0±0.1	2.1±0.2	0.9±0.2	0.1±0.1	0.1±0.1	2.1±0.2	1.3±0.2	0.29	<0.001	0.48
Net $iAUC_{0\text{-}180min}$	-0.1±0.1	0.0±0.1	0.7±0.2	0.3±0.1	-0.1±0.1	0.1±0.1	1.1±0.2	0.7±0.2	0.08	<0.001	0.27
Plasma insulin (mU/L)											

Fasted	5.3±1.0	4.8±0.8	6.9±1.1	6.2±1.0	4.1±1.0	4.5±0.8	4.1±1.1	4.5±1.0	0.22	0.41	0.13
Peak	11.7±1.9	33.2±6.9[2]	55.9±7.3[2]	93.3±16.8	9.7±1.9	31.7±6.9	36.5±7.3	72.7±16.9	0.34	<0.001	0.006
Time to peak (min)	35±13	32±3[3]	33±2[3]	36±3	32±4	48±3[3]	46±2[3]	42±3	0.001	0.91	0.038
Return to baseline (min)	73±26	132±8	112±12	160±11	109 ±18	149 ±8	163±12	166±11	0.011	0.068	0.14
Net $iAUC_{0\text{-}60min}$	1.8±0.5	16.5±3.8	27.1±4.4	46.2±9.3	2.3±0.5	15.5±3.8	20.9±4.4	39.8±9.3	0.59	<0.001	0.26
Net $iAUC_{0\text{-}180min}$	0.2±0.6	8.3±1.9	10.7±2.4	25.8±6.2	1.5±0.6	9.6±1.9	11.6±2.4	26.6±6.2	0.77	<0.001	0.99
Plasma glucagon											
(pg/mL)											
Fasted	9.7±1.4	10.9±1.6	9.8±1.4	10.3±1.5	8.7±1.4	9.1±1.6	8.1±1.4	9.6±1.5	0.51	0.27	0.69
Peak/Nadir	11.2±1.5	36.4±5.8	10.7±1.4	22.6±3.1	10.2±1.5	33.1±5.8	9.1±1.4	22.1±3.1	0.71	<0.001	0.73
Time to peak (min)	68±22	57±17	68±24	54±9	65±22	51±17	56±24	48±9	0.83	0.65	0.98
Return to baseline (min)	54±16	176±153	71±29	176±51	126±39	391±153	133±29	277±51	0.11	0.011	0.69
Net $iAUC_{0\text{-}60min}$	-0.7±0.0	16.3±2.6	-4.6±0.7	6.3±1.5	-0.1±0.0	13.5±2.6	-2.4±0.7	6.3±1.5	0.98	<0.001	0.33

Net $iAUC_{0\text{-}180min}$	-0.9±0.3	10.8±1.3	-3.9±0.8	5.2±1.3	-0.5±0.3	9.9±1.3	-3.2±0.8	5.1±1.3	0.95	<0.001	0.92

Blood glucose (mmol/L), plasma insulin (mU/L) and glucagon (pg/mL) concentrations fasted (baseline), peak, time to peak (min), return to baseline (min), Net $iAUC_{0\text{–}60min}$ (change from baseline area under the curve during the first hour), Net $iAUC_{0\text{–}180min}$ (change from baseline area under the curve during the three hours) following drink ingestion containing (i) flavored water (C, control, ~2 kcal), (ii) 30 g whey protein (P), (iii) 30 g glucose (G) or (iv) 30 g whey protein + 30 g glucose (GP) in younger and older men. Effects of age and drink condition and the interaction effect were determined using a mixed-effect model using G and GP for glucose and P, G and GP for insulin and glucagon. [1] The age × drink-condition interaction, $P = 0.031$ (time to peak glucose is longer after G in older than younger men). [2] The age × drink-condition interaction, $P = 0.006$ (peak insulin concentration is higher after G than P in younger but not older men). [3] The age drink-condition interaction, $P = 0.038$ (time to peak insulin is longer after P and G in older than younger men). Drink-condition effects: GP vs. G reduced peak ($P < 0.001$) and Net iAUC ($P < 0.001$) glucose concentrations and the peak occurred earlier ($P < 0.001$) in both older and younger men: P, G, GP vs. C increased Net iAUC insulin concentrations ($P < 0.001$); G vs. C decreased and P, GP vs. C, P vs. GP increased Net iAUC lucagon concentrations ($P < 0.001$). Age effects: older vs. younger later peak ($P = 0.007$) and return to baseline ($P = 0.030$) glucose concentrations; older vs. younger later peak ($P = 0.001$) and return to baseline ($P = 0.01$) insulin concentrations.

5.5.2 Plasma insulin

Baseline insulin concentrations did not differ between age groups ($P = 0.22$) or study days ($P = 0.41$, Table 5.1).

5.5.2.1 Interaction effects

The peak insulin concentration was higher after G than P in younger but not older men ($P = 0.006$). Time to peak insulin was longer after P and G in older than younger men ($P = 0.038$). The age by drink-interaction effect was non-significant for all other outcomes.

5.5.2.2 Drink-condition effects

Plasma insulin concentrations (Net iAUC) increased compared with the control following glucose (G), whey protein (P) and their combined ingestion (GP, $P < 0.001$). Co-ingestion of whey protein with glucose (GP) had a more than additive effect on the increase in plasma insulin concentrations (Net iAUC0–180/min GP vs. G plus P: 28.3 ± 4.7 vs. 21.3 ± 3.2 mU/L*min, $P = 0.002$, Figure 5.1). Time to peak and time to return to baseline (see age effects below) did not differ between P, G and GP ($P > 0.05$).

5.5.2.3 Age effects

Peak insulin concentrations were (non-significantly) lower after G and GP in older than younger men ($P = 0.34$). Peak plasma insulin concentrations occurred later in

older than younger men (P = 0.001). The time taken to return to baseline after the caloric drinks was longer in older than younger men (P = 0.01). GP increased plasma insulin concentrations compared with G plus P; 22 ± 1% in the older men (Net iAUC0–180min 27 ± 6 vs. 21 ± 5 mU/L*min, P = 0.02) and 27 ± 1% in the younger men (26 ± 6 vs. 19 ± 3 mU/L*min, P = 0.04). Peak plasma insulin concentrations were 50 ± 1% increased by GP compared with G in the older men and 41 ± 1% in the younger men (P < 0.001, Table 5.1).

5.5.3 Plasma glucagon

Baseline glucagon concentrations did not differ between age groups (P = 0.51) or during the study days (P = 0.27, Table 5.1).

5.5.3.1 Interaction effects

The age by drink-interaction effect was non-significant for all outcomes.

5.5.3.2 Drink-condition effects

Plasma glucagon concentrations (Net iAUC) decreased following glucose when compared with the control (P < 0.001), increased following combined GP (P < 0.001) and increased even more after whey protein alone (P < 0.001, Table 5.1, Figure 5.1). The increase in glucagon concentrations from baseline was greater after P than GP (P < 0.001).

Whey protein increased plasma glucagon concentrations by 27 ± 1% more than G

over 3 h on average ($P < 0.001$): 36 ± 1% more than G in the first hour ($P < 0.001$), 26 ± 1% more than G in the second hour ($P < 0.05$) with a comparable increase in the third hour ($P = 0.85$). Peak glucagon concentrations were higher after P than GP in both age groups to a similar degree ($P < 0.001$, age by drink-condition effect, $P = 0.58$, Figure 5.1). Time to peak/nadir after G and GP and nadir after G did not differ between treatments ($P = 0.65$). The time taken to return to baseline after the drinks was longer after P than GP or G ($P = 0.01$).

5.5.3.3 Age effects

Glucagon concentrations were not affected by age ($P = 0.71$).

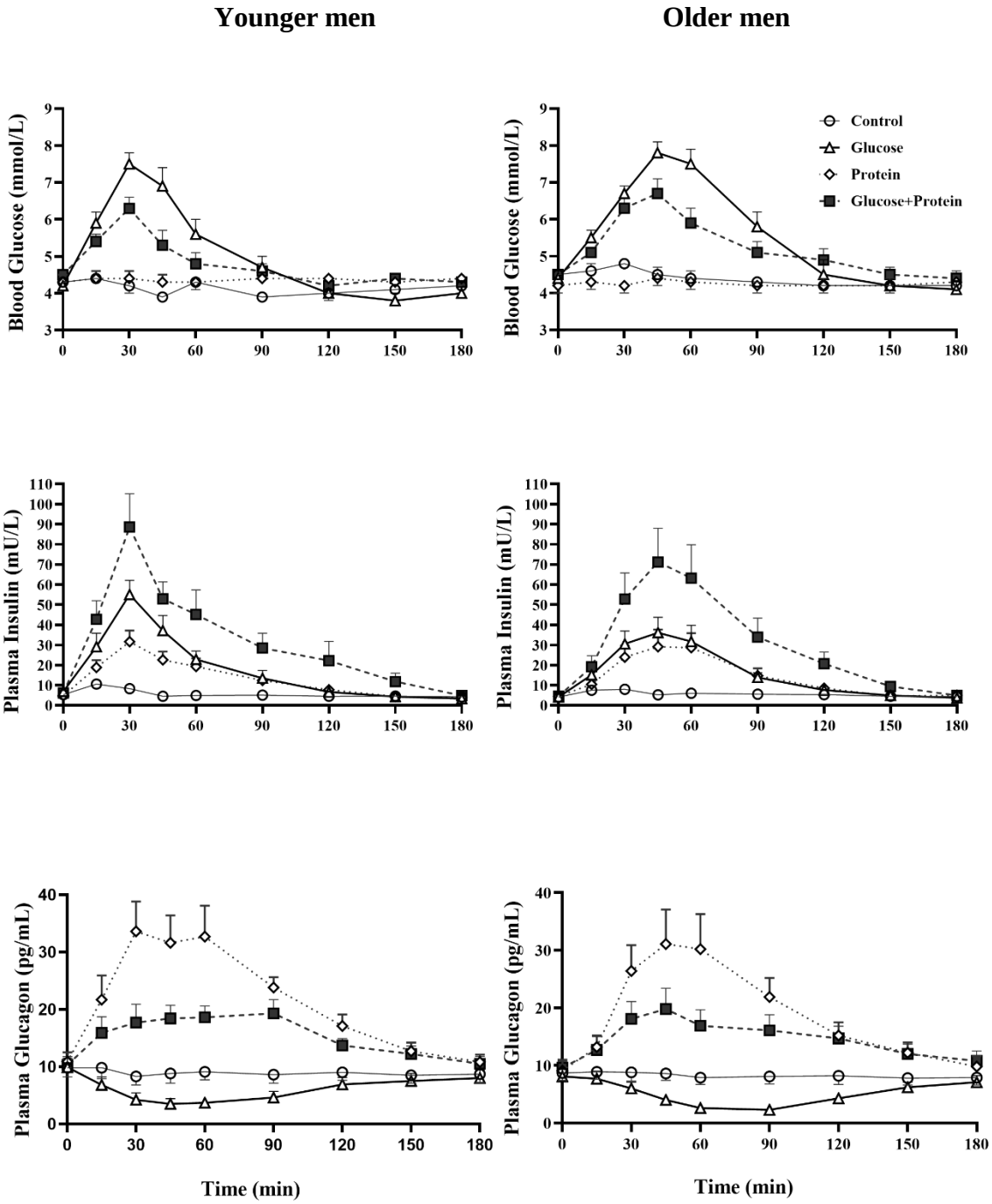

Figure 5.1: Mean (± SEM) blood glucose (mmol/L), plasma insulin (mU/L) and glucagon (pg/mL) concentrations following drink ingestion containing (i) flavored water (C, control, ~2 kcal), (ii) 30 g whey protein (P), (iii) 30 g glucose (G) or (iv) 30 g whey protein plus 30 g glucose (GP) in younger and older men. Effects of age and drink condition and the interaction effect were determined using a mixed-effect model with baseline concentrations as covariates and post hoc Bonferroni correction.

T = 0 min refers to the point immediately before the drink consumption. Age by drink-condition interaction effects: peak insulin (P = 0.006) was higher after G than P in younger but not older men; time to peak glucose (P = 0.03) and insulin (P = 0.038) was longer after G in older than younger men. Drink-condition effect: P < 0.001 Net iAUC glucose GP < G; P = 0.002 Net iAUC insulin GP > G; P < 0.001 peak glucagon P > GP. Age effect: time to peak glucose (P = 0.007) and insulin (P = 0.001) occurred later in older than younger men; time to return to baseline glucose (P = 0.03) and insulin (P = 0.01) occurred later in older than younger men.

5.5.4 Gastric emptying

A number of data points could not be included in the analysis due to poor quality of the samples, particularly in the young men and on the control treatment day, thus the graphs/values obtained were insufficient to determine gastric emptying. Data relating to gastric emptying in these participants were excluded from analysis. After exclusion of these data, gastric emptying data on all three protein and/or glucose drink days were evaluated in 5 younger and 10 older participants who had data from all 3 days.

Gastric emptying was slower after the protein ($_{AUC0\text{-}180/min}$ gastric retention: 35 ± 2%*min) than the glucose (30 ± 1%*min) drink and slower after the combined glucose and protein drink (40 ± 3%*min) than either protein or glucose alone (P = 0.001). Gastric emptying was non-significantly slower in older than younger men (T50 GP: 72 ± 13 vs. 53 ± 7 min, P: 47 ± 5 vs. 39 ± 5 min, G: 38 ± 2 vs. 33 ± 2, P = 0.29) (Figure 5.2).

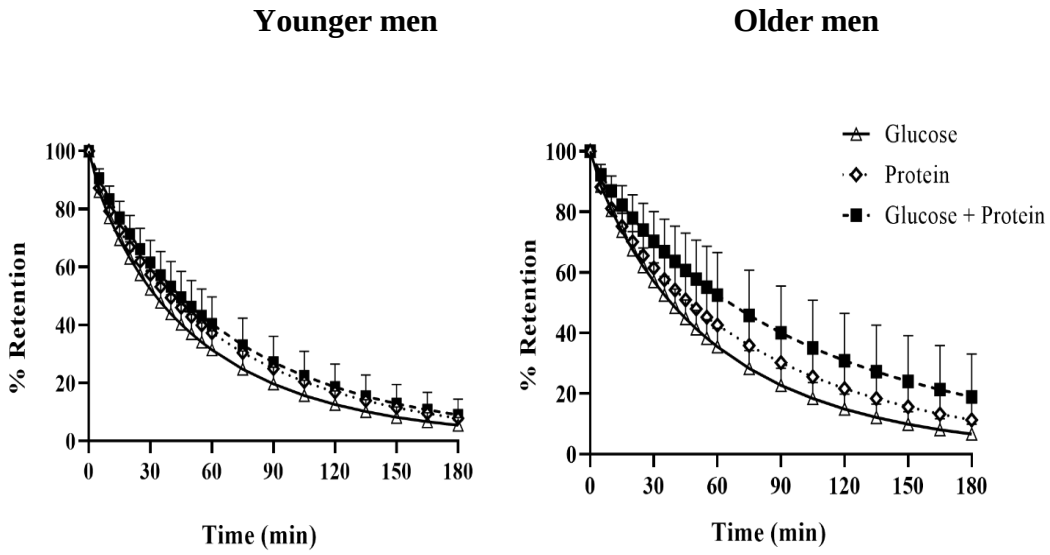

Figure 5.2: Intragastric retention (%) following drink ingestion containing (i) 30 g whey protein (P), (ii) 30 g glucose (G) or (iii) 30 g whey protein plus 30 g glucose (GP) in younger (n = 5) and older (n = 10) men. Effects of age and drink condition and the interaction effect were determined using a mixed-effect model with baseline concentrations as covariates and post hoc Bonferroni correction. T = 0 min refers to the point immediately before the drink consumption. Drink-condition effect: $P <$ 0.001 gastric emptying of GP was slower than P and both emptied slower than G.

5.5.5 Energy intake

Ad libitum energy intake three hours following preload drink ingestion was comparable between age groups ($P = 0.37$) and drink condition ($P = 0.95$; C, P, G, GP: older: 1008 ± 89, 1053 ± 106, 1047 ± 97, 988 ± 995 kcal; younger: 994 ± 89, 1003 ± 106, 960 ± 97, 939 ± 99 kcal). For GP, the mean energy intake was lower (not significant) compared with all the drinks.

5.5.6 Perceptions of appetite

Mean baseline perceptions of appetite did not differ between age groups (older vs. younger: hunger: 42 ± 9 vs. 49 ± 9 mm, p = 0.56; fullness: 6 ± 3 vs. 9 ± 3 mm, *P* = 0.55) or study days (*P* > 0.05).

The protein and protein plus glucose drink suppressed hunger compared with the control during the 3 h following the drinks in the younger ($_{AUC0\text{-}180/min}$ C, P, G, GP: 12 ± 5, 4 ± 5, 8 ± 5, 1 ± 4 mm*min) but not the older (2 ± 5, 10 ± 5, 2 ± 5, 8 ± 4 mm*min) men (age by drink-condition interaction *P* = 0.02, Figure 5.3).

Fullness increased less following the glucose drink when compared with the other drinks in the younger ($_{AUC0\text{-}180/min}$ C, P, G, GP: 4 ± 4, 5 ± 3, 0 ± 3, 7 ± 3 mm*min) but not the older (7 ± 4, 6 ± 3, 11 ± 3, 8 ± 3 mm*min) men (age by drink-condition interaction *P* = 0.01, Figure 5.3).

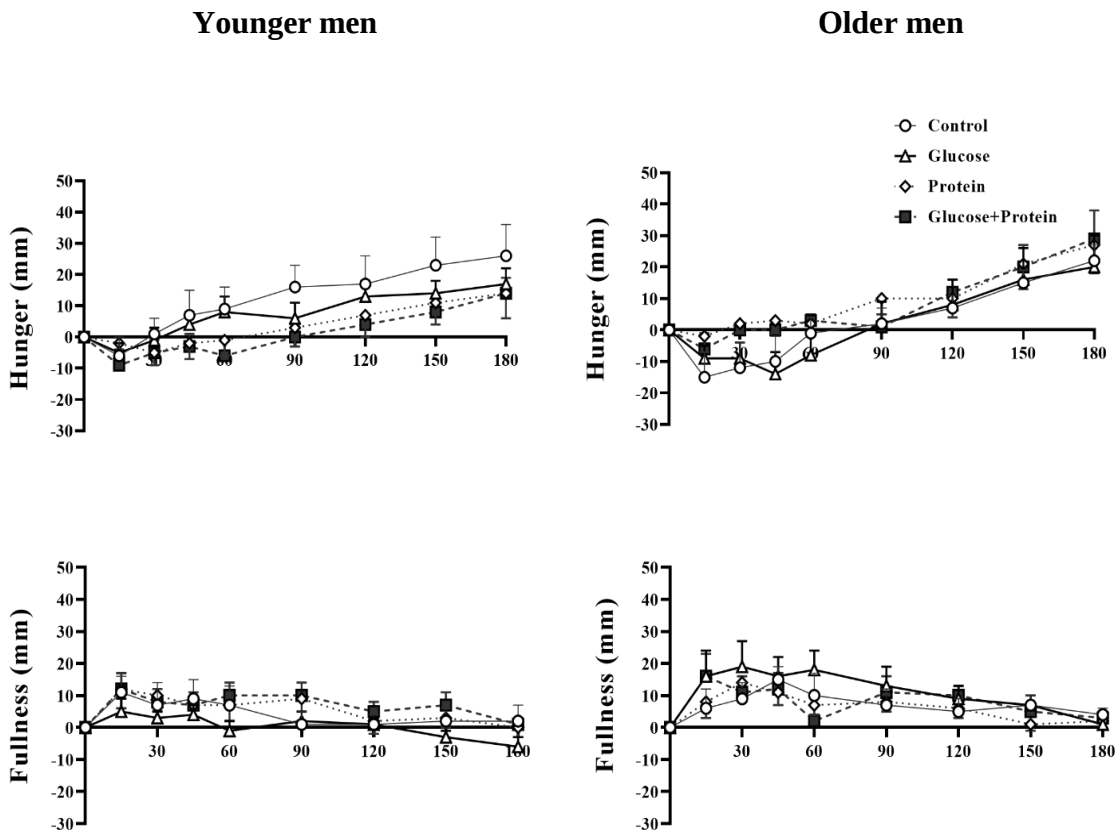

Figure 5.3: Mean ± SEM visual analogue score (VAS) of hunger (mm) and fullness (mm) following drink ingestion containing (i) flavored water (C, control, ~2 kcal), (ii) 30 g whey protein (P), (iii) 30 g glucose (G) or (iv) 30 g whey protein + 30 g glucose (GP) in younger and older men. Effects of age and drink condition and the interaction effect were determined using a mixed-effect model with baseline concentrations as covariates and post hoc Bonferroni correction. T = 0 min refers to the point immediately before the drink consumption. Age by drink-condition interaction effects: $P = 0.02$ hunger was suppressed by P and GP in younger but not older men; $P = 0.01$ fullness was less following G compared with the other drinks in younger but not older men.

5.6 Discussion

The main findings of this study are: (i) the addition of 30 g of whey protein to 30 g of glucose in drink form substantially attenuated the increase in blood glucose concentrations induced by glucose alone; (ii) the magnitude of the whey-induced reduction in blood glucose was not affected by age, with comparable reductions in older to those in younger adult men; (iii) the stimulation of plasma insulin concentrations by whey protein was not reduced by ageing, unlike the insulin response to glucose; (iv) whey protein suppressed hunger less in older than younger men.

These findings are consistent with previous observations, in which the ingestion of various proteins, including oral whey protein in doses ranging from 9 to 55 g, reduced the rise in oral carbohydrate-induced blood glucose concentration in younger adults (44, 86, 138, 350, 351, 353, 354). Those findings are extended by demonstrating that these effects of whey protein are maintained into old age; all older men in this study were 68 years or older with a mean age of 78 years. Previous studies have largely included young and middle-aged adults, with none, as far as we know, studying the effects of whey protein, or other proteins, in any participants much over 60 years. In contrast to this finding, some other responses to dietary protein are affected by ageing. Whey protein ingested alone, in doses of 30–70 g, has a reduced ability to suppress appetite and food intake in older adults compared with young adults (9, 12, 15) and produces greater increases in circulating concentrations of cholecystokinin (CCK) and glucose-dependent insulinotropic polypeptide (GIP) in older adults than younger adults, with comparable increases in circulating insulin (90). Intraduodenal infusions of whey protein, at rates spanning the normal range of gastric emptying, also have different effects on feeding, gut hormone release and glucose metabolism in healthy older adults compared with young adults (9).

The reduced insulin concentrations and slightly higher glucose concentrations observed after ingestion of glucose alone in this study in older adults compared with younger participants are consistent with reported changes in glucose metabolism that accompany human ageing (356-357). There are varying reports as to whether the increase in circulating concentrations of the incretin hormones glucagon-like peptide-1 (GLP-1) and GIP in response to glucose ingestion is affected by ageing (229). Indeed, GIP secretion in response to glucose ingestion may be increased in older people (90, 357, 229), perhaps in compensation for the age-related reduction in the ability of GIP to stimulate insulin secretion (363). Ageing is, however, associated with islet dysfunction and impaired insulin secretion, with disordered pulsatile insulin secretion in both the fasting and hyperglycemic state (357).

Ageing-associated defects in insulin secretion have been demonstrated largely in studies where hyperglycemia has been induced by oral or intravenous glucose; they may not be present in response to protein administration. The results of the present study suggest that they are not. This would be consistent with differing mechanisms of insulin release after glucose and protein ingestion, as also would be the more than additive increases in plasma insulin after combined glucose and protein compared with either alone in this study. Nuttall et al., reported qualitatively similar at-least-additive increases in insulin concentrations after higher, 50 g, doses of protein and glucose. Ingested protein, as with glucose, promotes insulin secretion by stimulating the release of the incretins GLP-1 and GIP. The effects of increasing age on protein-induced increases in circulating incretin concentrations appear similar to those in response to glucose, with equivalent increases in GLP-1 and greater increases in GIP in older and younger participants after whey protein ingestion (90).

Amino acids released by protein digestion also stimulate insulin secretion by direct action on the pancreatic islet cells (142). In contrast to islet responses to glucose, those to amino acids may be less affected, or not at all, by ageing. Some amino acids also stimulate insulin release by acting as substrates in the Krebs cycle to create ATP, which acts directly on the beta cells to increase their insulin secretory response to glucose (142). Support for the existence of this glucose-sensitizing effect of whey protein is provided by greater-than-additive, apparently synergistic, increases in plasma insulin concentrations after combined glucose/whey protein drinks in the present study.

The ingestion of proteins or their component amino acids causes increases in circulating concentrations of both insulin and glucagon, with their opposing effects on blood glucose concentrations and slowing of gastric emptying (90, 346), with resultant slight increases, no effect or slight decreases in blood glucose concentration when ingested on their own, depending on the type of protein or amino acid (346, 351, 352). Circulating glucagon concentrations after the three drinks in this study were not affected by ageing. Glucose alone suppressed plasma glucagon concentrations equally in older and younger participants in this study, consistent with the results of some (142, 364) but not all (365) previous studies. Whey protein alone increased plasma glucagon concentrations markedly and comparably in the two age groups, as previously reported (90). The combination whey protein/glucose drink increased glucagon concentrations equally in both age groups, with the increase approximately half that following whey protein alone. The post whey protein/glucose glucagon increase suggests a stronger stimulatory effect of whey protein on glucagon secretion than the inhibitory effect of glucose, at least with 30 g (120 kcal) of each.

Whey is a dairy protein present in milk along with casein. Relative to casein and most other proteins, whey has higher concentrations of the amino acids leucine, isoleucine, lysine, threonine and tryptophan (93), which are among the most effective amino acids in increasing circulating insulin concentrations and reducing blood glucose when co-ingested with glucose (346, 352), and has greater effects in inhibiting blood glucose increases when co-ingested with carbohydrates (44, 351). In non-elderly people with type 2 diabetes, 17 g whey protein preloads administered alone 15 min before a meal acutely reduced post-prandial glycemia and, when taken twice daily for 12 weeks combined with guar, had sustained effects to slow gastric emptying, reduce postprandial glucose and modestly reduce HbA1C (366). Whey protein supplements are relatively low cost with few, if any, side effects in older people (63). The finding in this study that the increase in circulating insulin concentrations and the reduction in hyperglycemia after whey protein ingestion was unaffected by ageing and persists in those over 70 years and into their 80s, provides encouragement for further studies into the use of whey protein supplements in the prevention and treatment of hyperglycemia in older people.

Whey protein ingestion may have beneficial effects in older people in addition to those on post-prandial glucose concentrations. There is evidence that it can lead to reductions in blood pressure, blood triglyceride levels, inflammation and oxidative stress (128), of which harmfully elevated levels are more prevalent in older adults than younger adults. Another beneficial effect of whey protein in older people is likely to be that on skeletal muscle mass and function. With increasing age there is on average a progressive loss of muscle mass and function, with approximately 5% of muscle mass lost per decade after age 30 years. When excessive, this muscle loss

leads to sarcopenia, with associated substantial increases in morbidity including rates of frailty and mortality (63). Various nutritional measures have been proposed to counteract these adverse effects of ageing, including the use of protein supplements, which has been shown to increase muscle mass and strength and may also reduce morbidity and mortality, with the greatest benefits in the most under-nourished and sarcopenic older people (63).

As with its effects on glucose metabolism, whey protein is a particularly good form of protein to exert this anabolic effect on muscle because of its relatively high content of branched chain amino acids, particularly leucine, which are the most effective amino acids in promoting muscle protein synthesis (249). Some of this effect may be due to the anabolic actions on muscle of the increased insulin secretion following whey protein ingestion. Due to age-related anabolic resistance (63, 270), older adults appear to require 30–35 g/serve of protein to stimulate muscle protein, compared with 20 g or less in younger adults (63), and a minimum of 25–30 g protein intake per meal for older people has been recommended by the PROT-AGE study group (97). This provides the rationale for our use of a 30 g whey protein dose in the present study.

Weight loss in older people, particularly if involuntary, is often associated with adverse outcomes (71) and therefore not necessarily desirable. Protein is the most satiating macronutrient in young adults, and whey protein drinks of 30–70 g suppress appetite ratings and ad libitum energy intake at subsequent meals in healthy young adults. Increasing age is, however, associated with a marked reduction in the satiating effects of whey protein, with no reduction in appetite ratings or ad libitum energy intake by older people at meals 35–510 min after 30 g whey protein drinks by older

people and only minimal effects after 70 g (63). Energy intake at the test meal in this study was not reduced significantly by any of the drinks in either age group, with a slightly greater suppression after the combined whey protein–glucose drink in the younger than the older participants (5.5 vs. 2%). In older people, increasing the dietary content of whey protein may preserve or increase skeletal muscle at the expense of fat gain (128), without reducing appetite and energy intake.

The longer times taken for plasma glucose and insulin concentrations to rise to peak and then return to baseline after the nutrient drinks in the older participants than the younger participants are probably due to a combination of slightly slower gastric emptying and delayed post-gastric effects in the older participants. Gastric emptying of nutrient drinks was non-significantly slower in the older participants than the younger participants, consistent with the results of previous studies, where the slowing of gastric emptying of whey protein with age has been minor when present (63, 229). Post-gastric delays probably played a greater role. Circulating glucose and insulin concentrations increase more slowly in the older men than the younger men, even when the stomach is bypassed by infusing glucose alone (28) directly into the duodenum, and insulin concentrations increase more slowly in the older men than the younger men after intraduodenal whey protein infusion (13). The mechanisms responsible for these post-gastric ageing-related changes are unknown.

Our study has several limitations. The number of participants was relatively small. Nevertheless, the results regarding glucose and insulin results were clearcut and statistically significant. We only studied men, therefore the observation may not apply to women. The results do not necessarily apply for older people ingesting doses of whey protein/glucose other than 30 g, as used in this study, although the

qualitatively similar results observed with a range of protein doses in previous studies of younger participants suggest that they will. While encouraging and suggestive, the ability of co- or pre-ingested whey protein to reduce blood glucose increases in older people after ingestion of carbohydrate in forms other than pure glucose, for example as part of a mixed meal, seems likely but will need to be established (147). Similarly, the effects in older people of non-whey proteins and proteins in non-drink form require further study.

5.7 Conclusions

In conclusion, 30 g of oral whey protein, a dose likely to have beneficial effects on skeletal muscle mass and function in older people, substantially attenuated the rises in blood glucose induced by 30 g of glucose alone in older men without diabetes. This extent of this reduction was not affected by ageing. The plasma insulin and glucagon responses to protein ingestion are preserved in older participants, in contrast to the insulin response to glucose, which is reduced. These findings provide a rationale for future studies of protein supplements/preloads in older people, in whom disorders of glucose metabolism such as type 2 diabetes and of muscle metabolism such as sarcopenia are prevalent and often serious.

CHAPTER 6: EFECTS OF CO-INGESTING GLUCOSE AND WHEY PROTEIN ON BLOOD GLUCOSE, PLASMA INSULIN AND GLUCAGON CONCENTRATIONS, AND GASTRIC EMPTYING, IN OLDER MEN WITH AND WITHOUT TYPE 2 DIABETES

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Contribution	Conducted the clinical trial, contributed to the overall design of the manuscript, literature review, data analysis and interpretation, drafting and revision of the manuscript.		
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By signing the Statement of Authorship, each author certifies that:

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6.1 Abstract

Co-ingestion of dietary protein with, or before, carbohydrate may be a useful strategy to reduce postprandial hyperglycaemia in younger and older men without type 2 diabetes (T2D), but its effect in older people with T2D is not known. Blood glucose, plasma insulin and glucagon concentrations were measured for 180 min following a drink containing either: 30 g glucose (G, 120 kcal), 30 g whey protein (P, 120 kcal), 30 g glucose plus 30 g whey protein (GP, 240 kcal), or control (~2 kcal) in older men with T2D (n = 10, 77 ± 1 years; 31 ± 1.7 kg/m^2) and without T2D (n = 10, 78 ± 2 years; 27 ± 1.4 kg/m^2). Mixed model analysis was used. GP vs. G (i) markedly reduced the increase in blood glucose concentrations ($P < 0.001$) and (ii) had a synergistic effect on the increase in insulin concentrations ($P < 0.001$), in men both with and without T2D. Glucose concentrations were higher in men with, when compared to without, T2D whereas insulin and glucagon concentrations were largely unaffected by the presence of T2D. Gastric emptying was faster in men with than without T2D. We conclude that the ability of whey protein to reduce carbohydrate-induced, postprandial hyperglycaemia is retained in older men with, when compared to without, T2D and that whey protein supplementation may be a useful strategy in the prevention and management of T2D in older people.

6.2 Introduction

Type 2 Diabetes mellitus (T2D) becomes more prevalent with increasing age and the impairment of glucose tolerance and hence hyperglycaemia tends to progress over time after diagnosis (340, 367-369). Lifestyle (dietary and exercise) measures are beneficial in improving glycaemic control and metabolic outcomes in T2D, alone and when combined with medications (370-373). Lifestyle measures remain important in older people with T2D (129, 374, 375) and the optimisation of dietary modifications in elderly people with T2D is therefore likely to have substantial benefits in the large number of older people with this condition.

Co-ingestion of protein with carbohydrates increases the insulin secretory response to carbohydrate and so blunts the increase in blood glucose concentrations following ingestion of carbohydrate alone in people both with and without T2D (136, 143, 144, 376, 377). This effect is particularly evident when the ingested protein is whey, a major protein, along with casein, in milk and dairy products (44, 86, 95, 352, 378-385).

We have reported recently the results of a study (135) in older and younger men without T2D, that the inhibitory effect of 30 g whey protein (a dose chosen due to its likely beneficial effects on muscle anabolism in older people) (97) on the increase in blood glucose concentrations after 30 g of glucose ingestion was preserved in older, when compared to younger, men. The stimulation of plasma insulin concentrations by whey protein was not reduced by ageing, unlike the insulin response to oral glucose intake (135). The present study was conducted to determine whether the effects of glucose and whey protein co-ingestion, compared to their ingestion alone,

on blood glucose, plasma insulin and glucagon concentrations, gastric emptying of the drinks, perceptions of appetite and subsequent energy intake present in older men without T2D (135) are also present in those with T2D.

6.3 Materials and methods

6.3.1 Participants

This randomized, double-blind, cross-over study included 10 older men with T2D (age range 69-84 years; 77 ± 1 years; body weight: 89 ± 6 kg; height: 168 ± 3 m; body mass index (BMI): 31.4 ± 1.7 kg/m^2) and 10 older men without T2D (age range 68-87 years, 78 ± 2 years; body weight: 82 ± 2 kg; height: 176 ± 2 m; BMI: 27.3 ± 1.4 kg/m^2). Body weight and BMI did not differ significantly between groups (P > 0.05). Participants were recruited by online advertisement and by flyers placed on notice boards at the University of Adelaide, Australia.

Study exclusion criteria were the following: smokers; vegetarians; intake of any illicit substance; intake of > 20 g alcohol on a daily basis; significant gastrointestinal symptoms or history of gastrointestinal disease including known gastroparesis, or surgery (other than appendectomy or cholecystectomy), proteinuria; any other illness deemed significant by the investigator (including chronic illnesses not explicitly listed above); current use of medications which are likely to affect gastrointestinal function or appetite (e.g. opiates, anticholinergics, levodopa, calcium-channel antagonists, beta blockers, clonidine, nitrates, tricyclic antidepressants, selective serotonin re-uptake inhibitors, phosphodiesterase type 5 inhibitors, sumatriptan, metoclopramide, domperidone, cisapride, tegaserod, or erythromycin); use of non-prescribed medications (including vitamins and herbal supplements) which may

affect appetite, body weight, gastrointestinal function or energy metabolism (e.g. green tea extracts, Astragalus, St John's Wort etc.); individuals who are found to be unable to comprehend the study protocol.

In participants with T2D further exclusion criteria were treatment with insulin or other diabetes medications apart from metformin and/or a dipeptidyl 4 (DPP4) inhibitor.

Inclusion criteria included being weight stable (< 5% fluctuation in their body weight) at study entry, as assessed by self-reported weight in the preceding 3 months, willingness to maintain usual physical activity level throughout the study and for participants with T2D having a HbA1c between 6% and 8.5% at the time of screening. The HbA1c of participants with T2D is presented in Table 6.1. Mean HbA1c of participants without T2D was 5.3%.

Table 6.1: Participant characteristics of older men with T2d (n = 10)

Age (years)	Time since Diabetes Diagnosis (years)	Screening HbA1c (%)	Diabetes medication
77	22	6.1	-
84	17	6.4	-
69	13	7.8	Metformin
78	22	7	Metformin
75	9	8.1	Metformin
82	6	7.9	Metformin/ Sitagliptin

73	16	7.3	Metformin/Empagliflozin
78	8	7.2	-
76	2	6.2	-
80	10	6.1	Metformin

The Royal Adelaide Hospital Human Research Ethics Committee approved the protocol which was conducted in accordance with the Declaration of Helsinki. The study was registered with the Australian New Zealand Clinical Trial Registry (www.anzctr.org.au, registration number ACTRN12619000420145). All participants provided written informed consent prior to their study inclusion.

6.3.2 Protocol

The protocol was identical to that of our previous study that compared younger and older men without T2D, and the results in the older men without T2D have been published previously (135). The study had a randomized (using the method of randomly permuted blocks; www.randomization.com), double blind study design where each participant was studied on four occasions, separated by ~7–10 days, and received a drink of either 30 g glucose (G, 120 kcal) (glucose monohydrate- Sigma-Aldrich, Missouri, USA), 30 g whey protein (P, 120 kcal) (Bulk Nutrients, Tasmania, Australia), 30 g glucose plus 30 g whey protein (GP, 240 kcal), or lime flavoured water (control, C; ~2 kcal) to determine the effects on blood glucose, plasma insulin and glucagon concentrations, gastric emptying, perceptions of appetite and energy intake at a buffet-style meal.

The study drinks were equivolaemic (~250 mL) and prepared by a research assistant

who was not involved in analysis of the study results. The drinks were stirred continuously at low speed on a stirring plate to ensure that all ingredients were dissolved evenly throughout and served in a covered cup to achieve blinding. Both the investigators conducting the study and the participants were blinded to the drink composition. The drinks were flavoured with varying amounts of distilled water, sodium chloride, light lime cordial (Bickford's Australia Pty Ltd, South Australia) and 100 mg [^{13}C] sodium acetate to match for taste.

Participants were instructed (i) to consume the same meal on the night before each study day at around 1900h, (ii) to fast overnight from solids and liquids and, (iii) to refrain from strenuous physical activity and alcohol for 24 hours prior to their attendance at the laboratory at the Clinical Research Facility, Adelaide Health and Medical Sciences Building, the University of Adelaide.

Upon arrival at the facility at 0830am, participants were seated in a chair. A cannula was inserted in an antecubital vein for blood sampling. A heated pad was used so that the blood samples were arterialised. Participants were instructed to consume the test drink within 2 minutes.

6.3.3 Measurements

6.3.3.1 Blood glucose, plasma insulin and plasma glucagon concentrations

Blood samples were collected into ice-chilled, EDTA-coated tubes for the measurement of blood glucose, plasma insulin and glucagon concentrations at t = 0 (baseline, after overnight fasting immediately before consumption of the study drink), 15, 30, 45, 60, 90, 120, 150 and 180 min. No inhibitors were added (362).

Plasma was obtained by centrifugation for 15 min, at 3200 rpm at 4°C, and samples were stored at -80°C for further analysis of insulin and glucagon concentrations. Blood glucose concentrations (mmol/L) were measured using a glucose analyser by the glucose oxidase method (YSI 2900 Stat Plus, Yellow Springs Instruments, Yellow Springs, Ohio, USA). Intra- and inter-assay coefficient of variations (CVs) were ≤ 2%. Total plasma insulin concentrations (milliunits per liter) were measured by enzyme-linked immunosorbent assay (ELISA) immunoassay (10-1113; Mercodia, Uppsala, Sweden). The sensitivity of the assay was 1.0 mU/L. Intra- and inter-assay CVs were 2.9% and 11.6%. Plasma glucagon concentrations were measured by ELISA immunoassay (10-1271-01, Mercodia, Uppsala, Sweden). The lower limit of quantification (LLOQ) was 1.5 pmol/L, the detection limit was 0.75 pmol/L. Intra- and inter-assay CVs were 9.3% and 7.5%.

6.3.3.2 Gastric emptying

Gastric emptying (% intragastric retention at t = 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 90, 105, 120, 135, 150, 165 and 180 min) of the drink was measured with ^{13}C-sodium acetate breath test (359). Breath samples consisted of the participant 's end-expiratory breath, collected into a 100mL foil bag. The quantity of $^{13}CO_2$ (disintegrations per min) was quantified using a non-dispersive infrared spectrometer (FANci2, Fischer ANalysen Instrumente, Germany) (360). Intragastric retention was calculated using the Wagner-Nelson method (361) which has been shown to be of comparable accuracy to scintigraphy in the measurement of gastric emptying of liquid food ingestion (242, 362).

6.3.3.3 Energy intake

At 180 min, each participant was presented with a standardised, cold, buffet-style meal, in excess of what they were expected to consume and were allowed to eat freely for 30 min (180–210 min) until comfortably full in a room by themselves to limit external distractions. The total energy content of the buffet-style meal was 2457 kcal; 19% protein, 50% carbohydrates, 31% fat. The buffet-style meal consisted of palatable food items including sliced bread, cheese, ham chicken, lettuce, tomato, cucumber, banana, apple, yogurt, custard, margarine, mayonnaise, iced coffee, orange juice, fruit salad, and water (16). Food consumed (g) was quantified by weighing the food before and after consumption. Energy intake was calculated as absolute (kcal) at the buffet meal using commercially available software (Foodworks version 8; Xyris Software Pty Ltd.) (15).

6.3.3.4 Perception of hunger and fullness

Perceptions of hunger and fullness were rated using a 100 mm visual analog scale questionnaire (t = 0, 15, 30, 45, 60, 90, 120, 150 and 180 min).

6.4 Data and statistical analysis

Statistical analyses were performed using SPSS software (version 25; IBM, Armonk, NY, USA). Sample size was based on statistical power functions of between-groups contrasts of older men with and without T2D with overall $P = 0.05$, statistical power of 0.8 and anticipated drop-out rate of ~10%, and significance levels adjusted to account for the 4 comparisons (control, whey protein, glucose, whey protein plus glucose). Calculations were performed for the primary outcome of area under the

curve (AUC) of blood glucose concentrations, assuming a within- participant SD of 0.5 mmol/l, and a between-participants SD of 1.4 mmol/l (14, 15, 90) to detect a difference between groups of 1.5 mmol/L, and between treatments of 0.4 mmol/L.

For measures of blood glucose, plasma insulin and glucagon concentrations, net incremental area under the curve (Net iAUC), peak, time to peak and time to return to baseline was calculated. Net iAUC was calculated from baseline using the trapezoidal rule, and then divided by time (min) to present a weighted average value over the time interval.

Mixed-effects model was used to determine the main effects of age and drink-condition, and their interaction effect, on blood glucose, plasma insulin and glucagon concentrations with the drink-condition as the within- participant factor and age as the between- participant factor, including baseline values at each treatment visit as a covariate. Post hoc comparisons, which were adjusted for multiple comparisons with Bonferroni correction, were performed when there were significant drink-condition or interaction effects. A repeated measures ANOVA (G and GP for glucose; G, P and GP for insulin and glucagon) was used to determine peak concentrations, time to peak and time to return to baseline for blood glucose, plasma insulin and glucagon concentrations in those drink conditions associated with changes from baseline. When the time to return to baseline was between 2 blood sampling time points, an interpolated value was estimated assuming a linear relationship between the 2 time points. If a blood glucose or plasma insulin or glucagon concentration did not return to baseline by 180 minutes the time to return to baseline was calculated using a linear extrapolation from the values at 150-180 mins. We hypothesised that insulin concentrations following both G and P drinks

would increase, and to determine if the effect on insulin of the combination drink is synergistic or additive, we compared the effect of combined whey protein and glucose (GP drink) on the rise in plasma insulin concentrations to that of the sum of the effects of glucose and whey protein alone (G drink + P drink) on insulin concentrations, using a paired t test. The inhibition of the increase in blood glucose concentrations following glucose ingestion by whey protein, when compared to glucose ingestion alone was calculated as the difference between Net iAUC of G and GP as a percentage of Net iAUC of G. The increase in plasma insulin concentrations following GP when compared to G ingestion alone was calculated as the difference between Net iAUC of GP and G as a percentage of Net iAUC of G. The increase in plasma glucagon concentrations following P when compared to G ingestion alone was calculated as the difference between Net iAUC of P and G as a percentage of Net iAUC of G. Statistical significance was accepted at $P < 0.05$. Data are presented as mean values ± SEM.

6.5 Results

The study protocol was well tolerated by all participants and no adverse effects were reported.

6.5.1 Blood glucose concentrations

6.5.1.1 Interaction effects

Peak blood glucose concentrations occurred earlier after GP than G in older men without T2D ($P = 0.008$), and occurred later in men with T2D than without T2D following ingestion of the GP drink ($P = 0.005$). The group (diabetes status) by drink

interaction effect was non-significant for all other outcomes (Table 6.2).

6.5.1.2 Drink-condition effects

Blood glucose concentrations increased after overnight fasting following glucose ingestion, alone (G, $P < 0.001$) and when combined with whey protein (GP) ($P < 0.001$) compared to control, to peak mean concentrations of 6.9 - 12.5 mmol/L (Table 6.2). Glucose concentrations did not change from baseline following the C and P drinks ($P > 0.05$).

Co-ingestion of glucose with whey protein, when compared to glucose ingestion alone, reduced the peak (GP vs. G: 8.7 ± 0.3 vs. 10.3 ± 0.5 mmol/L, $P < 0.001$) and Net $iAUC_{0\text{-}60min}$ (1.9 ± 0.2 vs 2.6 ± 0.2 mmol/L, $P < 0.001$) glucose concentrations, without significant effect of diabetes status on this inhibitory effect ($P > 0.05$).

The inhibition of the increase in blood glucose concentrations following glucose ingestion by whey protein, when compared to glucose ingestion alone was particularly evident during the first two hours following the drink ingestion; 30 ± 7 % in the first hour and 31 ± 9 % in the second hour (Figure 6.1).

6.5.1.3 Group effects

Overnight fasting ($P < 0.001$), peak ($P < 0.001$), Net $iAUC_{0\text{-}60min}$ ($P = 0.005$) and Net $iAUC_{0\text{-}180min}$ ($P < 0.001$) glucose concentrations were higher in men with T2D than without T2D (both, Table 6.2). Inhibition of the increase in blood glucose concentrations following glucose ingestion by whey protein, when compared to

glucose ingestion alone (see above), did not differ significantly between groups; older men with vs. without T2D 20 ± 11 % vs. 41 ± 6 % in the first hour (P = 0.12) and 15 ± 13 % vs. 51± 9 % in the second hour (P = 0.14).

Table 6.2: Blood glucose, plasma insulin and glucagon concentrations following control drink (C), 30g whey protein drink (P), 30g glucose drink (G) and 30g glucose plus 30g whey protein drink (GP) ingestion

	Older men without T2D				Older men with T2D				P value		
	C	**P**	**G**	**GP**	**C**	**P**	**G**	**GP**	**Group**	**Drink Condition**	**Interaction**
Blood glucose (mmol/L)											
Fasted	4.5 ± 0.4	4.2 ± 0.4	4.4 ± 0.5	4.5 ± 0.3	6.6 ±0.4	6.9 ± 0.4	7.1 ± 0.4	6.6 ± 0.3	<0.001	0.52	0.33
Peak	4.9 ± 0.4	4.7 ± 0.1	8.1 ± 0.7	6.9 ± 0.5	7.2 ± 0.4	7.6 ± 0.5	12.5 ±0.7	10.5 ± 0.5	<0.001	<0.001	0.21
Time to peak (min)	27 ± 7	38 ± 7	51 ± 4[1]	36 ± 3[1,2]	29 ± 7	32 ± 7	51 ± 4	51 ± 3[2]	0.09	0.049	0.049
Return to baseline (min)	60 ± 22	125 ± 23	132 ± 14	153 ± 49	81 ± 31	44 ± 12	186 ± 14	221 ± 49	0.22	0.69	0.83
Net $iAUC_{0\text{-}60min}$	0.1 ± 0.1	0.1 ± 0.1	2.1 ± 0.2	1.3 ± 0.2	0.0 ± 0.1	0.2 ± 0.1	3.1 ± 0.3	2.4 ± 0.3	0.005	<0.001	0.87

Net $iAUC_{0\text{-}180min}$	0.1 ± 0.1	0.07±0.08	1.1 ± 0.2	0.7 ± 0.2	-0.4 ±0.2	0.0 ± 0.2	2.5 ± 0.3	1.9 ± 0.2	<0.001	0.001	0.65
Plasma insulin (mU/L)											
Fasted	4.1 ± 1.7	4.5 ± 1.4	4.1 ± 1.4	4.5 ± 1.9	7.9 ± 1.7	8.2 ± 1.4	8.2 ± 1.4	8.5 ± 1.9	0.08	0.52	0.95
Peak	9.7 ± 2.9	31.7 ± 7.5	36.5 ± 7.9	72.7 ±14.5	15.1±2.9	41.2 ± 4.5	36.4 ±7.9	81.1 ±14.5	0.67	<0.001	0.07
Time to peak (min)	29 ± 7	48 ± 3	46 ± 6	42 ± 3[3]	39 ± 5	48 ± 3	49 ± 6	61 ± 3[3]	0.12	0.40	0.015
Return to baseline (min)	109 ± 18	149 ± 9	163 ± 12	166 ± 21	95 ± 18	163 ± 9	204 ± 12	222 ± 21	0.009	0.013	0.26
Net $iAUC_{0\text{-}60min}$	2.3 ± 0.5	15.5 ± 3.8	20.9 ± 4.4	39.8 ± 9.3	3.2 ± 0.9	17.5 ± 4.2	16.4 ±4.7	33.6 ± 8.9	0.76	<0.001	0.14
Net $iAUC_{0\text{-}180min}$	1.5 ± 0.6	9.6 ± 1.9	11.6 ± 2.4	26.6 ± 6.2	0.9 ± 0.6	11.9 ± 2.6	12.4± 2.9	31.2 ± 6.9	0.68	<0.001	0.68
Plasma glucagon											
(pg/mL)											
Fasted	8.7 ± 1.3	9.1 ± 1.5	8.1 ± 1.2	9.6 ± 1.3	9.6 ± 1.3	9.9 ± 1.5	9.2 ± 1.2	8.8 ± 1.3	0.78	0.56	0.70
Peak/Nadir	10 ± 1.3	33.1 ± 4.8	9.1 ± 1.4	22.1 ± 2.9	11.7±1.5	35.9 ± 4.8	11.2 ±1.4	25.1 ± 2.9	0.53	<0.001	0.94
Time to peak (min)	65 ± 20	51 ± 10	56 ± 21	48 ± 7	57 ± 20	69 ± 10	34.5 ± 21	37.5 ± 7	0.76	0.11	0.17
Return to baseline (min)	52 ± 14	391 ± 153	133 ± 29	277 ± 51	72 ± 14	185 ± 153	98 ± 34	155 ± 51	0.12	0.042	0.47

Net $iAUC_{0\text{-}60min}$	-0.1 ±0.2	13.5 ± 2.6	-2.4 ± 0.7	6.3 ± 1.5	0.4 ± 0.2	16.8 ± 2.1	-0.2 ± 0.6	8.8 ± 1.5	0.07	<0.001	0.12
Net $iAUC_{0\text{-}180min}$	-0.5 ±0.3	9.9 ± 1.3	-3.2 ± 0.8	5.1 ± 1.3	-0.1 ±0.3	12.1 ± 1.3	-1.7 ± 0.6	7.3 ± 1.4	0.09	<0.001	0.20

Blood glucose (mmol/L) and plasma insulin (mU/L) and glucagon (pg/mL) concentrations fasted (baseline), peak, time to peak (min), return to baseline (min), Net $iAUC_{0\text{-}60min}$ (change from baseline area under the curve during the first hour), Net $iAUC_{0\text{-}180min}$ (change from baseline area under the curve during the three hours) following drink ingestion containing (i) flavored water (C, control, ~2 kcal), (ii) 30 g whey protein (P), (iii) 30 g glucose (G), or (iv) 30 g glucose plus 30 g whey protein (GP) in older men with and without T2D. Effects of group and drink-condition and interaction effects were determined using a mixed-effect model using G and GP for glucose and P, G and GP for insulin and glucagon. Group x drink-condition interaction: $P = 0.049$ [1]Peak glucose concentrations occurred earlier after GP than G in older men without T2D; [2,3]Peak glucose and insulin concentrations occurred later in men with T2D than without T2D. Drink-condition effects: $P < 0.001$ G and GP vs. C higher glucose and G, P and GP vs. C higher insulin concentrations; $P < 0.05$ GP vs. G lower glucose and GP vs. G and P higher insulin concentrations; $P < 0.05$ G vs. C lower GP vs. C and P vs. C and GP higher glucagon concentrations. Group effects: $P<0.05$ older men with T2D vs. without T2D higher glucose concentrations.

6.5.2 Plasma insulin

6.5.2.1 Interaction effects

Peak blood insulin concentrations occurred later in men with T2D than without T2D following ingestion of the GP drink ($P = 0.015$). The group (diabetes status) by drink interaction effect was non-significant for all other outcomes (Table 6.2).

6.5.2.2 Drink-condition effects

Plasma insulin concentrations (peak, Net iAUC) increased after overnight fasting following G, P and GP, compared to control ($P < 0.001$, Table 6.2). Insulin concentrations (peak and Net iAUC) increased more ($P < 0.001$), and time to return to baseline was greater ($P = 0.013$), following GP than both G and P, with no difference between the increase after G and P over 3 hours ($P = 0.80$). GP increased insulin concentrations more than the sum of the increases after G and P administered separately (Net $iAUC_{0\text{-}180/min}$ GP vs. G plus P: 28.9 ± 4.8 vs. 22.7 ± 3.8 mU/L*min, $P = 0.002$).

6.5.2.3 Group effects

Baseline insulin concentrations were non-significantly higher in older men with T2D than without T2D ($P = 0.08$). Older men with T2D vs. without T2D showed non-significantly higher peak insulin concentrations after the P and GP drinks (Table 6.2). Insulin concentration time taken to return to baseline after drink ingestion was longer in men with T2D than without T2D ($P = 0.009$).

The higher increase in insulin concentrations over 3 hours by GP when compared to G plus P (see above) was comparable (P = 0.68 in the men with T2D (23 ± 1 %) and without T2D (22 ± 1%).

6.5.3 Plasma glucagon

6.5.3.1 Interaction effects

The group (diabetes status) by drink interaction effect was non-significant for plasma glucagon concentrations (Table 6.2).

6.5.3.2 Drink-condition effects

When compared to control, plasma glucagon concentrations decreased after overnight fasting following G, increased following GP and increased significantly more following P than G and GP (P < 0.001, Table 6.2, Figure 6.1). P increased glucagon concentrations by 26 ± 34 % more than did G over 3 hours (P < 0.001); 14 ± 22% more in the first hour (P < 0.001), 29 ± 41% more in the second hour (P < 0.001), with a comparable increase in the third hour (P= 0.28). Glucagon concentrations time taken to return to baseline was longer after P than GP and G (P = 0.042).

6.5.3.3 Group effects

Glucagon concentrations were not affected by the presence of diabetes (Table 6.2).

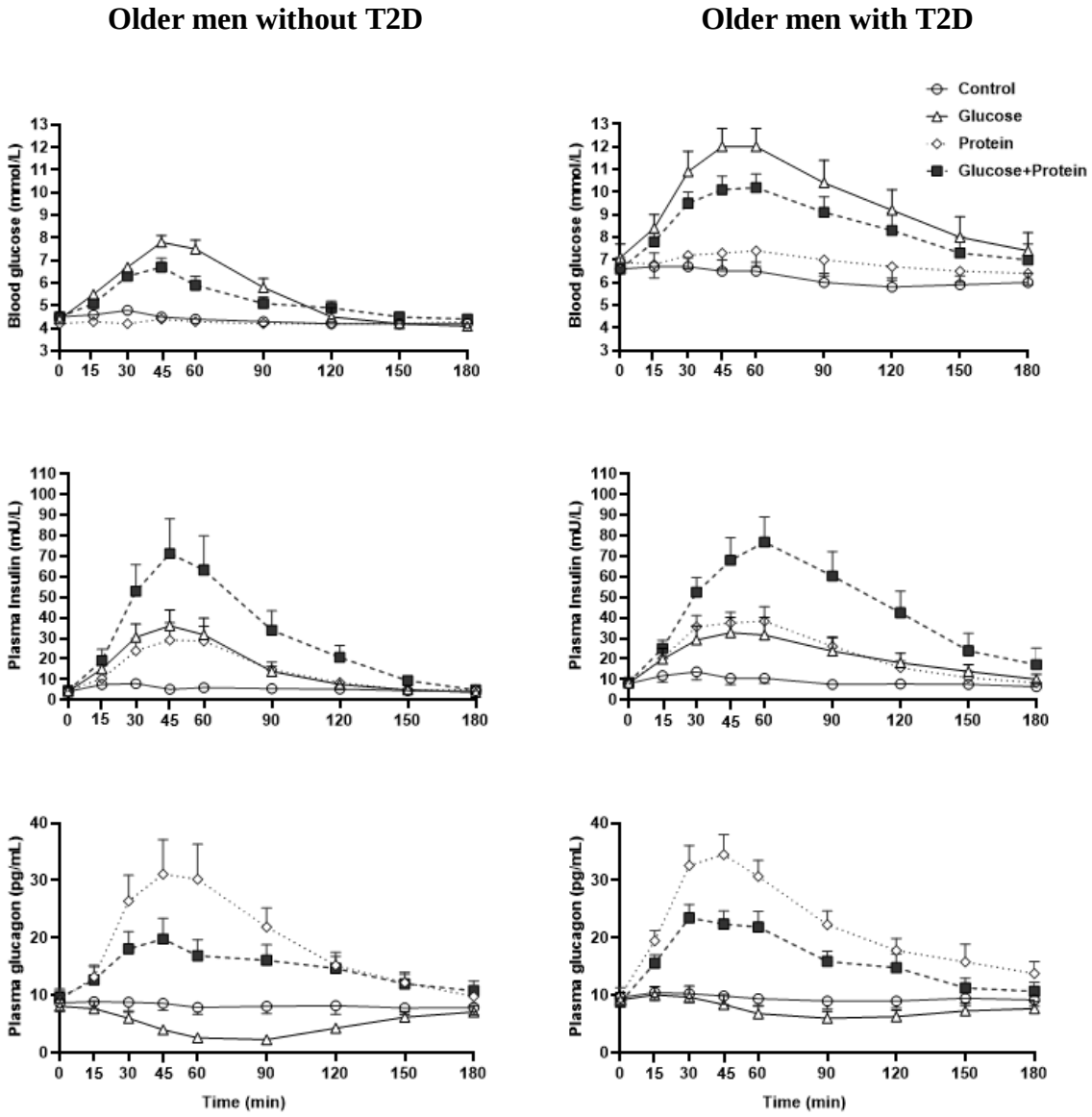

Figure 6.1: Mean (± SEM) blood glucose (mmol/L), plasma insulin (mU/L) and glucagon (pg/mL) concentrations following drink ingestion containing (i) flavored water (C, control, ~2 kcal), (ii) 30 g glucose (G), (iii) 30 g whey protein (P), or (iv) 30 g glucose plus 30 g whey protein (GP) in men with and without T2D. Effects of group and drink-condition and interaction effects were determined using a mixed-effect model using G and GP for glucose and P, G and GP for insulin and glucagon. Group x drink-condition interaction: $P = 0.049$ Peak glucose concentrations occurred earlier after GP than G in older men without T2D; Peak glucose and insulin concentrations occurred later in men with T2D than without T2D. Drink-condition

effects: $P < 0.001$ G and GP vs. C higher peak and Net iAUC glucose and G, P and GP vs. C higher insulin concentrations; $P < 0.05$ GP vs. G lower glucose and GP vs. G and P higher peak and Net iAUC insulin concentrations; $P < 0.05$ G vs. C lower GP vs. C and P vs. C and GP higher peak and Net iAUC glucagon concentrations. Group effects: $P < 0.05$ older men with T2D vs. without T2D higher peak and Net iAUC glucose concentrations.

6.5.4 Gastric emptying

6.5.4.1 Interaction effects

The group (diabetes status) by drink interaction effect was non-significant for $AUC_{0\text{-}180/min}$ gastric retention ($P = 0.23$).

6.5.4.2 Drink-condition effects

Gastric emptying was slower ($P = 0.007$) after P ($AUC_{0\text{-}180/min}$ gastric retention: 34 ± 2%*min) than G (30 ± 1%*min) and slower after GP (41 ± 3%*min) than either P or G (both $P < 0.001$) (Figure 6.2).

6.5.4.3 Group effects

Gastric emptying was faster ($P = 0.04$) in older men with T2D (31 ± 2%*min) than without T2D (38 ± 2%*min).

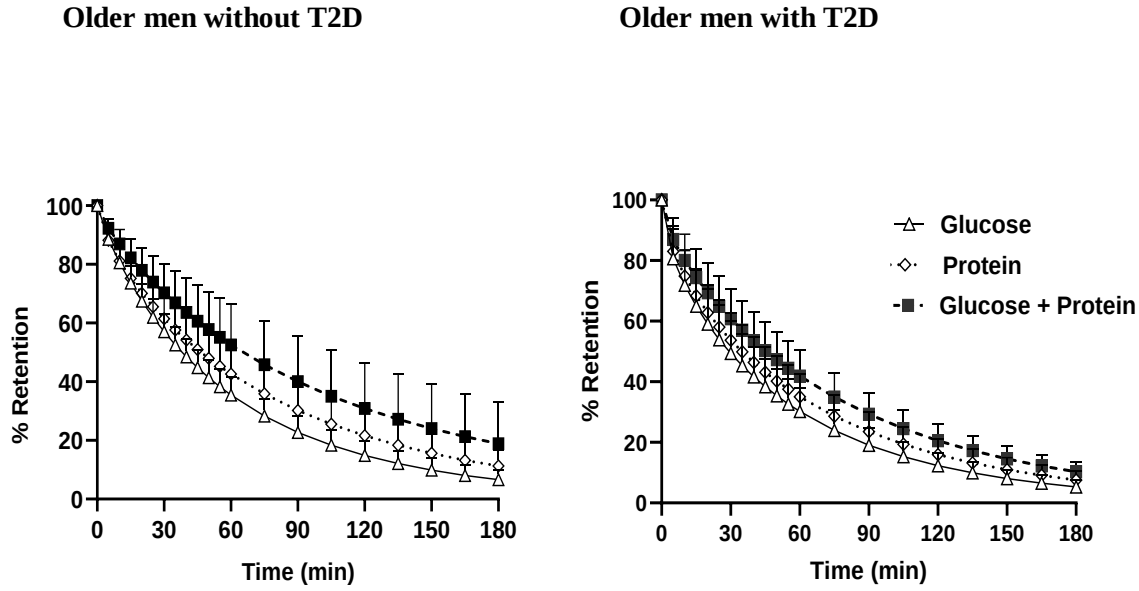

Figure 6.2: Intragastric retention (%) following drink ingestion containing (i) 30 g glucose (G), (ii) 30 g whey protein (P), or (iii) 30 g glucose plus 30g whey protein (GP) in men with (n = 10) and without T2D (n = 10). Effect of group and drink-condition and the interaction effect were determined using a mixed-effect model and post hoc Bonferroni correction. T = 0 min refers to the point immediately before the drink consumption. Drink condition effect: $P < 0.001$ GP vs. P and G, P vs. G slower gastric emptying.

6.5.5 Energy intake

Ad libitum energy intake three hours following drink ingestion was comparable between groups *(P* = 0.76) and drink-condition (*P* = 0.88); older men with T2D: C: 1015 ± 95 kcal, G: 997 ± 99 kcal, P: 997 ± 95 kcal, GP: 965 ± 115 kcal; older men without T2D: C: 1008 ± 89 kcal, P: 1053 ± 106 kcal, G: 1047 ± 97 kcal, GP: 988 ± 995 kcal.

6.5.6 Perception of hunger and fullness

Mean baseline perceptions of appetite did not differ significantly between study days ($P > 0.05$) or groups; older men without T2D vs. with T2D: hunger: 42 ± 9 mm vs. 26 ± 9 mm, $P = 0.21$; fullness: 6 ± 3 mm vs. 8 ± 3 mm, $P = 0.66$.

Hunger decreased immediately following all drinks in older men without T2D to steadily increase during the remainder of the study duration (Net $iAUC_{0\text{-}180/min}$ C: 2 ± 5, P: 10 ± 5, G: 2 ± 5, GP: 8 ± 4 mm*min, repeated measures time effects $P < 0.001$), whereas hunger did not decrease following ingestion of the drinks and increased steadily during the three hours in older men with T2D (Net $iAUC_{0\text{-}180/min}$ C: 5 ± 3, P: 7 ± 4, G: 7 ± 5, GP: 10 ± 5 mm*min, repeated measures time effects $P < 0.05$, Figure 6.3). Fullness increased immediately following G, P and GP in older men without T2D to decrease back to baseline during the remainder of the study duration (Net $iAUC_{0\text{-}180/min}$ C: 7 ± 3, P: 6 ± 3, G: 11 ± 5, GP: 8 ± 3 mm*min, repeated measures time effects $P < 0.05$), whereas fullness did not change significantly over time following drinks ingestion in older men with T2D (Net $iAUC_{0\text{-}180/min}$ C: 4 ± 2, P: 8 ± 3, G: 5 ± 4, GP: 9 ± 4 mm*min, repeated measures time effects $P > 0.05$).

Older men without T2D **Older men with T2D**

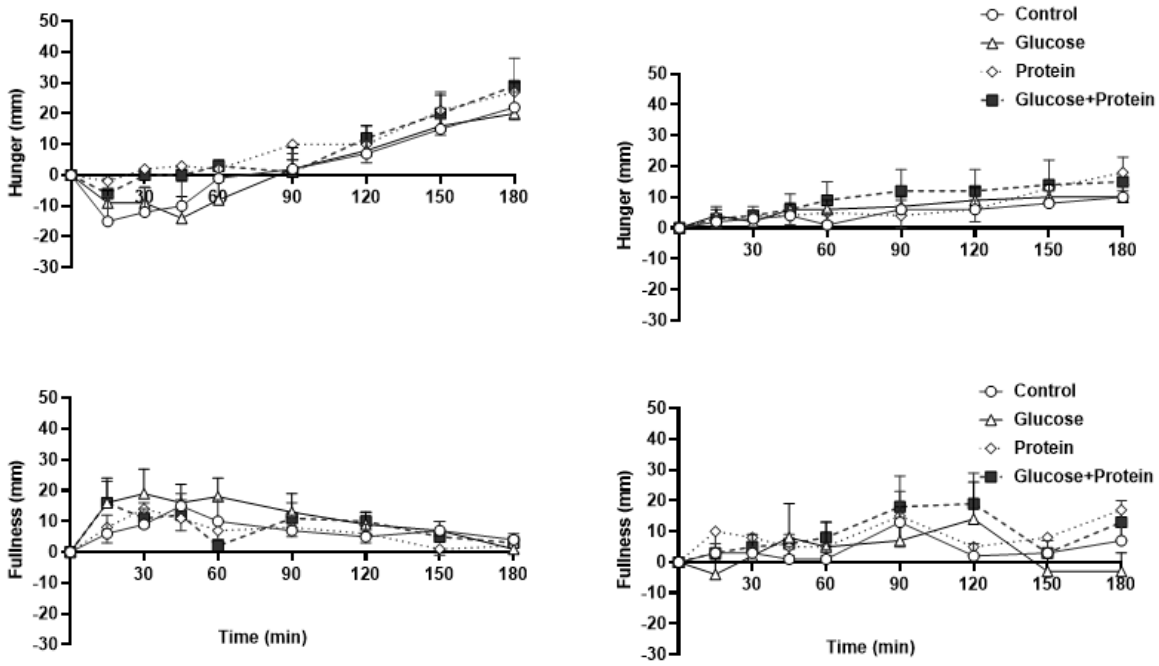

Figure 6.3: Mean ± SEM visual analogue score (VAS) of hunger (mm) and fullness (mm) following drink ingestion containing (i) flavored water (C, control, ~2 kcal), (ii) 30 g glucose (G), (iii) 30 g whey protein (P), or (iv) 30 g glucose plus 30 g whey protein (GP) in men with and without T2D. Repeated measures time effects: Hunger decreased immediately following all drinks in older men without T2D to steadily increase during the remainder of the study duration ($P < 0.001$), whereas hunger did not decrease following ingestion of the drinks and increased steadily during the three hours in older men with T2D ($P < 0.05$). Fullness increased immediately following G, P and GP in older men without T2D to decrease back to baseline during the remainder of the study duration ($P < 0.05$), whereas fullness did not change significantly over time following drinks ingestion in older men with T2D ($P > 0.05$).

6.6 Discussion

The main findings of this study which determined postprandial effects of glucose, whey protein and their combined ingestion are: (i) fasted and postprandial blood glucose concentrations were higher in older men with than without T2D; (ii) the addition of 30 g of whey protein to 30 g of glucose in drink form substantially attenuated the increase in blood glucose concentrations after glucose alone, to a similar extent in older men both with and without T2D; (iii) the stimulation of plasma insulin concentrations by whey protein was not affected by T2D, (iv) time to peak glucose and insulin concentrations occurred later in older men with than without T2D; (v) plasma glucagon concentrations increased after protein and decreased after glucose in older men both with and without T2D; (vi) gastric emptying of the caloric drinks was faster in men with than without T2D.

These results extend and are consistent with those of previous studies. The ability of combined orally ingested carbohydrate and protein ingestion to stimulate insulin secretion more than carbohydrate alone was first suggested in 1960 (146, 386), and later confirmed in people without (69) and with T2D (68, 348, 349). Nuttall *et al.* studied younger participants with T2D than in our study, and similarly reported at-least-additive increases in circulating insulin concentrations, albeit after higher (50 g) doses of protein and glucose than we used and with the protein in the form of a beef hamburger (68). Other studies have reported a 3-4 fold rise in insulin with a protein and carbohydrate meal as compared to glucose alone in people with T2D with a substantial reduction in postprandial glycaemia (143) similar to our study. They have, however, studied younger participants and administered the protein and carbohydrate using more complex study protocols e.g., as protein hydrolysate with

leucine, and phenylalanine together with continuous glucose infusions (376). Our study has investigated the effects of a simpler oral administration of whey protein and glucose in drink form on older men with and without T2D.

We have reported previously that co-ingesting 30 g whey protein with 30 g glucose substantially and significantly attenuated the increase in blood glucose concentration following 30 g glucose alone, to a similar degree in younger and older men without T2D (135). We now report that whey protein also inhibits glucose-induced blood glucose rises in older men with T2D. This is of possible therapeutic significance given the large numbers of older people with T2D.

Of note, although their diabetes was often longstanding (mean duration of known diabetes 12.5 years, range 2-22 years), none of the older men with diabetes in this study were receiving injectable treatment for their T2D i.e., insulin or a GLP-1 analog. It cannot be assumed that the glucose lowering effects of co-administered whey protein will be present in such people with T2D. Indeed this is probably unlikely, particularly in insulin-deficient people with T2D receiving insulin treatment, given that the marked whey-induced increases in plasma insulin concentrations suggest that this is the main mechanism by which whey protein inhibits glucose-induced hyperglycaemia. T2D is characterised by progressive beta cell failure with a substantial proportion of people with this condition eventually requiring insulin treatment (387). The natural history and time course of this failure is, however, variable, as illustrated by the participants in this study; their mean HBA1C was 7%, despite none being on insulin treatment a considerable time after diagnosis. We suspect that the blood glucose lowering effects of whey protein and other proteins will depend on the degree to which insulin secretory capacity and

action is retained, rather than the duration of T2D. There is, however, the possibility that the insulin secretory response to protein will be retained longer than that to carbohydrate in people with T2D, as we have found with the effects of ageing in people without T2D (135). It will be interesting to determine the effect of whey protein on glucose concentrations in older people with T2D on injectable hypoglycaemic medications.

Protein ingestion causes increases in circulating concentrations of both insulin and glucagon with their opposing effects on blood glucose concentrations in men with and without T2D with resultant slight increases, no effect or slight decreases in blood glucose concentration when ingested on their own. Circulating glucagon concentrations after the three caloric drinks in this study were not affected by the presence of T2D. Glucose alone suppressed plasma glucagon concentrations equally in both the participant groups in this study.

We and others have previously shown that gastric emptying is slower in older than younger adults without T2D (15, 215, 222, 325). In the present study the whey protein drink slowed gastric emptying more than an equicaloric glucose drink and the combined drink slowed emptying even more, consistent with previous reports (135, 147, 388). The men with T2D in this study had good glucose control (mean HBA1c 7%) and had faster gastric emptying than those without diabetes. The faster gastric emptying is consistent with previous reports in people with early and well-controlled T2D (218, 222, 389), where there is a positive relationship between the blood glucose increment after a standardized test meal and the rate of gastric emptying, but not necessarily in people with T2D which is poorly controlled or of longer duration (225, 226, 390). The reason for accelerated gastric emptying in early

and well-controlled T2D is not known.

The changes in appetite ratings following the drinks were less in older men with vs. without T2D. Previously a study in 11 people with T2D reported an increase in fullness and decrease in hunger ratings following ingestion of 15g of whey in drink form with a mixed nutrient meal vs. control drink plus meal – the study did not include a group without T2D (391).

Our study has several limitations. Although, the number of participants were relatively small, the glucose and insulin results were clearcut and statistically significant. We studied only men, so the observation may not apply to women. The results do not necessarily apply for doses of whey protein and glucose other than 30 g as used in this study. The ability of co- or pre-ingested whey protein to reduce blood glucose increases in older people with T2D after ingestion of carbohydrate in forms other than pure glucose, for example as part of a mixed meal, seems likely but will need to be established (147). Similarly the effects of non-whey proteins and protein in non-drink form in older people with T2D, require further study.

6.7 Conclusions

In conclusion, 30 g of oral whey protein, a dose likely to have beneficial effects on skeletal muscle mass and function in older people, substantially attenuated the rises in blood glucose concentrations induced by 30 g of glucose alone in older men with and without T2D, with no significant difference between the size of that effect between the two groups. The plasma insulin and glucagon responses to protein ingestion were preserved in older participants with T2D when compared to older men without T2D. These results support the further investigation and use of combined protein ingestion with carbohydrate as a part of the treatment of older people with

T2D.

CHAPTER 7: EFFECTS OF AGE ON BLOOD PRESSURE AND HEART RATE RESPONSES TO WHEY PROTEIN IN YOUNGER AND OLDER MEN

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Signature		Date	October 2022

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By signing the Statement of Authorship, each author certifies that:

i) the candidate's stated contribution to the publication is accurate (as detailed above);
ii) permission is granted for the candidate in include the publication in the thesis; and
iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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7.1 Abstract

Background: Postprandial falls in blood pressure (BP) are more common in older compared to younger individuals. The effects of protein compared to carbohydrates and fat on postprandial BP, and the relation to gastric emptying rates, are poorly studied.

Objective: To determine the effects of a whey protein compared to a control drink on systolic BP (SBP) and diastolic BP (DBP), and heart rate (HR) in healthy younger and older men, and to relate these effects to gastric emptying.

Design: A pooled analyses of two randomized, double-blind, cross-over studies.

Setting: Two acute clinical intervention studies with identical study design.

Participants: Nineteen older (age: 74 ± 1 years, body mass index: 26 ± 1 kg/m^2) and 13 younger (23 ± 1 years, 24 ± 1 kg/m^2) healthy men.

Intervention: A 70 g/280 kcal whey-protein or control (water with diet cordial, ~2 kcal) drink (450 ml).

Measurements: BP and HR were assessed with an automated device immediately before and at 3-min intervals after drink ingestion (0–180 min). Gastric emptying of the drinks was measured using 3D ultrasonography (0–180 min).

Results: Older versus younger men exhibited a greater fall in SBP (−23 ± 2 vs −15 ± 2 mmHg, $P = 0.001$) after whey-protein versus control, as BP did not change after the two drinks in younger men ($P > 0.05$). The nadir in SBP occurred later in the older than younger men (114 ± 11 vs 62 ± 14 min; $P < 0.001$), with SBP still apparently declining 180 min after whey-protein ingestion in the older men. The magnitude of the rise in HR was greater ($P < 0.05$) in the younger than older men.

Conclusion: Following ingestion of 70 g whey protein, healthy older men exhibited a sustained fall in BP, despite an increase in HR, whereas in younger men there was

no change in BP. BP may need to be monitored after high protein meals in older people at risk of postprandial hypotension.

7.2 Introduction

Falls and syncope are common in older people (155). Dizziness, syncope, and falls may reflect a substantial reduction in blood pressure (BP) induced by energy intake, so-called postprandial hypotension (PPH) (261, 392). PPH occurs often in older people and represents a major cause of morbidity and mortality (262). The prevalence of PPH ranges from ~13% in healthy older people (166, 393) to ~24–36% in nursing home residents (262) (160, 394) and ~43% in hospitalized geriatric patients (395).

We have reported that glucose, when infused directly into the duodenum, at rates within the normal range for gastric emptying (1–4 kcal/min), decreased postprandial systolic BP (SBP) and diastolic BP (DBP) in healthy older, but not younger, men, particularly during the first 60 min (396). Similarly, after an oral glucose load, which is used often in the diagnosis of PPH (155), the magnitude of the fall in SBP is greater when the rate of gastric emptying is relatively more rapid (227). Other groups have similarly reported a greater reduction in SBP and DBP after mixed macronutrient meals in healthy older compared to younger adults (159). The release of gut hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (397-399), and an impaired sympathetic nervous system response to compensate for the meal-induced splanchnic pooling are likely to be integral to the fall in SBP in older adults (228, 263).

There is increasing emphasis on the importance of protein, especially whey protein, consumption in older people, to prevent and manage malnutrition (97). Information relating to the effects of protein on postprandial BP is limited, with some studies reporting a reduction in BP after protein (174, 263), whereas in other studies no effect

was evident (173, 175).

PPH, defined as a sustained drop in SBP of ≥ 20 mmHg for ≥ 30 min (262), is commonly diagnosed through measurement of BP for 2 h in response to a 75 g glucose load (155). This timeframe, however, may not be sufficient to diagnose PPH following protein ingestion given its slower digestion time compared to glucose (400). For example, administration of protein, carbohydrate, and fat (at a rate of 3 kcal/min) resulted in comparable reductions in SBP and a rise in superior mesenteric artery (SMA) blood flow (changes in SMA flow typically occur after nutrient ingestion) in healthy older men, but these responses were relatively delayed for protein and fat compared to glucose (263, 401).

The current study represents an analysis of secondary outcomes of previously published studies (12, 15), to determine the effect of orally ingested whey protein on SBP, DBP, heart rate (HR), and gastric emptying in healthy older and younger men. We hypothesized that older, when compared to younger, men will exhibit greater falls in SBP and DBP, and a smaller increase in HR, following whey protein ingestion compared to control.

7.3 Methods

7.3.1 Participants

Nineteen healthy older (age: mean ± standard error of the mean [SEM]: 74 ± 1 years; body weight: 79 ± 2 kg; BMI: 26 ± 1 kg/m^2, 13 participants from the study described in Giezenaar et al. (12) and six participants from the study described in Giezenaar et al. (15) and 13 younger men (23 ± 1 years; 78 ± 2 kg; 24 ± 1 kg/m2 from Giezenaar

et al. (12) were included. Two older participants participated in both studies; only data from the most recent study (12) were included for these two participants. The exclusion criteria were smoking, alcohol abuse, use of illicit substances, (at risk of) diabetes mellitus (HBA1C > 6.0 mmol/L), major disease (i.e., cancer, Parkinson's, multiple sclerosis, etc.), gallbladder, or pancreatic disease, gastrointestinal surgery (apart from an uncomplicated appendectomy), significant gastrointestinal symptoms (abdominal pain, gastro-esophageal reflux, diarrhea, or constipation), use of medications known to potentially affect gastrointestinal motor function, known lactose intolerance, low plasma ferritin levels, or blood donation in the 12 weeks before the study, and cognitive impairment.

Four older men took anti-hypertensive medication (angiotensin-converting enzyme inhibitor, n = 1; antiarrhythmic, n = 1; beta blockers, n = 1; and angiotensin receptor blockers, n = 1). Participants were instructed not to take their medication on the morning of their study visit. The study protocols were approved by the Royal Adelaide Hospital Research Ethics Committee (120503a and 140407, clinical trial registration: ACTRN12612000941864 and ACTRN12614000846628, www.anzctr.org.au), and the studies were conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

7.3.2 Protocol

Both studies included in this pooled data analysis had an identical protocol and a randomized double-blind cross-over design (using the method of randomly permuted blocks; www.randomization.com) including 325 or 424 study days, separated by 3–14 days. The sub analysis related to two of these conditions, which were included in

both studies (control and 70 g whey protein).

On study days, participants fasted for ~14 h overnight, after consuming a standardized evening meal (beef lasagne [McCain Foods Pty Ltd, Australia], ~591 kcal), attended the laboratory at ~08:30 h, and ingested test drinks containing 70 g (280 kcal) whey protein or a control drink (~2 kcal) (12, 15). The drinks were prepared by a research assistant who was not involved in the analysis of the study results, flavored with diet lime cordial (Bickford's Australia Pty Ltd, South Australia) to match for taste, and served at room temperature. Drinks were presented in a covered cup to achieve blinding. Upon arrival, participants were seated in a chair, and a cannula was inserted for blood sampling (results not presented in this manuscript). An automated BP monitor (DINAMAP ProCare 100; GE Medical Systems, Milwaukee, WI) was used to measure SBP, DBP, and HR. Participants were studied for 180 min after drink ingestion, and were seated for the duration of the study.

7.3.3 Measurements

7.3.3.1 Blood pressure and heart rate

Measurements were taken three times immediately after the other at baseline (before drink ingestion), and every 3 min in the 3 h following the drink (0–180 min). Baseline BP and HR were calculated as an average of the three baseline measurements. T = 0 min refers to the point immediately after drink consumption.

7.3.3.2 Perceptions of light-headedness and drowsiness

At baseline, T = 0 min, and every 15 min after the drink, perceptions of light-headedness and drowsiness were assessed using a visual analog scale which consisted of a 100-mm horizontal line for each outcome. Participants were asked to place a mark indicating the strength of the sensation at the specified times.

7.3.3.3 Gastric emptying

At baseline, T = 0 min and every 15 min after drink ingestion, gastric volume was determined using 3-dimensional ultrasonography (Logiq 9, GE Healthcare Technologies, Australia), a method that has been validated against the "gold standard" scintigraphy for measurement of gastric emptying (230). Intragastric retention was calculated as total gastric volume minus baseline "empty" gastric volume at each time point, expressed as a percentage of the maximal gastric volume (100%), that is, 450 ml volume of the ingested drink. The time at which 50% of the drink was emptied from the stomach (50% gastric emptying time; T_{50}; min) was calculated.

7.4 Statistical analysis

Statistical analyses were performed using SPSS software (version 25; IBM, Armonk, NY). Interaction effects of age and treatment were determined using a two-way repeated-measures analysis of variance (ANOVA), with age as the between-participant factor, and treatment as the within- participant factor. Effects of age, time, and their interaction were determined within treatment groups. For SBP, DBP, and HR, areas under the curve (AUC) were calculated from baseline to 180 min, using the trapezoidal rule. Cumulative changes from baseline were calculated for

SBP, DBP, and HR (0–60 min, 60–120 min, and 120–180 min) by subtracting baseline values from the value at each time point, such that larger negative values indicate larger excursions below baseline, and larger positive values correspond to excursions above baseline. Nadir and time to nadir of SBP and DBP, and peak and time to peak of HR, light-headedness, and drowsiness were determined between 0 and 180 min. Assumptions of normality were verified for all outcomes before statistical analysis. Statistical significance was accepted at $P < 0.05$. Data in the text and tables are presented as mean values ± SEM.

7.5 Results

The protocol was well tolerated in all participants. Ultrasound images were of poor quality in four older and two younger men and therefore all gastric emptying data were excluded from analyses for these participants.

7.5.1 Systolic blood pressure

Baseline SBP was higher in older than younger men (older vs younger: control, protein drink: 130 ± 3, 131 ± 3 vs 116 ± 3, 120 ± 4 mmHg; age main effect $P = 0.005$).

In older, but not younger men, there was a fall in SBP from baseline following the protein drink but not control. As the older men had higher baseline SBPs than younger men, absolute SBPs remained higher after control (older vs younger: 129 ± 3 vs 115 ± 3 mmHg, $P = 0.006$), but were similar after protein (123 ± 3 vs 118 ± 3 mmHg, $P = 0.27$), in older compared to younger men (AUC0–180min interaction

effect of age by drink $P = 0.010$). The greatest difference in the SBP response to treatments between the two age groups occurred in the third hour after drink consumption: there was a fall in SBP (cumulative change from baseline 120 to 180 min; interaction effect of age by drink $P = 0.015$) following the whey protein drink compared to control in the older (protein vs control: −11 ± 2 vs 0 ± 2 mmHg, $P = 0.001$), but not younger (2 ± 3 vs 1 ± 2 mmHg, $P = 0.84$), men. In older (protein vs control nadir: −23 ± 2 vs −15 ± 2 mmHg, $P = 0.001$), but not younger (−13 ± 2 vs −13 ± 2 mmHg, $P = 0.96$), men SBP maximum decrease was larger after the protein compared to control drink (interaction effect of age by drink $P = 0.033$; Figure 7.1). Furthermore, the nadir in SBP occurred later in the older than younger men, independent of the study drink (older vs younger: 114 ± 11 vs 62 ± 14 min; age main effect $P < 0.001$), and SBP was still apparently falling at the end of the study period (3 h after drink ingestion) in the older men.

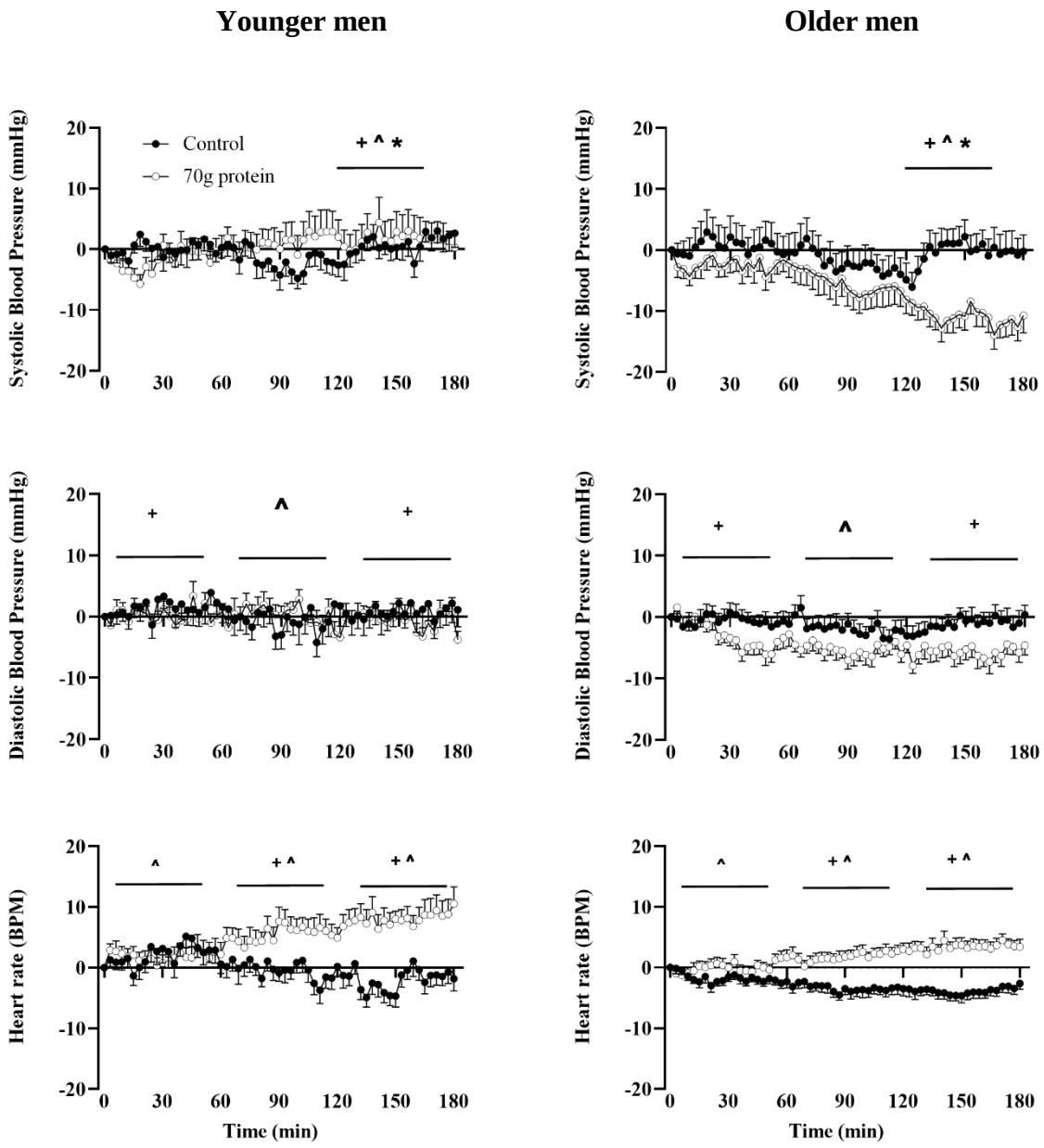

Figure 7.1: Mean (±standard error of mean [SEM]) cumulative changes from baseline of systolic and diastolic blood pressure (mmHg) and heart rate (beats per minute [BPM]) in 13 healthy younger and 19 healthy older men following control (~2 kcal), and protein (70 g whey protein, 280 kcal), drinks. Effects of drink condition, age, and the drink by age interaction effect were determined for cumulative changes from baseline between 0–60 min, 60–120 min, and 120–180 min by repeated-measures ANOVA with post hoc Bonferroni corrections. +$P < 0.05$, the

overall effect of drink condition; ^$P < 0.05$, the overall effect of age. *$P < 0.05$, post hoc study drink effect within the age group, control is significantly different from protein condition

7.5.2 Diastolic blood pressure

Baseline DBP was higher in older compared to younger men (older vs younger: control, protein drink: 76 ± 2, 76 ± 2 vs 67 ± 1, 67 ± 2 mmHg; age main effect, $P < 0.001$).

In older, but not younger men, there was a fall in DBP from baseline in response to the protein drink but not control. Accordingly, absolute DBPs were higher after control (older vs younger: 75 ± 2 vs 67 ± 2 mmHg, $P = 0.005$), but not after protein (71 ± 1 vs 67 ± 2 mmHg, p = 0.09), in older compared to younger men ($AUC_{0\text{-}180/min}$ interaction effect of age by drink, $P = 0.037$).

The maximum fall in DBP was greater in older than younger men, independent of the study drink (older vs younger: −14 ± 1 vs −11 ± 1 mmHg, age main effect, $P = 0.050$; Figure 7.1). Between 60 and 120 min, there was a further fall in DBP in the older (cumulative change from baseline 60 to 120 min: −4 ± 1 mmHg), but not younger (0 ± 2 mmHg), men, independent of study drink (age main effect, $P = 0.047$).

7.5.3 Heart rate

Baseline HR was not significantly different between age groups (older vs younger: control, protein drink: 59 ± 2, 59 ± 2 beats per minute (BPM) vs 65 ± 4, 65 ± 3 BPM;

age main effect, $P = 0.15$).

Independent of the study drink, older compared to younger men had lower absolute HRs (older vs younger: $AUC_{0\text{-}180/min}$ 58 ± 2 vs 66 ± 3 BPM; age main effect, $P = 0.013$). Regardless of the age group, absolute HR was higher after the protein drink compared to the control drink (control vs protein: 60 ± 2 vs 65 ± 2 BPM; drink main effect, $P < 0.001$).

HR increased less from baseline in older than younger men during the first (cumulative change from baseline 0 to 60 min: older vs younger: control: −2 ± 1 vs 2 ± 1 BPM, protein: 0 ± 1 vs 2 ± 1 BPM; age main effect, $P = 0.021$), second (60–120 min: control: −3 ± 1 vs 0 ± 1 BPM, protein: 2 ± 1 vs 6 ± 2 BPM); $P = 0.016$), and third (120–180 min: control: −4 ± 1 vs −2 ± 1 BPM, protein: 3 ± 1 vs 8 ± 2 BPM; $P = 0.029$) hour after drink consumption, independent of the study drink. The maximum increase in HR was less in older than younger men, independent of the study drink (older vs younger: 6 ± 1 vs 15 ± 3 BPM; age main effect, $P < 0.001$, Figure 7.1).

7.5.4 Postprandial hypotension

In response to the control drink, 26% of older (5/19) and 23% of younger (3/13) men experienced a ≥ 20 mm Hg decrease from baseline in SBP of at some time during the following 3 h. Following the definition of PPH as a decrease of ≥ 20 mm Hg sustained for ≥ 30 min in SBP after nutrient ingestion; 10% of older (2/19) and 0% of younger men had PPH after control drink ingestion.

In response to the whey protein drink, 58% older (11/19) and 23% of younger (3/13) men demonstrated a fall of ≥ 20 mm Hg in SBP at some time during the 3 h, and 10% of older (3/19) and 0% of younger men had PPH after whey protein drink ingestion. In two of those three older men, the onset of that fall was more than 120 min after whey drink consumption. In two additional older men, there was a fall of ≥ 20 mm Hg in the six BP measurements before the end of the study period (from T = 165 to 180 min after whey ingestion). As participants were only followed until T = 180 min, these two participants may have had a sustained decrease in SBP ≥ 20 mm Hg for ≥ 30 min.

7.5.5 Perceptions of light-headedness and drowsiness

There were no age, treatment, or interaction effects on AUC or cumulative changes from baseline for perceptions of light-headedness or drowsiness (Figure 7.2). Regardless of the age group, perceptions of drowsiness increased after administration of the protein drink (time main effect, $P = 0.031$), and peak drowsiness was higher after the protein compared to the control drink ($P = 0.011$).

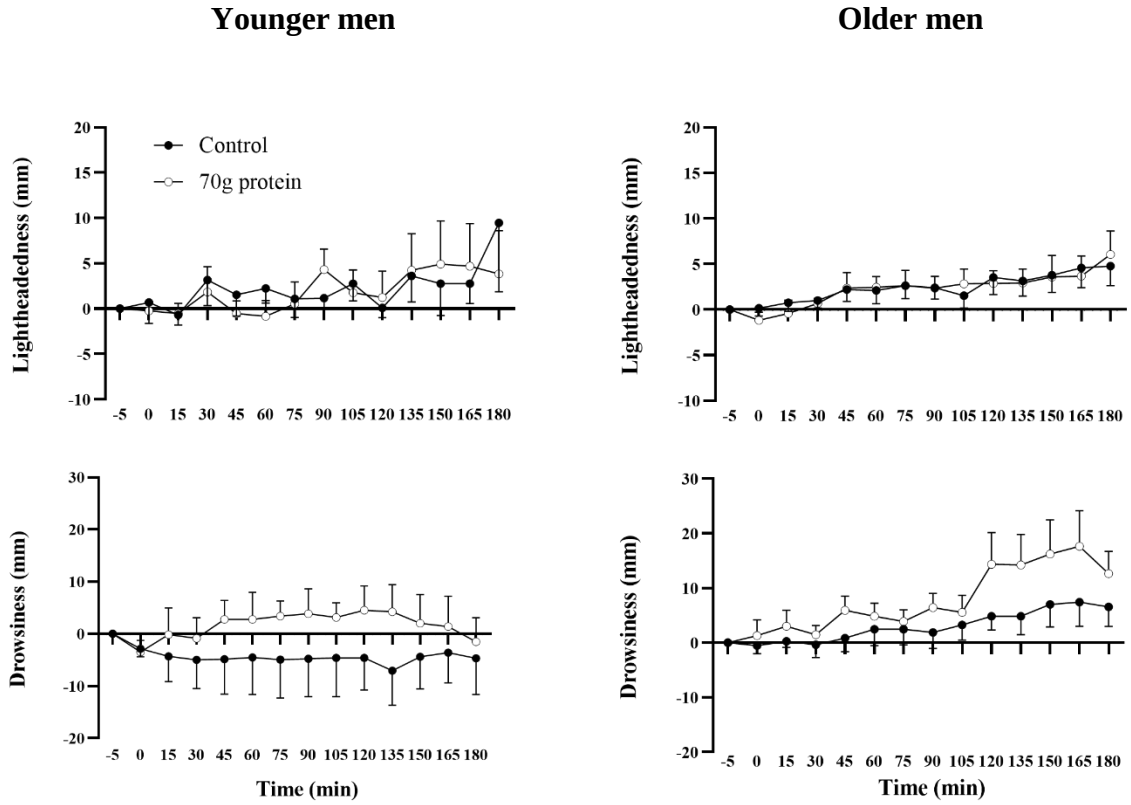

Figure 7.2 Mean (±standard error of mean [SEM]) cumulative changes from baseline for perceptions of light-headedness and drowsiness in 13 healthy younger and 19 healthy older men following control (~2 kcal), and protein (70 g whey protein, 280 kcal), drinks. Effects of age, time, and age by time interaction effect within the study drink conditions were determined by repeated-measures ANOVA. Drowsiness: the overall effect of time: $P = 0.031$. Data are mean (± standard error of mean [SEM]) [Color figure can be viewed at wileyonlinelibrary.com]

7.5.6 Gastric emptying

In older men, gastric emptying of the protein drink (T_{50}; older vs younger: 70 ± 6 min vs 58 ± 9 min, $P = 0.019$), but not control (12 ± 1 vs 12 ± 1 min, $P = 0.15$), was

slower, when compared to the younger men (interaction effect of age by study drink, $P = 0.027$; Figure 7.3).

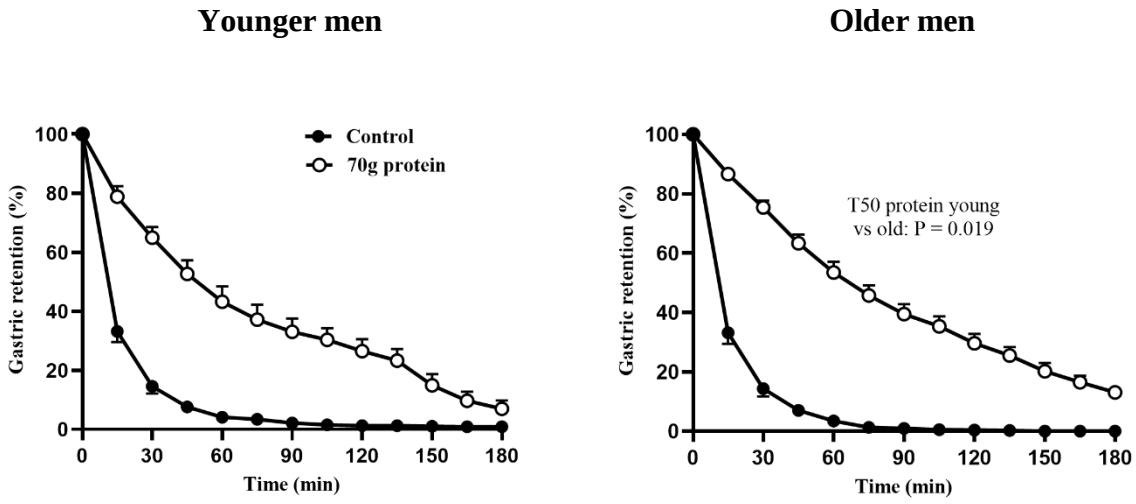

Figure 7.3: Mean (±standard error of mean [SEM]) gastric retention (%) in 11 healthy younger and 14 healthy older men following control (~2 kcal), and protein (70 g whey protein, 280 kcal), drinks. Effects of drink-condition, age, and the drink-by-age interaction effect were determined by repeated-measures ANOVA with post hoc Bonferroni corrections. Interaction effect of age by drink condition: $P = 0.027$ [Color figure can be viewed at wileyonlinelibrary.com]

7.6 Discussion

Our study has established that, in response to a 280 kcal (70 g) whey protein drink (450 ml) ingestion, healthy older men exhibit a substantial decrease in SBP and DBP, whereas younger men maintained their BP at a level comparable to that after an iso-volumic, non-caloric, drink. Furthermore, in the older participants, the fall in BP was sustained until at least 180 min after consumption of the drink. There was an increase in HR after the protein drink, but this increase in HR was greater in the younger than older men. The perception of drowsiness, but not light-headedness, was greater after

protein irrespective of age. The use of protein supplements to aid undernutrition in older people is increasing, and PPH is particularly common in institutionalized older patients, who are more likely to take nutritional supplements (97). It is certainly not appreciated that PPH is a potential outcome of the use of protein supplements in this group; with emergent increased rates of falls, syncope, and death (155). All our participants were seated, and orthostatic hypotension was not assessed. Posture likely affects postprandial BP (402) and it is possible that the lowering of BP that occurred in the older participants may have been caused partially by the upright seated position during the study.

Our observations also indicate that, in studies evaluating BP after protein administration, BP should be monitored for a minimum of 3 h. PPH is defined as a sustained fall in BP $\geq$ 20 mm Hg within 2 h of a meal (262). This definition is based on the outcome of studies using high-carbohydrate meals or glucose drinks to diagnose PPH, and our observations suggest that the definition should be modified to increase this time frame—at least after a high protein (i.e., more than 30 g) meal or supplement.

The current findings are consistent with our previous observations, which showed a decrease in BP in response to intraduodenal glucose infusion in older, but not younger, adults (396). We have also reported a more delayed hypotensive response after an intraduodenal infusion of protein compared to glucose in healthy older people (263). This difference may be attributed to differences in time for digestion of carbohydrates and protein (400) to stimulate an increase in superior mesenteric artery (SMA) blood flow response (401); that is, the fall in BP may be triggered by amino acids, the digestion products of protein. In the younger participants, the rise

in HR induced by the protein drink was greater than in the older participants, which is likely to contribute to the maintenance of BP despite a substantial increase in splanchnic blood flow in both age groups (396). The increase in splanchnic blood flow after the protein drink may be reflected by the increase in perceptions of drowsiness which occurs in both age groups. In contrast with our results, another study with a very similar study design, in which BP was measured every 5 min for 150 min after 75 g oral loads of glucose, whey protein, and cream, older hypertensive patients showed a fall in SBP and DBP after glucose, but not after fat and protein. Furthermore, HR increased after glucose and fat but was not affected by protein intake (173). In the current study, participants were seated throughout the study (i.e., 3 h), whereas the participants in the described study (173) were in a semi-recumbent position, reducing effects of potential orthostatic hypotension. In patients with autonomic failure, the fall in postprandial BP was also larger after carbohydrates and fat (403), and in healthy young participants, two iso-caloric loads containing 92 g of either carbohydrates or protein resulted in increased cardiovascular output and ventricular contractility after carbohydrates compared to protein (404). These studies may suggest that even though we reported a fall in BP after protein, this fall may not be as large as a fall in response to carbohydrates.

As shown in this study, gastric emptying of protein was slower in the older compared to younger participants (12, 15). Slower gastric emptying should, intuitively, be associated with increased, and more sustained, gastric distension which is known to attenuate the postprandial fall in BP (228). In older people, this may, in part, represent a compensatory mechanism to reduce the magnitude of a fall in BP after a meal.

This study has several potential limitations. The study represents a pooled data analysis from two separate studies, and whereas the methodology in terms of inclusion criteria, timeline, and drink composition were identical in both studies, there is the inherent potential for bias. We evaluated the effects of a relatively large protein dose (70 g), representative of a normal daily intake of protein— a meal typically contains 15–30 g of protein. Protein supplements used to manage undernutrition in older people typically contain 10–25 g protein, and therefore, use of these may not necessarily be associated with falls in BP as large as those observed in this study. We only studied whey protein, and therefore, the results may not apply to other protein sources. We only studied healthy men, and therefore, the results may not be translatable to women. Given that there was a fall in BP in response to whey protein in healthy older men, it is likely that the fall would be greater in populations at risk of PPH (e.g., diabetic, hospitalized and geriatric patients, and patients with neurological disorders). This warrants investigation as these are patient groups that are most likely to benefit from increased protein intake through the use of protein supplements. We did not measure autonomic nerve function or orthostatic BP, and therefore we were unable to determine whether nerve dysfunction may be an underlying factor in the age-related results on BP and gastric emptying reported in this study. We acknowledge that the inclusion of participants with hypertension may have affected the BP responses in older adults.

In summary, healthy older men exhibited a sustained fall in systolic and diastolic BP, despite an increase in HR, following ingestion of 70 g whey protein, whereas in younger men the increase in HR was greater and there was no change in BP. In future research, it should be investigated whether the substitution of carbohydrates and/or fat by protein slows gastric emptying, and thereby attenuates the postprandial fall in

BP in older people. In conclusion, these observations suggest that postprandial BP after protein supplements or high protein meals may need to be monitored in older people, particularly those at particular risk of PPH.

CHAPTER 8: BLOOD PRESSURE AND HEART RATE RESPONSES FOLLOWING DIETARY PROTEIN INTAKE IN OLDER MEN

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Overall percentage	75%		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	October 2022

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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i) the candidate's stated contribution to the publication is accurate (as detailed above);
ii) permission is granted for the candidate in include the publication in the thesis; and
iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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8.1 Abstract

Postprandial hypotension (PPH) occurs frequently in older people > 65 years old. Protein-rich supplements, particularly whey protein (WP), are increasingly used by older people for various health benefits. We have reported that 70 g WP drinks cause significant, and in some cases marked, falls in blood pressure (BP) in older men. The effects of lower, more widely used, doses (~30 g) on systolic (SBP) and diastolic (DBP) blood pressure and heart rate (HR) are not known. In a randomized order, eight older men (age: 72 ± 1 years; BMI: 25±1 kg/m^2) after overnight fast ingested a drink containing (i) a non-caloric control (~2 kcal), (ii) 30 g of whey protein (120 kcal;'WP30'), or (iii) 70 g of whey protein (280 kcal; 'WP70'). The BP and HR were measured in this pilot study with an automated device before and at 3-min intervals for 180 min following drink ingestion. Drink condition effects were determined by repeated-measures ANOVA. The SBP decreased after both WP drinks compared to the control (P = 0.016), particularly between 120 and 180 min, with no difference in the effects of WP30 and WP70. The SBP decreased by ≥ 20 mm Hg in more than 50% of people after both WP drinks (WP30: 63%; WP70: 75%) compared to 38% after the control. The maximum fall in the SBP occurred during the third hour, with the nadir occurring latest after WP70. The DBP decreased non-significantly by several mmHg more after the WP drinks than after the control. The maximum HR increases occurred during the third hour, with the greatest increase after WP70. The SBP decreased after both WP drinks compared to the control, with the effects most evident between 120 and 180 min. Accordingly, ingestion of even relatively modest protein loads in older men has the potential to cause PPH.

8.2 Introduction

Human ageing is associated with reductions in skeletal muscle mass and strength, which are associated with increased rates of falls and nursing home admissions, as well as other adverse outcomes (111, 306, 310-315). Falls may also be caused by dizziness and/or syncope due to postprandial hypotension (PPH), a substantial reduction in blood pressure (BP) caused by ingestion of nutrients (261, 392), which has been defined as a decrease in systolic blood pressure (SBP) of ≥ 20 mm Hg for ≥ 30 min within 120 min of consumption of a meal (262). PPH occurs frequently in older people and is associated with increased morbidity and mortality (262).

A strategy increasingly adopted to prevent or treat under-nutrition, weight loss, and sarcopenia in older people is to increase consumption of high-energy, protein- rich supplements (97). These supplements are often rich in whey protein (15), a component of milk, which is high in essential amino acids, particularly leucine, which are rapidly digested to increase postprandial amino acid availability and stimulate muscle protein accretion (7, 319).

Oral ingestion of whey protein or whey-protein-rich supplements has the potential to reduce the BP to a degree that is harmful in some older people, predisposing to falls and other adverse effects. Compared to younger adults, older people exhibit greater decreases in the BP after meals (405). Ingestion of all three macronutrients (carbohydrate, protein, fat) decreases the post-prandial BP in older people (263, 264). Carbohydrate and protein lower the BP to a similar degree, although the fall in the BP may occur earlier after carbohydrate ingestion than protein ingestion (263). The hypotensive effects of protein are likely to be mediated by amino acids produced by

digestion, explaining the latency and time of onset of changes in the BP and HR after protein loads. We have recently reported that ingestion of a 70 g whey protein drink is associated with a substantial decrease in the BP in healthy older men; the majority of older men studied had a decrease in the systolic BP (SBP) of 20 mm Hg or more, with the greatest reduction occurring 2–3 h after drink ingestion (264).

Overall, 70 g of protein is more than most older people ingest at one time; amounts of ~30 g are probably sufficient to induce muscle synthesis in older people, particularly if ingested twice a day (63). It is not clear whether the hypotensive effects of whey protein drinks are dose dependent in older people and whether whey doses less than 70 g also cause a substantial fall in the BP. We have reported that ingestion of a whey protein drink as a preload in combination with guar in a much lower dose of 16.4 g has no effects on the BP in healthy older people for up to 2 h (406). Two hours may not, however, have been long enough to detect the maximum hypotensive effects of the drink in that study.

This study compared the effects over 3 hours of 30 g and 70 g whey protein drinks on the BP and heart rate (HR) in older men. This pilot study is a subset (n = 8 males) of a larger study and represents an analysis of secondary outcomes measured in men (BP and HR) in a previously published study (14) that described the effect of orally ingested whey protein on energy intake, gastric emptying, and plasma gut-hormone concentrations in older men (n = 8) and women (n = 8).

8.3 Materials and methods

8.3.1 Participants

Eight older (mean age: 73 ± 1 years; body weight: 77 ± 4 kg; body mass index (BMI): 26 ± 1 kg/m^2) men were recruited by advertisement.

Exclusion criteria included alcohol intake of > 2 standard drinks on > 5 days per week; smoking; intake of any illicit substance; use of prescribed or non-prescribed medications that may affect appetite, body weight, gastrointestinal function, or energy metabolism; being vegetarian; known lactose intolerance or food allergies; epilepsy; gallbladder, pancreatic, cardiovascular, or respiratory diseases; significant gastrointestinal symptoms (abdominal pain, gastroesophageal reflux, diarrhea, or constipation) or surgery; any other illness deemed significant by the investigator; low plasma ferritin levels; donation of blood in the 12 weeks prior to the study days; undernourished condition (score < 24 on the Mini Nutritional Assessment (407); or depression (score ≥ 11 on the Geriatric Depression Questionnaire (327), impaired cognitive function (score < 25 on Mini Mental State (326), and inability to comprehend the study.

The Royal Adelaide Hospital Human Research Ethics Committee approved the proto-col, which was conducted in accordance with the Declaration of Helsinki. The study was registered with the Australian New Zealand Clinical Trial Registry (www.anzctr.org.au (accessed on 15 October 2021; registration number ACTRN12612000941864). All participants provided written informed consent prior to their study inclusion.

8.3.2 Protocol

The protocol was similar to that of our previous study comparing older and younger men (15). Each participant was studied on 3 occasions, separated by 3–14 days, to determine the effects of drinks (~450 mL) containing (i) a non-caloric control (~2 kcal; 'C'), (ii) 30 go f whey protein (120 kcal; 'WP30'), or (iii) 70 g of whey protein (280 kcal; 'WP70') on the SBP, DBP, and HR in a randomized (using the method of randomly permuted blocks;www.randomization.com; accessed on 3 August 2018), double-blind, cross-over design.

The drinks were prepared by dissolving whey protein isolate (Fonterra Co-Operative Group Ltd., Palmerston North, New Zealand) in varying volumes of demineralized water and diet lime cordial (Bickford's Australia Pty Ltd., Salisbury South, SA, Australia), to achieve the desired composition, by a research officer who was not involved in the data analysis on the morning of the study day. The drinks were matched for taste and served in a covered cup so that both the participant and the investigator were blinded to the treatment.

Participants consumed a standardized meal (beef lasagne (McCain Foods Pty Ltd., Wen-douree, Victoria, Australia), ~591 kcal) on the night before each study day at ~1900 h. They were instructed to fast overnight ~12 h, taking no solids and liquids except water.

8.3.3 Measurements

Participants were seated in an upright position on a wooden chair with arms, where they remained throughout the study day. They were asked to sit quietly for at least 15 min, and the BP was measured 3 times at 3-min intervals using an automatic BP-measuring device (DINAMAP Pro Care 100; GE Medical Systems, Milwaukee, WI, USA). Participants were then instructed to consume the drink within 2 min, and the BP and HR were measured every3 min for a further 180 min. When SBP measurements between consecutive samples varied by ≥ 10 mm Hg, repeat measurements were taken.

8.4 Data and statistical analysis

Responses to the drinks were calculated for the first, second, and third hour following drink ingestion as the mean of individual measurements (mean 0–60 min, mean60–120 min, mean 120–180 min, respectively). All data are presented as mean values ± standard error of the mean (SEM), and statistical significance was accepted at $P < 0.05$. Statistical analyses were performed using SPSS software (version 24; IBM, Armonk, NY, USA). The maximum decrease from baseline was calculated as the difference between the minimum value and baseline and the time of nadir that was analyzed for the SBP, DBP, and HR.

The baseline blood pressure was calculated as an average of −9, −6, and −3 min readings. T = 0 min refers to the point immediately after drink consumption. Mixed effects model analysis including treatment as a fixed effect, and an unstructured covariance structure was used to determine the effect of protein load on the SBP,

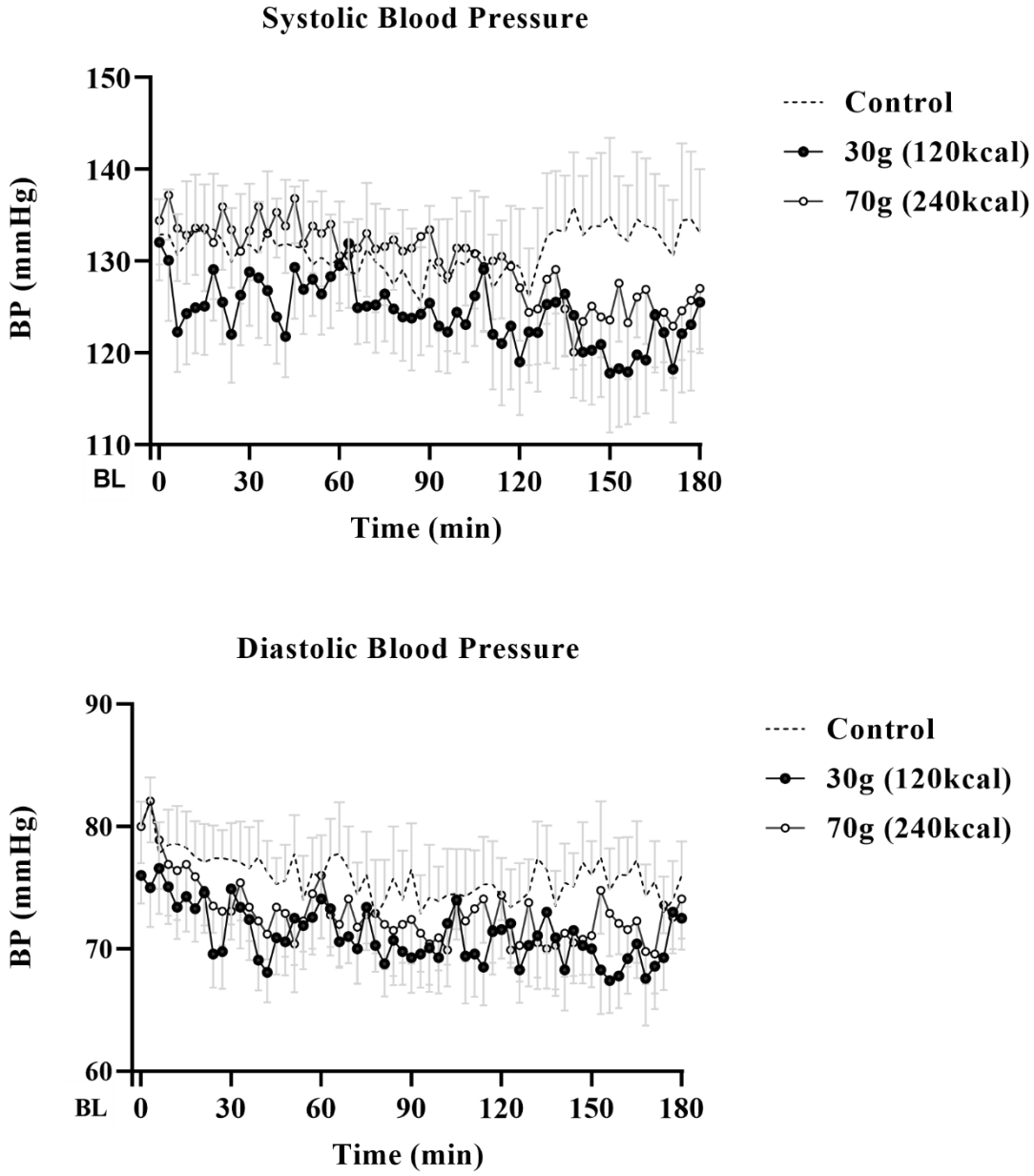
DBP, and HR baseline levels and each of the three mean outcomes, along with the maximum decrease and the time to nadir. When significant treatment effects were present, Bonferroni corrected post hoc tests were performed to determine which specific drink conditions differed.

8.5 Results

The study protocol was well tolerated by all participants.

8.5.1 Systolic blood pressure (SBP)

The baseline SBP was comparable on the 3 study days (C: 133 ± 4 mm Hg; WP30: 132 ± 4 mm Hg; WP70: 134 ± 5 mm Hg; $P = 0.95$). During the first 2 hours, SBPs after all drinks were not significantly different (mean 0–60 min, mean 60–120 min; C: 132 ± 6, 129 ± 6 mm Hg, respectively; WP30: 127 ± 4, 124 ± 5 mm Hg, respectively; WP70: 134 ± 5, 131 ± 6 mm Hg, respectively; $P = 0.12$, $P = 0.09$, respectively). In contrast, between 120 and180 min, the SBP was lower after both whey protein drinks than after the control drink ($P = 0.016$; C vs. WP30 $P = 0.02$; C vs. WP70, $P = 0.20$), with mean SBP changes from baseline of −10 mmHg after both whey protein drinks during the third hour compared to 0 mm Hg after the control drink (mean 120–180 min; C: 133 ± 7 mm Hg; WP30: 122 ± 6 mm Hg; WP70: 125 ± 5 mm Hg; Figure 8.1A, Table 8.1).


Systolic Blood Pressure
150
140
130
120
110
BP (mmHg)
BL
0
30
60
90
120
150
180
Time (min)
Control
30g (120kcal)
70g (240kcal)
Diastolic Blood Pressure
90
80
70
60
BP (mmHg)
BL
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Time (min)
Control
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70g (240kcal)

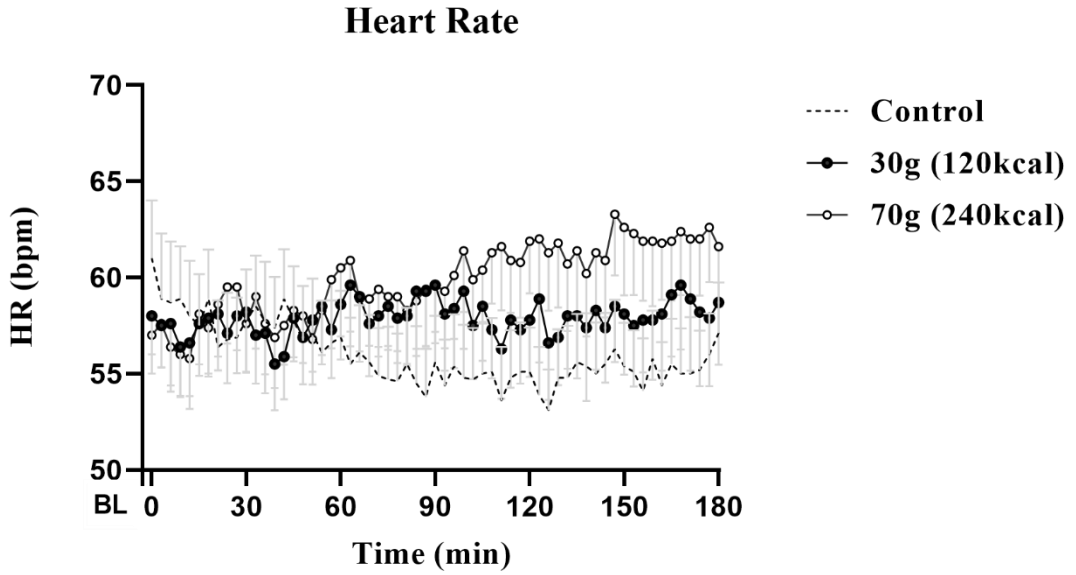

Figure 8.1: Mean (±SEM) systolic (A) and diastolic (B) blood pressure (mmHg) and heart rate (bpm) (C) following ingestion of drinks containing (i) flavored water (control, ~2 kcal) or (ii) 30 g (120 kcal) of whey protein (WP30) or (iii) 70 g (280 kcal) of whey protein (WP70) in older (n = 8) men. The base-line blood pressure represented as 'BL' in the figures was calculated as an average of measurements at −9, −6, and −3 min. T = 0 min refers to the point immediately after drink consumption. Drink condition effects were determined by using mixed model analysis. SBPs were lower after the whey protein drinks when compared to the control drink during the third hour ($p < 0.05$). The mean fall in the DBP was greater after 30 g and 70 g of whey protein drinks than after the control drink. The HR was higher after the whey protein drinks when compared to the control drink during the second ($P = 0.007$) and third ($P = 0.005$) hours, with the greatest increase after WP70 ($P = 0.002$).

Table 8.1: Blood pressure and heart rate after drink ingestion

	Control	WP30	WP70	*P*
SBP (mmHg)				
Mean 0-60 min	132 ± 6	127 ± 4	134 ± 5	0.123
Mean 60-120 min	129 ± 6	124 ± 5	131 ± 6	0.092
Mean 120-180 min	133 ± 7	122 ± 6	125 ± 5	0.016
Max change from baseline	-15 ± 2	-24 ± 2	-25 ± 4	0.020
Time to Nadir(min)	90 ± 8	111 ± 22	136 ± 11	0.084
DBP (mmHg)				
Mean 0-60 min	77 ± 7	73 ± 2	75 ± 3	0.235
Mean 60-120 min	75 ± 3	71 ± 3	72 ± 3	0.414
Mean 120-180 min	75±3	70±23	72±3	0.202
Max change from baseline	-13 ± 2	15 ± 1	-17 ± 2	0.13
Time to nadir (min)	89 ± 19	98 ± 19	108 ± 11	0.74
HR (bpm)				
Mean 0-60 min	55 ± 3	58 ± 3	59 ± 3	0.057
Mean 60-120 min	53 ± 2	60 ± 3	60 ± 3	0.007
Mean 120-180 min	53 ± 2	58 ± 3	62 ± 3	0.005
Max change from baseline	2 ± 1	6 ± 1	10 ± 2	0.002
Time to nadir (min)	71 ± 25	112 ± 17	120 ± 17	0.181

Mean (± SEM) systolic blood pressure (SBP, mm Hg), diastolic blood pressure (DBP, mm Hg) and heart rate (beats per minute, bpm) following ingestion of drinks containing (i) flavored water (control, ~2 kcal) or (ii) 30g (120 kcal) whey protein (WP30) (iii) 70g (280 kcal) whey protein (WP70) in older (n = 8) men. P value

treatment effect of mixed model analysis.

Maximum decreases in the SBP from baseline (C: −15 ± 2 mm Hg; WP30: −24 ± 2 mm Hg; WP70: −25 ± 4 mm Hg; $P = 0.02$) occurred from ~2 h after drink ingestion onward, with the nadir (C: 118 ± 5 mm Hg; WP30: 108 ± 5 mm Hg; WP70: 110 ± 4 mm Hg) occurring latest after the 70 g whey protein drink (C: 90 ± 8 min; WP30: 111 ± 22 min; WP70: 136 ± 11 min; $P = 0.08$). The SBP decreased by ≥ 20 mm Hg in more than 50% of participants after both whey protein drinks (C: 3/8 (38%); WP30: 6/8 (75%); WP70: 5/8 (63%)).

8.5.2 Diastolic blood pressure (DBP)

The baseline DBP was not different on the study days (C: 80 ± 2 mm Hg; WP30: 76 ± 2 mm Hg; WP70: 80 ± 3 mm Hg; $P = 0.35$). The mean fall in the DBP was greater after 30 g and 70 g whey protein drinks than after the control drink, which did not achieve statistical significance during any period. Mean decreases in the DBP from baseline during the third hour were −6/ −8 mm Hg after WP30/WP70 compared to −4 mmHg after the control drink (mean 0–60 min, mean 60–120 min, mean 120–180 min; C: 77 ± 7, 75 ± 3, 75 ± 3 mm Hg, respectively; WP30: 73 ± 2, 71 ± 3, 70 ± 3 mm Hg, respectively; WP70: 75 ± 3, 72 ± 3, 72 ± 3 mm Hg, respectively; $P = 0.23$, $P = 0.41$, $P = 0.20$, respectively; Figure 8.1B, Table 8.1). Maximal changes in the DBP from baseline (C: −13 ± 2 mm Hg; WP30: −15±1 mm Hg; WP70: −17 ± 2 mmHg; $P = 0.13$) occurred ~1.5–2 h after drink ingestion, with the nadir occurring latest after the 70 g whey protein drink (C: 89 ± 19 min; WP30: 98 ± 19 min; WP70: 108 ± 11 min; $P = 0.74$).

8.5.3 Heart rate (HR)

The baseline HR was not different between the study days (C: 61 ± 3 bpm; WP30: 58 ± 2 bpm; WP70: 57 ± 2 bpm; $P = 0.56$). The HR was higher after the whey protein drinks when compared to the control drink during the last 2 hours (mean 0–60 min, mean 60–120 min, mean 120–180 min; C: 55 ± 3, 55 ± 2, 55 ± 2 bpm, respectively; WP30: 58 ± 3, 60 ± 3, 58 ± 3 bpm, respectively; WP70: 59 ± 3, 60 ± 3, 62 ± 3 bpm, respectively; $P = 0.05$, $P = 0.007$, $P = 0.005$, respectively), with the greatest increase after WP70 and during the third hour (change from baseline to 120–180 min: C: −5 ± 1 bpm; WP30: 0 ± 1 bpm; WP70: +5 ± 1 bpm; $P < 0.05$; Figure 8.1C, Table 8.1). The maximal increase in the HR from baseline (C: 2 ± 1 bpm; WP30: 6 ± 1 bpm; WP70: 10 ± 2 bpm; $P = 0.002$) occurred ~1.5–2 h after drink ingestion (C: 71 ± 25 min; WP30: 112 ± 17 min; WP70: 120 ± 17 min; $P = 0.181$).

8.6 Discussion

We demonstrated that healthy older men exhibit a decrease in the SBP after ingestion of both 30 g and 70 g whey protein drinks, which was similar in degree after both whey protein drinks and the greatest between 120 and 180 min after their ingestion. Previously, we reported that protein, when administered directly into the duodenum and thereby bypassing gastric effects, lowers blood pressure comparably to glucose and fat, with the onset of the hypotensive response being earlier after glucose ingestion (263). In addition, the type of protein may affect lowering of blood pressure, and certain proteins (e.g., in ancient wheat) have an effect on endothelial reactivity (408).

Protein supplements, usually in drink form, are increasingly administered to older people, particularly the institutionalized elderly, who are at a high risk of postprandial hypotension. While we have shown that protein loads of 70 g can lead to substantial falls in the SBP (264), this amount of protein is greater than usually taken at one time by older people. Lower doses are more likely to be used in supplements for older people, and there is evidence that ~30 g doses, once or twice daily, are likely to be beneficial for weight, nutrition, and muscle preservation (63). Our observation suggests that care may need to be taken to monitor and prevent the hypotensive effects of protein supplements in susceptible older people, even when used in doses as low as 30 g and for 3 hours or even longer after protein supplement ingestion; our observations finished at 3 h when the SBP was possibly still reduced. The heart rate (HR) increased after both whey protein drinks, with the greatest increase after the 70 g drink. These dose-responsive HR increases help to maintain cardiac output and hence the BP in the face of diversion of blood to the gut to aid protein absorption and digestion. The greater compensatory increase in the HR (and thus cardiac output) after the 70 g than the 30 g drink is likely to be a factor in the two doses lowering the BP to a similar degree. This might also suggest that if the compensatory HR increases after protein (or other nutrient) ingestion are impaired in older people, the decrease in BP after nutrient ingestion will be greater and potentially more likely to lead to falls and other adverse effects. This is supported by our previous finding that in young men, there were greater increases in the HR and lesser decreases in the SBP than in older men after 70 g whey protein drinks (264). Older people taking medications such as beta-blockers, which limit the heart rate and heart rate responses, or with cardiac conditions leading to bradycardia may be at even greater risk of post-protein BP falls. Following a standard mixed macronutrient breakfast meal, 16 participants over 75 years old showed a significant fall in blood

pressure, which may, in some less robust elderly persons, contribute to falls, while the younger control group did not show a significant decrease in postprandial blood pressure (159).

Our study has some limitations. The study was only conducted in healthy men, with a relatively small number of participants. The results may not be translatable to women. While our observation period of 3 hours was longer than in most previous studies, the BP was possibly still decreasing and the HR was still higher than on the control day 3 h after the protein drinks. A longer study duration would have helped determine the full time course of the effects of protein drinks. Since only whey protein was studied, the observation cannot be applied to other protein sources. A greater protein-induced BP fall than observed in this study might occur in older people at greater risk of postprandial hypotension than our participants, for example, institutionalized, frail older people. A possible safety issue for older people adopting a program of post-protein supplement or postprandial exercise might be excessive BP drops leading to falls.

Our result suggest that if the intention is to give an older person a nutritional supplement drink containing 30–70 g of whey protein to preserve or even enhance muscle mass and function, excessive post-protein BP is a possibility, particularly in those most at risk. Consideration should be given to monitoring for this and/or advising measures (such as care when standing) to reduce the harmful effects of excessive postprandial BP decreases. It would also be appropriate in future studies to examine the effects on the BP in older people of combining protein and other nutrients with exercise.

8.7 Conclusions

The SBP decreased after both WP drinks but was not dose dependent compared to the control, with the effects most evident between 120 and 180 min. Accordingly, ingestion of even relatively modest protein loads in older men has the potential to cause PPH.

CHAPTER 9: ACUTE EFFECTS OF WHEY PROTEIN, ALONE AND MIXED WITH OTHER MACRONUTRIENTS, ON BLOOD PRESSURE AND HEART RATE IN OLDER MEN

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By signing the Statement of Authorship, each author certifies that:

i) the candidate's stated contribution to the publication is accurate (as detailed above);
ii) permission is granted for the candidate in include the publication in the thesis; and
iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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9.1 Abstract

Background: Caloric supplements are increasingly used by older people, aiming to increase their daily protein intake. These high caloric drinks, rich in glucose and whey-protein in particular, may result in potential harmful decreases in blood pressure (BP). The effect of ingesting whey-protein with glucose and fat on BP is unknown. It has also been assumed that the maximum fall in systolic blood pressure occurs within 2 h of a meal.

Methods: This study aimed to determine in older men, the effects of whey-protein, alone and mixed with other macronutrients, on systolic (SBP) and diastolic (DBP) blood pressure and heart rate (HR) in older men for 3 h. Thirteen older men (age 75 ± 2 yrs; body mass index (BMI) 25.6 ± 0.6 kg/m^2) ingested a drink on separate study days: (i) 70 g whey-protein (P_{280}); (ii) 14 g whey-protein, 28 g carbohydrate, 12.4 g fat (M_{280}); (iii) 70 g whey-protein, 28 g carbohydrate, 12.4 g fat (M_{504}); or (iv) a non-caloric control drink (C).

Results: SBP decreased after all three nutrient drinks compared to the C, with the greatest reduction after the M_{504} drink (P = 0.008). Maximal decreases in SBP (C: -14 ± 2 mm Hg, P_{280}: -22 ± 2 mm Hg, M_{280}: -22 ± 4 mm Hg, M_{504}: -24 ± 3 mm Hg) occurred about 2 h after drink ingestion and this fall was sustained thereafter (120-180 min: P_{280} and M_{504} vs. C $P < 0.05$). Maximum DBP decreases and HR increases occurred after M_{504}, with no differences between the effects of the P_{280} and M_{280} drinks.

Conclusions: The effects of whey-protein containing drinks to lower BP and

increase HR appear to be primarily dependent on their energy content rather than macronutrient composition and may persist for at least 3 h after ingestion. Pure whey-protein drinks may represent the best approach to maximize protein intake without increasing the potential for deleterious BP falls in older people.

9.2 Introduction

Older people are increasingly encouraged to take high protein nutritional supplements to reduce the age-associated loss of muscle mass and function (97). Whey protein is often part of these supplements, given that it is high inessential amino acids and appears to be effective in stimulating muscle protein formation (7).

Ingestion of nutrients can lead to postprandial reductions in blood pressure (BP), in older people (even when apparently healthy) (227, 396), in part, caused by postprandial diversion of blood to the gut, which can lead to syncope, falls and, in some cases, stroke or death (262, 409, 410). This so-called postprandial hypotension (PPH) has been defined as a fall in systolic blood pressure (SBP) greater than 20 mm Hg during the 2 h following nutrient ingestion i.e. it is assumed that the maximum fall in SBP occurs within 2 h. We have recently reported, in a cohort of healthy older men, that ingestion of a 70 g (280 kcal) whey-protein drink decreased SBP substantially when compared to a non-caloric control drink (264). In the majority of men in that study magnitude of the SBP decrease was greater than 20 mmHg after the 70 g whey protein drink (11/19 compared to 5/19 after the control drink) (262). Furthermore, the hypotensive effect of a whey protein drink was prolonged, with a sustained reduction in SBP being evident at 3 h after ingestion. It has been suggested that hypertensive men may be of particular risk of blood pressure falls following food intake (411). The current study aimed to determine in older men the effects of whey protein, when ingested in a lower quantity but with carbohydrate and fat as occurs frequently in a "real world" setting, on blood pressure and heart rate for 3 h. We hypothesized that the hypotensive effects of whey protein containing drinks would be dependent on the energy rather than protein content of the drink and often persist

for more than 2 h.

9.3 Materials and methods

9.3.1 Participants

Thirteen older men were recruited by advertisement. Participant characteristics are detailed in Table 9.1

Table 9.1: Participant characteristics

Age (years)	75 ± 2
Height (m)	1.75 ± 0.01
Weight (kg)	79 ± 2
BMI (kg/m^2)	25.6 ± 0.6

Mean and standard error of mean of 13 older men

Exclusion criteria were smoking; being vegetarian; alcohol intake of > 2 standard drinks on > 5 days per week; use of prescribed or non-prescribed medications which may affect appetite, body weight, gastrointestinal function or energy metabolism; intake of any illicit substance; known lactose intolerance or food allergy(s); epilepsy; gallbladder, pancreatic, cardiovascular or respiratory diseases; significant gastrointestinal symptoms including as abdominal pain, gastroesophageal reflux, diarrhea, or constipation or surgery; any other illness deemed significant by the investigator; low levels of plasma ferritin; blood donation in the previous 12 weeks; undernutrition (score < 24 on the Mini Nutritional Assessment (407); depression (score ≥ 11 on the Geriatric Depression Questionnaire (327); impaired cognitive function (score < 25 on Mini Mental State (326); or inability to comprehend the study. Anti-hypertensive medication were taken by four older men (anti-arrhythmic

n = 1; angiotensin converting enzyme inhibitor, n = 1; beta blockers, n = 1; angiotensin receptor blockers n = 1). Participants were instructed to not take medication on the morning of their study visit.

The study was conducted in accordance with the Declaration of Helsinki and Royal Adelaide Hospital Human Research Ethics Committee approved the protocol. The study was registered with the Australian New Zealand Clinical Trial Registry (www. anzctr. org. au, registration number ACTRN12614000846628). All participants provided written informed consent prior to their study inclusion.

9.3.2 Protocol

Each participant was studied on four occasions in a randomised, double-blind, placebo-controlled design (using randomly permuted blocks; www. randomization. com), separated by 3–14 days, to determine the effects of drinks (~ 450 mL) containing either: (i) 70 g whey protein (280 kcal; 'P_{280}'); (ii) 14 g whey protein, 28 g carbohydrate, 12.4 g fat (280 kcal; 'M_{280}'); (iii) 70 g whey protein, 28 g carbohydrate, 12.4 g fat (504 kcal; 'M_{504}'); or (iv) an iso-palatable control drink (~ 2 kcal; 'control') on SBP, DBP and heart rate (HR). The BP and HR data are secondary outcomes of a published study which included results relating to energy intake, appetite, gastric emptying and plasma gut hormone concentrations (16). Sample size and power calculations for the original study were based on the primary outcomes of energy intake and gastric emptying (16). The drinks were prepared by homogenizing olive oil (Bertolli Australia Pty Ltd., Unilever Australasia, Sydney, NSW, Australia) and dissolving whey protein isolate (Fonterra Co-Operative Group Ltd., Palmerston North, New Zealand) and dextrose, in varying volumes of demineralized water and

diet lime cordial (Bickford's Australia Pty Ltd., Salisbury South, SA, Australia), to achieve the desired composition, on the morning of the study day, by a research officer, who was not involved in the data analysis. The drinks were stirred continuously at low speed on a stirring plate to ensure even mixing, were matched for taste and served in a covered cup. Both the investigator and the participant were blinded to the drink condition.

Participants were provided with a standardized meal [beef lasagne (McCain Foods Pty Ltd, Wendouree, Victoria VIC), Australia), ~591 kcal] to consume on the night before each study day at ~1900 h. They were instructed to fast overnight ~ 12 h from solids and liquids except water, and to refrain from strenuous physical activity until they attended the laboratory at ~0830 h.. The recruitment of participants started in March 2014, and the study was completed in December 2014.

9.3.3 Measurements

On arrival to the clinical research facility lab, level 4 at Adelaide Health and Medical Sciences, The University of Adelaide, Australia, participants were seated in an upright position where they remained throughout the study. An intravenous cannula was inserted for blood sampling. Participants sat quietly for 15 min then had 3 baseline measures of BP (Dinamap machine) and heart rate at 3-min intervals before drinking the test drink within 2 min. BP and HR were measured every 3 min until t = 180 min. Participants did not receive any other drink or food throughout the study.

9.4 Data and statistical analysis

Statistical analyses were performed using SPSS software (version 24; IBM, Armonk, NY). Drink-condition effects (control, P_{280}, M_{280}, M_{504}) were determined by using two way repeated-measures ANOVA. Significant effects were followed by Bonferroni corrected post-hoc tests to determine which specific drink conditions were different. Statistical significance was accepted at $P < 0.05$. All data are presented as means ± SEMs. Baseline blood pressure represented as BL in the figures was calculated as an average of -9, -6 and -3 min readings. T = 0 min refers to the point immediately after drink consumption.

9.5 Results

The study protocol was well tolerated by all participants. No participant reported symptoms of dizziness, faintness or any other adverse events during the study.

9.5.1 Systolic blood pressure (SBP)

Baseline SBP values were not different on the four study days (Mean control: 128 ± 3 mmHg, P_{280}: 129 ± 3 mm Hg, M_{280}: 130 ± 5 mm Hg, M_{504} 127 ± 4 mm Hg, P = 0.95). SBP did not change over the three hours after the control drink. SBP was lower after all three nutrient drinks compared to the control drink, particularly during the second (60-120 min P = 0.018) and third (120-180 min P = 0.022) hours, with the greatest reduction after the M_{504} drink (P = 0.008) (Figure 9.1). SBP following M_{504} ingestion was lower when compared to the P_{280} drink between 0–60 min (P = 0.044). There was no significant difference between SBP readings after the P_{280} and M_{280} drinks ($P > 0.05$). Following drink ingestion, a decrease in SBP > 20 mm Hg occurred at some time in 3/13 for control, 7/13 for P280, 6/13 for M280, and 9/13 for M_{504}.

Maximal SBP decreases from baseline (control: -14 ± 2 mmHg, P_{280}: -22 ± 2 mmHg, M_{280}: -22 ± 4 mmHg, M_{504} -24 ± 3 mm Hg; $P = 0.11$) occurred about two hours after the drinks (time baseline to nadir SBP: control: 99 ± 16 min, P_{280}: 119 ± 13 min, M_{280}: 116 ± 12 min, M_{504}:119 ± 15 min; $P = 0.86$) and was sustained thereafter following the nutrient drinks (average 120-180 min control: 128 ± 3 mmHg, P_{280}: 118 ± 2 mm Hg, M_{280}: 120 ± 4 mm Hg, M_{504}: 114 ± 3 mm Hg).

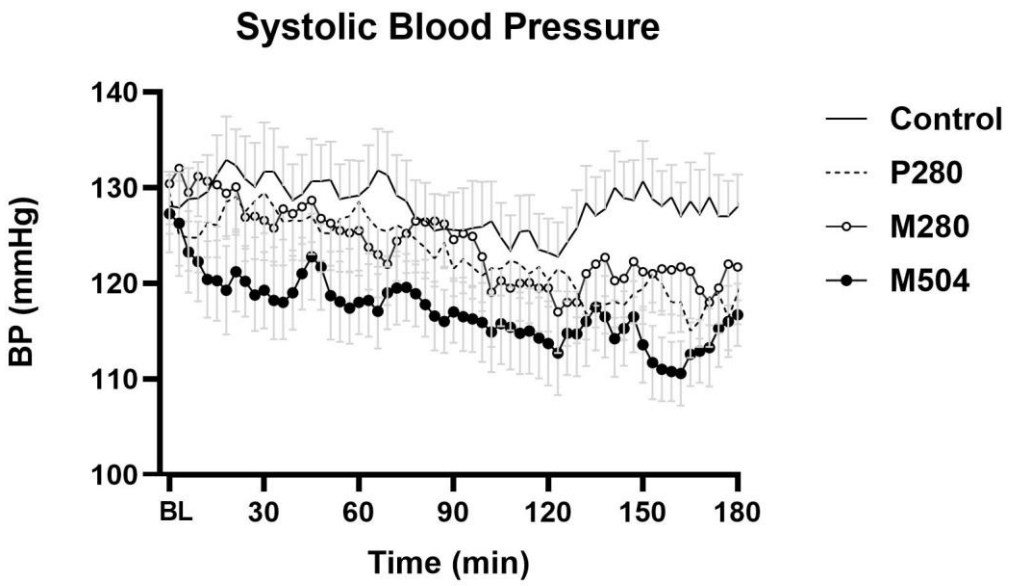

Figure 9.1: Mean (± SEM) systolic blood pressure (SBP; mmHg) following drink ingestion containing (i) flavored water (control, ~ 2 kcal) or (ii) 70 g whey protein (280 kcal; 'P_{280}'); (iii) 14 g whey protein, 28 g carbohydrate, 12.4 g fat (280 kcal; 'M_{280}'); (iv) 70 g protein, 28 g carbohydrate, 12.4 g fat (504 kcal; 'M_{504}') in older (n = 13) men. Drink-condition effects were determined by using repeated-measures ANOVA. Baseline blood pressure represented as BL in the figure was calculated as an average of -9, -6 and -3 min readings. SBP was lower after the M_{504} drink when compared to control ($P = 0.019$) during the second (60-120 min $P = 0.035$) and third (120-180 min $P = 0.005$) hour

9.5.2 Diastolic blood pressure (DBP)

Baseline DBP values were not different on the four study days (Mean control: 74 ± 2 mmHg, P_{280}: 74 ± 2 mm Hg, M_{280}: 75 ± 3 mm Hg, M_{504} 73 ± 2 mm Hg, $P = 0.94$). DBP was lower after all three nutrient drinks compared to the control drink, with the greatest reduction after M504 (Figure 9.2).

DBP did not change over the three hours after the control drink. DBP was less after M_{504} ($P < 0.001$) and P_{280} ($P = 0.026$) when compared to control – the drink-condition effect was significant during all three hours following drink ingestion (0–60 min $P = 0.002$, 60-120 min $P = 0.004$, 120-180 min $P = 0.003$). There was no difference between the effects of M_{280} and P_{280} on DBP ($P > 0.05$). Maximal DBP decreases from baseline (control:-12 ± 2 mm Hg, P_{280}: -15 ± 1 mm Hg, M_{280}: -15 ± 2 mm Hg, M_{504}: -15 ± 1 mm Hg, $P = 0.17$) occurred on average between one to two hours after drink ingestion (time baseline to nadir: control: 80 ± 17 min, P_{280}: 108 ± 16 min, M_{280}: 71 ± 12 min, M_{504}: 99 ± 15 min, $P = 0.24$) and were sustained during the third hour for P_{280} and M_{504} (average control: 74 ± 1 mm Hg, P_{280}: 68 ± 1 mm Hg, M_{280}: 71 ± 1 mm Hg, M_{504}: 66 ± 1 mmHg)

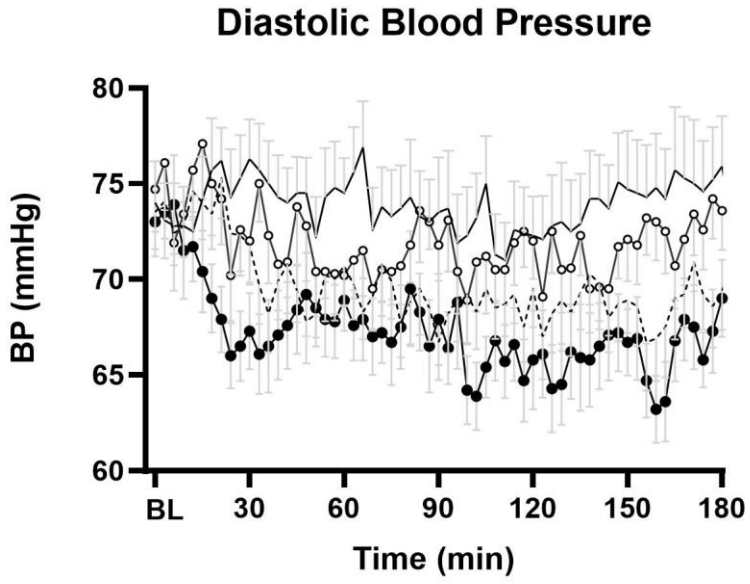

Figure 9.2: Mean (± SEM) systolic blood pressure (DBP; mmHg) following drink

ingestion containing (i) flavored water (control, ~ 2 kcal) or (ii) 70 g whey protein (280 kcal; 'P_{280}'); (iii) 14 g whey protein, 28 g carbohydrate, 12.4 g fat (280 kcal; 'M_{280}'); (iv) 70 g protein, 28 g carbohydrate, 12.4 g fat (504 kcal; 'M_{504}') in older (n = 13) men. Drink-condition effects were determined by using repeated-measures ANOVA. Baseline blood pressure represented as BL in the figure was calculated as an average of -9, -6 and -3 min readings. DBP was lower after the M_{504} ($P < 0.001$) and P_{280} ($P = 0.018$) drinks when compared to control during the second ($P = 0.012$) and third ($P = 0.035$) hour. There was no statistically significant difference between the effects of M_{280} and P_{280} on BP.

9.5.3 Heart rate (HR)

Baseline HR values were not different on the four study days (Mean control: 58 ± 2 bpm, P_{280}: 59 ± 3 bpm, M_{280}: 59 ± 2 bpm, M_{504} 59 ± 3 bpm, $P = 0.95$). HR decreased over 3 h after the control drink and increased after the M_{504} ($P < 0.001$) when compared to control, and did not change significantly after either the M_{280} or P_{280} drinks (0-180 min average control: 57 ± 1 bpm, P_{280}: 60 ± 1 bpm, M_{280}:60 ± 2 bpm, M_{504}:63 ± 1 bpm; Figure 9.3). The drink-condition effect was significant during all the three hours (0–60 min $P = 0.001$, 60-120 min $P < 0.001$, 120-180 min $P < 0.001$) hour following drink ingestion. Maximal HR increase from baseline (control: 3.8 ± 1 bpm, P_{280}:9 ± 2 bpm, M_{280}:12 ± 4 bpm, M_{504}: 13 ± 2 bpm, $P < 0.001$.) occurred on average between one to two hours after drink ingestion (time baseline to peak: control: 48 ± 14 min, P_{280}: 116 ± 15 min, M_{280}: 83 ± 14 min, M_{504}: 127 ± 16 min, $P = 0.003$) and were sustained during the third hour for P_{280} and M_{504} (average control: 55 ± 1 bpm, P_{280}: 61 ± 1 bpm, M_{280}: 60 ± 1 bpm, M_{504}: 64 ± 1 bpm).

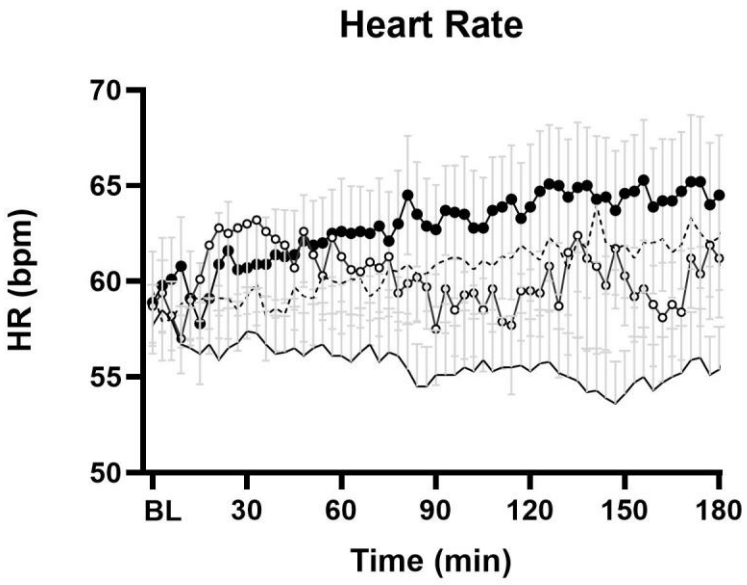

Figure 9.3: Mean (± SEM) Heart Rate (HR; bpm) following drink ingestion containing (i) flavored water (control, ~ 2 kcal) or (ii) 70 g whey protein (280 kcal; 'P_{280}'); (iii) 14 g whey protein, 28 g carbohydrate, 12.4 g fat (280 kcal; 'M_{280}'); (iv) 70 g protein, 28 g carbohydrate, 12.4 g fat (504 kcal; 'M_{504}') in older (n = 13) men. Drink-condition effects were determined by using repeated-measures ANOVA. Baseline Heart rate represented as BL in the figure was calculated as an average of -9, -6 and -3 min readings. HR increased after the M_{504} ($P < 0.001$) and P_{280} ($P = 0.017$) drinks when compared to control (0-180 min)

9.6 Discussion

The major observation in this study is that in healthy older men following ingestion of nutrient drinks containing 280 or 504 kcal energy as pure whey protein or as mixed macronutrients, the magnitude of the decrease in BP is dependent on the energy content, rather than, the protein content of the drinks. Furthermore, while the onset of the BP reduction was evident soon after nutrient drink ingestion, the hypotensive effect was sustained for at least 3 h. Both observations are of clinical relevance in a

'real world' setting. There is no advantage as far as the risk of postprandial hypotension by consuming protein with other macronutrients.

This is also consistent with the context that the rate of gastric emptying of nutrients, whether carbohydrate, fat or protein is primarily dependent on their caloric content.

Heart rate increased after ingestion of the nutrient drinks compared to the control drink, with the greatest and more sustained increase after the highest energy load drink. The resultant increase in cardiac output represents a compensatory mechanism for the potential postprandial fall in BP caused by diversion of blood to the gut. The nutrient-induced increases in HR were inadequate, however, to compensate fully for this diversion in these older men. We have reported smaller increases in HR and larger decreases in BP in healthy older than younger men after a pure 70 g whey protein drink (264) indicating that an age-related reduction in the ability to increase the heart rate after food ingestion contributes to the greater reduction in BP observed in older than younger adults after nutrient ingestion.

The nutrient drink-induced reduction in BP were substantial- a decrease of 20 mmHg SBP or more occurred at some time in 77% of the 504-kcal drink days, compared to 31% of the control drink days. None of the participants reported symptoms of dizziness or faintness, but they were seated throughout the study and not permitted to get up and walk around. A fall in systolic BP of 20 mmHg or more is clearly e associated with symptoms and an increased likelihood of falls and injury, as reflected in one definition of postprandial hypotension (262). It is not known whether the risk of symptoms is influenced by the baseline systolic pressure and this should be evaluated. However, it should be recommended that current criteria for definition of

hypertension do not take into account the relationship of the blood pressure measurement to meal ingestion. Our observations suggest that caution should possibly be exercised for several hours (certainly more than two) at least in older people mobilising after nutrient containing drinks in relation to the risks of syncope and falls. Individuals at risk may potentially benefit from more frequent, lower calorie drinks or meals. Further studies should evaluate this strategy (176). The fall in BP may also be dependent on whether food is already present in the stomach and/or small intestine at the time a nutrient supplement is ingested.

Limitations of this study include that it was only conducted in men. While women tend to have a lower baseline BP that would intuitively predispose them lower BP levels after a meal, we and others have shown that the magnitude of the postprandial fall in BP is related directly to baseline BP such that the fall is greater in those who are hypertensive (229, 411). Further limitations include that the participants remained sitting to standardize study conditions and the number of participants was relatively small. Nevertheless, the observed decreases in BP and HR were clear cut. Because, the effects of the drinks on BP were still evident at 3 h, when each study ceased, it would be of relevance to determine the total duration of the postprandial hypotensive effect. Our study was designed to clarify phenomenology of 'real world' relevance rather than mechanisms. In relation to the later, evaluation of autonomic function would be of interest. We also only evaluated the acute effects of drinks, although we have no reason to believe that their effects on BP will be modified by chronic use.

Our observations suggest that if the intention is to give an older person a nutritional supplement drink containing as much whey protein as possible to preserve, or

potentially enhance, muscle mass and function, administering it in as pure a form as possible (i.e., with the minimum amount of fat and carbohydrate) may potentially reduce the BP the least and so minimize the risk of postprandial hypotension. If, on the other hand, the intention is to provide a mixed macronutrient supplement to provide a specific amount of energy, it should be appreciated the total energy content of the supplement, rather than its macronutrient composition will be the major determinant of its hypotensive effect.

9.7 Conclusions

The hypotensive effect of mixed macronutrient drinks is dependent on overall energy intake rather than macronutrient composition of a drink and may be sustained for at least 3 h. Pure whey protein drinks may, accordingly represent the best approach to maximise protein intake while minimising the potential for deleterious BP falls in older people.

CHAPTER 10: CONCLUSIONS OF THE THESIS

The results of the physiological studies presented in this thesis provide new knowledge about the short term effects in older people, obese and non-obese, and with and without type 2 diabetes mellitus, of whey protein in drink form, on feeding behaviour, glucose metabolism and post-ingestion blood pressure. Disturbances of these factors are common in older people and can have important effects on wellbeing and quality of life. The impact of whey and other increasingly used protein supplements on these factors is therefore of relevance and importance.

Obesity, T2D and postprandial blood pressure falls (leading to postprandial hyopotension [PPH] when severe) are all prevalent in older people and associated with increased morbidity, mortality and health care costs. Existing and growing evidence indicates that nutrition-based strategies play an important role in the management of these conditions in older people. As outlined in chapter 2, because of an increasing awareness of weight loss, under-nutrition and sarcopaenia in older people protein supplements are gaining more attention and use in older people as a generally well tolerated and cost-effective way of preserving muscle mass and function. Whey protein appears to be a particularly effective protein for this purpose, due to its relatively high concentration of branched chain amino acids, particularly leucine.

The studies presented in this thesis have extended previous work by our group to now examine the effects on whey on feeding behaviours in older people over a longer time period than previously (chapter 3), in obese older people (chapter 4) on glucose metabolism in older people with and without T2D (chapter 5 and 5) and on blood pressure and pulse rate (chapters 7-9).

The main findings of these studies can be summarised as follows:

Chapter 3: Energy intake was suppressed by whey protein drinks in a protein load-responsive fashion at breakfast and particularly, at lunch, but not at dinner, and suppression of energy intake by protein was less in healthy older than younger men.

Chapter 4: The 30g whey protein drink did not suppress appetite or energy intake in obese younger or older men.

Chapter 5: The addition of 30g of whey protein to 30g of glucose substantially attenuated the increase in blood glucose concentrations induced by glucose alone; the magnitude was not affected by age. The stimulation of plasma insulin concentrations by whey protein was not reduced by ageing, unlike the insulin response to glucose. Whey protein suppressed hunger less in older than younger men. Glucagon concentrations were unaffected by age.

Chapter 6: The addition of 30 g of whey protein to 30 g of glucose substantially attenuated the increase in blood glucose concentrations induced by glucose alone; the magnitude was not affected by the presence of T2D and the stimulation of plasma insulin concentrations by whey protein.

Chapter 7: Older men exhibited a greater fall in SBP after whey-protein versus control than the younger men, with no BP change after the two drinks in younger men. The nadir in SBP occurred later in the older men, still declining 180 min after whey-protein ingestion. The magnitude of the rise in HR was greater in the younger men.

Chapter 8: Older men exhibited a decrease in SBP after ingestion of 30g and 70g of whey protein to a similar degree and greatest between 120 and 180 mins. HR was increased maximally after 70g particularly in the third hour and DBP decreased non significantly after protein drinks.

Chapter 9: SBP decreased after all three nutrient drinks with the greatest reduction after the M504. Maximal decreases in SBP occurred about 2 hour after drink ingestion and sustained thereafter. Maximum DBP decreases and HR increases occurred after M504, with no differences between the effects of the P280 and M280 drinks.

Collectively, the studies described in this thesis have produced clear-cut and exciting results. Overall the results of these studies suggest that whey protein has minimal suppressive effects on appetite and food intake in older obese and non-obese people, that the effects of a single dose of oral whey are relatively short-lived, that the effect of whey to lower blood glucose concentrations when co-ingested with carbohydrates persists in older people, both with and without type 2 diabetes. Whey protein, in a dose of 30gm, a dose likely to be effective in helping preserve muscle function in older people, lead to decreases in blood pressure, more in older people than young, and this could potentially cause adverse effects in predisposed older people, particularly if consumed with other macronutrients, as the BP lowering effect of the nutrient drinks studied is dependent on their total energy content rather than their macronutrient composition. These are novel findings that significantly add to the pre-existing body of literature.

Although each chapter has clearly mentioned its limitations, overall the studies presented in this thesis have several strengths and limitations. The participant numbers were relatively small, and most studies included only men, and therefore, we cannot comment on the effect of gender. We studied only men, as they appear to have the greatest ability to regulate energy intake in response to energy manipulation, and in women particularly the menstrual cycle may have a confounding effect on appetite and energy intake. We determined the effects of whey protein ingestion, a protein source which is frequently used in high energy supplements due to its high leucine content and 'rapid' digestion, when compared to other proteins such as casein. Whey, when compared to casein or soy, protein results in greater muscle protein synthesis in young and older men. It has to be determined whether our observations apply to other proteins. A strength of the studies was their randomised cross-over design in which the participants acted as their own control.

Further work will be required to determine if the short-term effects of whey found in the studies in this thesis persist with longer term use, with different doses and in different participant groups. Nevertheless, these results should provide guidance for further studies of whey and its use/ingestion by older people.

GLOSSARY

ACTRN; Australian New Zealand Clinical Trial Registry

ACE; Angiotension converting enzymes

ANOVA; Analysis of variance

AUC; Area under the curve

BP; Blood pressure

BMI; Body mass index

BPM; Beats per minute

CCK; Cholecystokinin

CI; Confidence interval

CT; Computerised tomography

CV; Coefficient of variation

DBP; Diastolic blood pressure

DPP-IV; Dipeptyl peptidase-4

EDTA; Ethylenediaminetetraacetic acid

ELISA, Enzyme-linked immunosorbent assay

GE; Gastric emptying

GIP;Ggastric inhibitory polypeptide/glucose-dependent insulinotropic peptide

GLP-1; Glucagon-like peptide-1

HR; Heart rate

HbA1C; Glycosylated haemoglobin

IAAO; Indicator amino acid oxidation

iAUC; Incremental area under the curve

LLOQ; Lower limit of quantification

MRI; Magnetic Resonance Imaging

MPS; Muscle protein synthesis

NHANES; National health and nutrition examination survey

OC; Older men control

OP; Older men protein

PPH; Postprandial hypotension

PYY; Peptide tyrosine tyrosine

SBP; Systolic blood pressure

SD; Standard deviation

SEM; Standard error of the mean

SGLT-1; Sodium-glucose cotransporter-1

SGLT-2; Sodium-glucose cotransporter-2

2D ultrasound- Two dimensional ultrasound

3D ultrasound; Three dimensional ultrasound

T2D; Type 2 Diabetes

T_{50}; 50% gastric emptying time

VAS; Visual analogue scale

WHO; World health organisation

YC; Younger men control

YP; Younger men protein

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