School of Public Health

Overweight and obese Australian adults and fibre supplementation: Its effects on vitamin, mineral and antioxidant status.

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DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Human Ethics (For projects involving human participants/tissue, etc): The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research (2007) – updated March 2014. The proposed research study received human research ethics approval from the Curtin University Human Research Ethics Committee (EC00262), Approval Number (HR41/2011).

Signature:

Jenny-Lee McKay

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"The more that you read, the more things you will know. The more that you learn, the more places you'll go." – Dr. Seuss

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"Wear gratitude like a cloak and it will feed every corner of your life."

— Rumi

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Jenny-Lee McKay

ABSTRACT

Background: Obesity and its associated comorbidities continues to be one of the biggest threats to human health globally. In most cases, overweight or obesity is due to an imbalance between excessive energy intake compared to energy expenditure. Furthermore, obesity typically comprises of diets that are 'nutritionally poor', especially in terms of micronutrients. Excess body weight has also been shown to alter the absorption, distribution, metabolism, and/or excretion of micronutrients within the body, and may impact the nutritional status of individuals with overweight and obesity.

Micronutrients play a critical role in almost every biochemical pathway and therefore maintaining nutritional sufficiency in terms of micronutrients is essential to supporting healthy metabolic functioning. Alternatively, deficiencies in key nutrients may negatively affect the health of individuals already compromised by excessive body weight or other metabolic syndrome risk factors.

A high dietary fibre intake has been well documented within the literature as beneficial to health. A diet high in fibre promotes bowel regularity and general gastrointestinal health; it also has a protective affect against cardiovascular disease, type 2 diabetes, some cancers (especially colorectal, breast and stomach cancers), hypertension, and obesity, which is itself a significant risk factor for developing these conditions. Current recommendations for Australia, Canada, Europe and the USA are that 25–30 g/day of fibre are to be consumed, from fibre-rich sources such as fruit, vegetables, legumes and whole grains, however most people find it difficult to consume enough dietary fibre through increased fruit and vegetable intake.

In those that have difficulty meeting their daily requirements, a daily dietary fibre supplement may be an easy and cost effect measure to increase fibre intake. Therapeutic doses of some fibres (psyllium and PGX) are now also being used as an easy and effective weight management support for individuals with overweight and obesity. In the past, high intakes of dietary fibre supplementation have been linked to deficiencies of calcium, iron, trace metals, and certain vitamins due to its role in metabolism and potential binding of nutrients within the food matrix. Therefore, before fibre supplementation can be recommended as a treatment to assist with weight management outcomes and metabolic syndrome risk factors, the long-term

impact of higher daily doses of fibre on the micronutrient status of the individual needs to be determined.

Aims: The aim of this study was to assess the nutritional status of a cohort of overweight and obese Australian adults enrolled in a long-term weight management trial, with respect to the clinical reference intervals of key micronutrients, to determine baseline micronutrient status. This study also aimed to ascertain the impact of 15g per day of psyllium or PGX fibre supplementation over 3 months on the (serum) micronutrient status in this group.

Methods: Serum micronutrient and dietary intake data was extracted at baseline and at 3 months from a larger weight management study with 159 Australian adults with overweight and obesity or a body mass index between 25-40 kg/m2, and aged between 18 and 65 years. Serum data was compared to the clinical micronutrient reference intervals for associations between BMI and micronutrient status, whereas dietary intake data was compared to the NHMRC Nutrient Reference Values for Australia and New Zealand. Associations between serum and dietary micronutrient data after 3 months psyllium or PGX fibre supplementation was also analysed. The micronutrients examined in this study include Vitamins A, B_9, B_{12}, C, D and E and minerals calcium, iron, iodine, magnesium, potassium, sodium and zinc.

Results: Significant negative associations between BMI and micronutrients' vitamin D ($p=0.044$), magnesium ($p=0.010$), potassium ($p=0.023$) and serum folate ($p=0.025$) were found. Baseline serum micronutrient levels for participants prior to supplementation were compared to the clinical reference intervals for Australians established by the Australasian Association of Clinical Biochemists (AACB). Most micronutrient levels were found to be lower than clinical recommendations. No significant differences between control (rice flour), psyllium and PGX fibre supplement groups for micronutrient status were found after 3 months at $p>0.05$.

Conclusions: The results of the present study supports the concern that being overweight and obese may compromise micronutrient status of an individual, as a likely result of the poor dietary choices that promote weight gain. In this instance, a large proportion of the study sample fell below the AACB clinical reference intervals for calcium, vitamin D, vitamin A, potassium, magnesium, folate, zinc and sodium. Therefore, it is imperative that any weight loss strategy, such as fibre supplementation, does not further impair the nutritional status of overweight and obese individuals. The results from this study suggest that supplementing 15g per

day of either psyllium or PGX fibre for 3 months does not have any deleterious effects for micronutrient status; however, many nutritional deficiencies manifest over a long period of time, therefore further studies of a greater duration than 3 months are needed to determine whether high-dose fibre supplementation would impair the micronutrient status of individuals with overweight and obesity in the long term.

LIST OF PUBLICATIONS

Paper 1/ Chapter 3:

McKay, J., Ho, S., Jane, M.. Overweight & obese Australian adults and micronutrient deficiency. BMC Nutr 6, 12 (2020). https://doi.org/10.1186/s40795-020-00336-9

Paper 2/Chapter 4:

McKay, J., Ho, S., Jane, M.. Micronutrient status of individuals with overweight and obesity following 3 months' supplementation with PolyGlycopleX (PGX®) or psyllium (PSY). (Submitted to Journal of Human Nutrition and Dietetics on 06/10/2020)

Other publications

Jane, M., McKay, J., Pal, S. (2019). Effects of daily consumption of psyllium, oat bran and polyGlycopleX on obesity-related disease risk factors: A critical review. Nutrition (Burbank, Los Angeles County, Calif.), 57, 84–91. https://doi.org/10.1016/j.nut.2018.05.036

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ABBREVIATIONS AND ACRONYMS

PGX: PolyGlycopleX®
DF: Dietary fibre
BMI: Body mass index
BP: Blood pressure
CVD: Cardiovascular disease
HDL-C: High density lipoprotein cholesterol
LDL-C: Low density lipoprotein cholesterol
SCFA: short-chain fatty acid
MS: Metabolic syndrome
NRVs: Nutrient reference values
NHMRC: National Health and Medical Research Council
RDIs: Recommended dietary intake
AI: Adequate intake
UL: Upper level
CVD: Cardiovascular disease
RBC: Red blood cell
TBW: Total body water
CNS: Central nervous system
CHD: Coronary heart disease
NIDDM: Non-insulin dependent diabetes mellitis
NSP: Non starch polysaccharide
DNA: Deoxyribose nucleic acid
ELISA: Enzyme-linked immunoabsorbant assay
ANCOVA: One-way analysis of covariance
FAAS: Flame atomic absorption spectroscopy
SEM: Standard error of mean
AACB: Australasian Association of Clinical Biochemists
RE: Retinol equivalents

CHAPTER ONE

INTRODUCTION AND OVERVIEW

1.1 Background

Food habits affect every biochemical and physiological process in the human body. An imbalance or lack of essential macro and micronutrients can have a significant effect on mood, behaviour, energy levels, and intellectual and physical performance (1). In the past, little attention was paid to dietary fibre in food. It was simply referred to as roughage or bulk and was measured as crude fibre. However, in the last three to four decades, a great deal of research has brought new insights into the health benefits of dietary fibre. Epidemiological and cohort studies have consistently revealed that higher fibre intakes in a person's diet are correlated with lower body weight, body mass index (BMI), waist circumference (2, 3), improved plasma lipid profiles (1, 4) and improved glycaemia and insulinaemia (5), indicating the benefits and risk reduction for metabolic syndrome, cardiovascular disease (CVD) and type 2 diabetes (6). Dietary fibre recommendations for adults in Australia, Canada, Europe and the USA are 25–30 g/day to be consumed from fibre-rich sources such as fruit, vegetables, legumes and whole grains (7). However, it is estimated that in many Western Countries, including Australia and Canada, adults consume approximately 15–25 g of dietary fibre/day (8).

The benefits of dietary fibre are well known, however most people find it difficult to eat the required amounts of fibre through increased fruit and vegetable intake, as well as cereals and grains as shown in the Australian Health Survey: Consumption of food groups from the Australian Dietary Guidelines 2011-2012 (9). Fibre supplements may provide a cost effective and easy alternative for increasing the fibre content of a persons' diet without the need for other major dietary modifications. However, high intakes of fibre in human and animal studies have been linked to deficiencies of calcium, iron, trace metals, and vitamin D and E, due to the effect of fibre on the absorption and bioavailability of these nutrients (10). Thus the use of therapeutic doses of fibre in supplement form may impair absorption of micronutrients in the gut, especially if taken prior to or during a meal (10).

The aim of this introduction chapter is to define dietary fibre and how it has evolved with time, followed by a review of the literature regarding the effect of supplemented

dietary fibre on vitamin and mineral status, and to then highlight knowledge gaps. The limited understanding of this effect is reflected in this chapter.

Chapter 3 will aim to evaluate the nutritional status of a cohort of overweight and obese Australian adults enrolled in a long-term weight management trial, with respect to the clinical reference intervals of key micronutrients using baseline serum samples. This study also aimed to determine the impact of 15g per day of psyllium or PGX fibre supplementation over 3 months on the (serum) micronutrient status in this group (Chapter 4).

1.2 Micronutrients

Many micronutrients are essential for normal metabolic functioning via the regulation of key pathways, so bioavailability is important. For example, vitamin A is important for epithelial cell differentiation, immune function and antioxidant activity (11), vitamin B12 is critical for red blood cell formation and nerve axon myelination (11). Folate is integral in red blood cell formation (11), vitamin C is important for antioxidant and anti-atherogenic activity as well as endothelial integrity (12). Vitamin E provides antioxidant activity and protection of pancreatic b-cells (13), whereas calcium and vitamin D aid in bone mineral homeostasis (14), and protection against glucose intolerance and type 2 diabetes (2, 3). Iodine is involved in the production of thyroid hormones, which are crucial for all aspects of human metabolism (15). Iron assists in the formation of red blood cell haemoglobin used to transport oxygen to the cells (15), magnesium contributes to ionic regulation and modulation of glucose transport across membranes (16), potassium is important for cellular function, fluid and electrolyte balance and blood pressure maintenance (15), and zinc is crucial for enzymatic activity, protein and DNA synthesis, as well as cell-mediated immunity (17).

1.3 Nutrient Reference Values (NRVs)

It has long been reported that Australians do not consume enough fruit and vegetables in their diet, this is confirmed when looking at dietary intake data for the population. The usual nutrient intake for Australians was compared with Nutrient Reference Values (NRVs) for each age and sex group and the results were published in the ‘Australian Health Survey; Usual Nutrient Intakes 2011-2012’(18). Key

findings showed that 17% of males and 14% of females had inadequate usual intakes of vitamin A. One in eleven adult females (aged 19 and over) did not meet the requirement for Folate, however almost all males met the dietary requirement through intake. Insufficient B_{12} intake accounted for between 5-8% of females dependent on age and less than one percent of males. Less than 5% of the population did not meet their dietary needs for vitamin C and vitamin E. However, 73% of females and 50% of males aged two years and over did not meet their calcium requirements. Forty percent of 14-18-year-old females and 38% of 19-50-year-old females had inadequate iron intakes compared to only 3% of males. One in every three people aged two years and over (37% of males and 34% of females) did not meet their requirements for magnesium, however 76% of males and 42% of females aged two years and over exceeded the UL for sodium. From age 14 males have a much higher requirement of zinc than females due to its key role in the male reproductive system (19). According to the Australian Health Survey results 37% of men and one in ten women (9%) had inadequate usual zinc intakes (18).

1.4 Deficiency Diseases

Without adequate amounts of these vitamins and minerals biochemical functioning starts to become impaired and prolonged insufficient absorption or bioavailability may lead to deficiency diseases. A lack of vitamin A can lead to eye disorders such as keratomalacia or xerophthalmia as vitamin A is essential for maintaining normal vision (20). Chronic low vitamin D can result in bone disorders such as rickets or Osteomalacia (21). Insufficient vitamin C leads to scurvy, a condition most associated with defects in connective tissues which is famous for affecting sailors during the 16^{th} to mid-19^{th} century (22). Vitamin E is a lipid-soluble vitamin; deficiency in this nutrient can cause vision loss and muscle dysfunction and is more likely caused by irregularities in dietary fat absorption or metabolism, however this is rare in developed countries (23). Megaloblastic anaemia results from insufficient B_{12} and B_9 as both vitamins are essential for the formation, maturation and functioning of healthy RBCs (24, 25). Similarly vitamin B_9, most commonly known as folate, is responsible for the development of the foetal nervous system, so deficiency can result in neural tube defects in a developing foetus (25). Hypocalcaemia is caused by chronic low levels of dietary calcium absorption of bioavailability and deficiency long term can lead to bone disorders such as osteomalacia and osteoporosis.

Similarly to vitamin D, calcium is also involved with the development of nutritional ricketts (26). Iron deficiency anaemia is one of the most common mineral deficiencies in the world, and iron plays a vital role in haemoglobins' ability to transport oxygen around the body (27). When deficiency is severe pica can develop, defined as a craving for eating non-food items (28). Clinical manifestations of low magnesium are vague often going unnoticed and include muscle weakness, tremor, and fasciculations. However chronic magnesium deficiency is associated with vascular calcification and cardiovascular disease (29). Low serum sodium referred to as hyponatremia, is an excess of total body water (TBW) relative to total body sodium content. It is potentially life threatening affecting central nervous system (CNS) function (30). A severe potassium deficiency (hypokalaemia) can lead to heart dysrhythmias from interference to the electrical conductivity, affects acid-base balance and impairs renal concentrating ability (31). Lastly, an estimated 20% of the global population is zinc deficient (32). Those who consume a predominantly plant based diets are at higher risk of zinc deficiency due to inhibiting effects of phytate (33). Secondary deficiency can also occur due to malabsorption disorders, diabetes mellitis, taking diuretics, in burn patients and the elderly (34). Symptoms include alopecia, dermatitis, impaired immunity, night blindness, anaemia, lethargy, and impaired wound healing (35). The impact of a high fibre diet long-term through added supplementation therefore has the possibility for wide ranging implications on almost all cellular activity (36) .

1.5 Obesity

Overweight and obesity is a complex, multifactorial disease that impacts almost all metabolic functions, which can lead to the development of chronic diseases and reduced quality of life (7). Overweight is defined a body mass index (BMI) of 25-29kg/ m^2 and obesity is 30kg/m2 and over (37). The development of obesity typically occurs when the caloric intake continuously exceeds energy expenditure. The most recent data from the Australian Health survey 2014–15, indicates almost two-thirds (63%) of Australians aged 18 and over were overweight or obese (38). The burden of overweight and obesity on an individual's health projects across multiple organ systems and diseases (39). As excess weight increases, so does the risk of developing disorders that are associated with high mortality and morbidity, including type 2 diabetes, hypertension, coronary heart disease (CHD), dyslipidaemia, gallbladder

disease, and certain malignancies (40). The pattern of fat distribution is also important, with visceral fat being the most significant for poorer health outcomes (41). Metabolic Syndrome is defined as a cluster of conditions that occur together and further increase disease risk. These conditions include central adiposity, hypertension, reduced high-density lipoprotein (HDL), hypertriglyceridemia, and hyperglycaemia (40). A person is considered to have metabolic syndrome when three or more of these conditions occur together (40).

1.6 Obesity and micronutrient deficiency

Overweight and obese individuals typically consume more than the required amount of total energy compared to their energy expenditure, and the type of food consumed is usually of low nutritional density (8, 42). Over time this pattern of eating may lead to low micronutrient status; however poor nutritional status may also result from alterations in micronutrient absorption or metabolism in this population subgroup (42). Some studies have suggested the rates of obesity and micronutrient deficiencies are correlated and a pattern is emerging as obesity rates are increasing in some parts of the world where micronutrient deficiencies are more prevalent (43). 'High-calorie malnutrition' is a term being coined to describe this issue, as overweight and obese persons are not 'regularly' considered to be at risk of malnutrition due to excess weight giving the false pretence that nutritional needs are exceeded (42, 44). Vitamin D, vitamin E, vitamin B_6 and B_{12}, calcium, magnesium, potassium, iron, and zinc have all been indicated as having a negative correlation with obesity (45). However, the direction of causality it still under debate. Leptin hormone for example has been found to play a role in maintaining the consistency of adipose tissue mass through regulation of energy intake versus energy expenditure, as well contributing to other risk factors for obesity such as the inflammatory response (45). Changes in leptin hormone through nutritional deficiency may result in fat deposition such as increased abdominal fat which can lead to an increased risk of chronic disease (45). One food particulate that is commonly lacking in the diet of overweight or obese individuals is dietary fibre, often due to inadequate intake of fibre rich nutrient dense foods such as fruit and vegetables. A diet high in fibre has long been documented to have wide ranging health benefits, from increased satiety and reduced waist circumference to improvements in blood lipids, insulin sensitivity and reduced blood pressure (40).

1.7 Commercially available fibre supplements

Naturally occurring dietary fibre is derived from the edible part of plants, and is resistant to digestion and absorption in the small intestine, but available for fermentation in the large intestine (46, 47). Dietary fibre can be further classified as either soluble or insoluble depending on chemical structure and can have varying viscosity when in solution (48). There is evidence to suggest that increasing daily fibre consumption overall can assist with weight loss (49), as it may decrease energy absorption by lowering the bioavailability of fatty acids and proteins (50). Furthermore, fibre can ferment in the colon, increasing short chain fatty acid (SCFA) concentration, the effects of which may decrease cholesterol synthesis (46, 51), and also enhance satiety (52). The effects of fibre-rich diets on blood lipids, appetite control, energy intake and body weight may be related to the chemical structure of the fibre and their physicochemical properties (such as solubility, viscosity, water-holding capacity and fermentability), rather than on the amount of fibre ingested (48, 53, 54).

A variety of fibre supplements are readily available in most grocery stores, pharmacies and health food stores. Unprocessed psyllium packaged in 500 g bags appear to be the most common and cost-effective option. These can be added to water or juice, yogurt, used in cooking or added to breakfast cereals or muesli. For those that find consuming unprocessed fibre inconvenient or unpalatable, a flavoured powder is available. This type of fibre supplement usually consists of psyllium, as is the case with Metamucil®, a commercially available fibre supplement. Other psyllium-containing products include capsules and wafers. A recent addition to the fibre supplement market is PolyGlycopleX (PGX®), a proprietary complex of three natural polysaccharides (fibres), and packaged as granules in dose-size sachets. Currently PGX® can only be obtained at retail outlets in the United States and Canada, but can be purchased via the internet in other regions. It is designed to be consumed with liquid prior to a meal or can be added during the cooking process to increase the fibre content of a meal as it is flavourless and odourless.

1.8 Psyllium

Psyllium seed husk is a viscous, water-soluble fibre, gel-forming mucilage from the Plantago ovata plant and has advantages over other types of soluble fibre because it is less readily fermented and therefore causes less abdominal bloating (54). Psyllium has been shown to be an effective supplement, in adjunct to dietary intervention, in the control of body weight and body composition, cholesterol, triglycerides, glucose and insulin levels (55, 56). Psyllium has a long and established record as a blood cholesterol lowering agent and bowel regulator (57, 58). Pal et al. 2011 reported significant changes to BMI, weight and total body fat in 57 overweight or obese participants following supplementation of 21 g of psyllium fibre daily for 3 months (59). Statistically significant reductions in body weight (2.6%), body fat percentage and waist circumference were found for overweight and obese Australian adults following supplementation of 15 g of psyllium per day for 12-months (60, 61). Satiety is also important for weight loss and improvements have been shown with the addition of psyllium husk to a meal. A study by Karhunen et al. (2010) showed postprandial GLP-1 , ghrelin, insulin and glucose concentrations were significantly suppressed following psyllium-enriched meals (62). In addition, peptide yy (PYY) - assessed at two hours after consuming the fibre enriched meals was prolonged, meaning participants stayed fuller for longer (62).

1.9 PolyGlycopleX® supplement

PolyGlycopleX® (PGX®) is a non-starch polysaccharide complex consisting of three natural fibre components (glucomannan or konjac, sodium alginate, and xanthan gum), and is manufactured by a proprietary process known as EnviroSimplex® (63). Together these fibre components form a highly fermentable complex with a very high viscosity and high water-holding and gel-forming properties (64). PolyGlycopleX manifests such high viscosity due to the synergistic complexing of the three fibre sources in the proprietary processing, making it approximately seven times more viscous than psyllium (64, 65). The safety and efficacy of consuming PGX have been confirmed in safety studies and human clinical trials found no adverse events with participants reporting only mild gastrointestinal discomfort (eg flatulence, bloating, intestinal rumbling, or abdominal pain) (66). The gastrointestinal complaints experienced by the participants are well documented in the literature (67), and are considered normal consequences of increasing dietary intake of fruits and

vegetables and fibre in general (68). To date most studies on PGX have focused on blood lipids, insulin and glycaemic response with significant benefits shown (61). Some clinical trials have begun to evaluate the effect of PGX® on weight loss, however there is still limited studies to date. One study by Reimer et al 2014 found significant reductions in waist circumference and visceral adiposity after a 14-week trial period where Japanese adults with central adiposity consumed 15 g of PGX® per day (69). A 12 month clinical trial comparing PGX® to control (rice flour) found significant reductions in body fat percentage and body weight in overweight and obese Australian adults consuming 15 g of PGX® daily (60).

ABSORPTION METHODS

1.10 Vitamin and mineral absorption

Intestinal absorption, and therefore bioavailability, of a vitamin depends on the chemical form and physical state in which the vitamin exists within the food matrix (70). These properties may be influenced by the effects of food processing and cooking, as well as the food components themselves. The food matrix enhances vitamin absorption by stimulating the secretion of digestive enzymes and bile salts (71). Bile salts inhibit gastric emptying and delay intestinal transit, allowing for increased time at the absorption sites (71). Moreover the food matrix can also negatively affect the absorption of micronutrients, if it contains inhibitors which bind to the vitamin or mineral and render them unavailable (70).

It is suggested that dietary fibre binds with polyvalent mineral ions forming un-absorbable fibre-mineral complexes (72). The relationship between dietary fibre and micronutrient absorption or balance is controversial, with some studies reporting impaired mineral absorption or balance and other studies reporting no change or a positive balance with a high-fibre diet compared with a moderate-fibre diet (72, 73).

1.10.1 Fibre and micronutrient interaction

Dietary fibre has well documented health benefits, however as fibre consumption can retard or enhance nutrient absorption, the composition of the diet is an important factor in bioavailability. For example, the presence of adequate amounts of dietary fat is essential for the absorption of the fat-soluble vitamins A, D and E (70). Fibrous plant material can interfere with the physiological mechanisms of absorption, as is

evident by the poor bioavailability of β-carotene in a raw carrot compared with that in a cooked carrot due to cellulose fibre (74). Furthermore, certain types of dietary fibre may either interfere with the formation of mixed micelles in the intestinal lumen or effectively alter the normal diffusion and accessibility of micellar lipids to the absorptive surface of the intestinal mucosa (70). Such events may compromise the absorption of lipids, including the fat-soluble vitamins.

In some studies high fibre intakes were found to cause deficiencies of calcium, iron, trace metals and certain vitamins (2, 3). A study by Fardet (2010), found no significant changes in vitamins and minerals including potassium, calcium, phosphorous, iron and vitamin A, during a three phase high-fibre dietary intervention with diabetic patients (1). A study with 20 overweight type 2 diabetic patients evaluated the effects of psyllium in three different phases: phase 1 (1 week), phase 2 (treatment, 14 g fibre/day, 6 weeks) and phase 3 (4 weeks) (75). A significant increase in magnesium and vitamin E was found when phases 2 and 3 were compared: sodium also showed a significant increase between phase 2 and phases 1 and 3 (75).

The effect of guar gum (Guarina) on glycaemic response and blood lipids as well as some minerals, was assessed in a randomized, double-blind and cross-over study on 16 participants with non-insulin dependent diabetes mellitus, and found no significant changes to the sodium, potassium, chloride, magnesium and calcium levels of participants during the 8-week guar gum treatment (36). Behall et al (1989) did not detect any significant changes in mineral balance (calcium, magnesium, iron, copper and zinc), with the exception of a negative manganese balance, after 16 subjects with non-insulin-dependent diabetes mellitus (NIDDM) consumed cellulose, an insoluble fibre, or carboxymethylcellulose gum, karaya gum or locust bean gum, all soluble fibres (equivalent to 19.5 g of fibre per day) for 4 weeks (76). Results of a subsequent second study, where 31.7 g of guar gum per day was administered for 6 months on the same subjects showed that mineral balance was not affected for iron, copper, zinc, calcium, manganese or magnesium (42). A study investigating the effect of a fibre rich-diet on mineral absorption (calcium, magnesium, iron and zinc) in healthy young men (n=90), found that the addition of either inulin (soluble) or sugar beet fibre (partly soluble) to normal mixed diets significantly improved the absorption of calcium without adversely affecting the retention of the other minerals (73).

A prospective cohort study analysed the effect of non-starch polysaccharide (NSP) fibre on plasma micronutrient concentrations in a large group of free-living middle-aged women (n=283) consuming their usual diet. No association was observed between higher intakes of NSP and lower plasma concentrations of the micronutrients measured, after controlling for the higher nutrient concentrations found in high-fibre foods (77). The micronutrients examined included carotenoids, vitamin A, vitamin E, thiamine, riboflavin, vitamin B_6, vitamin B_{12}, folic acid, vitamin C and trace metals (77). These researchers concluded that current guidelines for increasing the population's NSP consumption can be safely applied amongst middle-aged women (77).

At the time the current study was developed, there was little data on the effects of high fibre consumption via supplement on the absorption of vitamins and minerals for any fibre type soluble or insoluble. The few studies that were available were not recent, had small sample sizes and have relatively short-term interventions. There is currently no evidence on the impact of high fibre intakes on the micronutrient status of overweight and obese Australian adults, as well as no previous research about the effects of PGX®, a novel fibre and how it affects micronutrient absorption in the gut. There is also very limited research on psyllium fibre and its effects on absorption of micronutrients. Long-term therapeutic doses of fibre taken by a subset of the population who may already be nutritionally disadvantaged being overweight or obese may have adverse effects. Micronutrient deficiencies can develop over time as a result of poor diet, and the current available literature sheds no light on how high doses of fibre taken for weight loss could contribute to this. Further research in this field is necessary so that fibre supplementation can be used for health benefits without adverse effects.

1.11 Summary

Obesity has now reached epidemic proportions globally, and projections indicate that it will continue to rise. A study by Walls et al. 2012 predicts that normal-weight Australian adults will constitute less than a third of the population by 2025, and the obesity prevalence will have increased by 65% (78). A diet high in dietary fibre has been shown to have positive health benefits including increased satiety and reduced waist circumference, improved blood lipids, insulin sensitivity and a reduction in blood pressure (60). Current recommendations for dietary fibre for Australian adults

are 25-30 g per day, however most people find it difficult to achieve this levels through increased fruit and vegetable consumption (18, 79).

Psyllium fibre is a viscous, water-soluble fibre, commercially available as psyllium husk and widely consumed as it is less readily fermented in the gut, causing less abdominal bloating than other dietary fibres (80). PolyGlycopleX® (PGX®) is a novel non-starch polysaccharide compound comprising three natural fibre components (glucomannan or konjac, sodium alginate, and xanthan gum) (64). It is a highly fermentable complex approximately seven times more viscous than psyllium due to the proprietary processing resulting in synergistic complexing of the three fibre sources (64). Current research has examined the effect of fibre supplementation on weight and metabolic syndrome risk factors in overweight and obese individuals, however there is limited research to date on the effect of fibre supplementation on the micronutrient status of overweight and obese. A fibre supplement has been proposed as an easy and effective way to increase dietary fibre intake to meet the RDI and potentially receive some of the known health benefits of dietary fibre. However high dietary fibre intakes have previously been linked to micronutrient deficiencies (81). Overweight and obese populations are already at risk of nutritional deficiency, so before therapeutic doses of dietary fibre in supplement form can be recommended to this subset of the population for weight loss and other health benefits, fibres' effect on micronutrient status of overweight and obese needs to be examined.

1.11.1 Aims

The aim of this study was to investigate whether a 12-week 15 g/d consumption of two different soluble fibres (psyllium and PGX®) impacted micronutrient status of A, B_9, B_{12}, C, D and E and minerals' calcium, iron, iodine, magnesium, potassium, sodium and zinc, in overweight and obese Australian adults, participating in a long-term fibre supplement clinical trial. A further aim was to add to the body of knowledge surrounding micronutrient status and potential deficiency for overweight and obese Australian adults.

1.11.2 Hypothesis

It was hypothesised that the micronutrient status for the overweight and obese Australian adults participating in this study would be insufficient at baseline. It was also hypothesised that some micronutrients may be affected following 3 months fibre supplementation of 15 g per day of either psyllium or PGX® fibre.

1.11.3 Objectives

The first objective of this project will be to evaluate to what degree, if at all, micronutrient status is affected by fibre supplementation over a 3-month period. The hypothesis that micronutrient status may already be insufficient in overweight and obese Australian adults will be tested by first establishing a baseline overview of micronutrient status.

Secondly, baseline serum results will be further correlated with BMI to identify any potential relationships between serum increases or decreases and a person's weight. Baseline serum micronutrient levels will then be compared with the clinical reference intervals as determined by the Australasian Association of Clinical Biochemists (AACB) and the self-recorded dietary intake data compared to the NRVs for Australia and New Zealand as determined by the National Health and Medical Research Council (NHMRC) (82, 83).

Lastly, the hypothesis that fibre supplementation may affect micronutrient status will be measured by looking at between group differences in serum and self-recorded dietary intake micronutrient levels following 3 months fibre supplementation of either psyllium, PGX® or control (rice flour). The 3-month serum micronutrient levels will be compared with the clinical reference intervals and the 3-month self-recorded dietary intake data compared to the NRVs for Australia and New Zealand.

1.12 Chapters

Chapter 2: will go through the study protocol, including the methods and material, participant selection criteria, clinical and statistical assessments as well as the aims and hypothesis.

Chapter 3: will be the first paper titled 'Comparing baseline micronutrients with the nutrient reference values to determine the nutritional status of this sample of overweight/obese individuals.

Chapter 4: will be the second paper titled ‘Between group comparisons in (blood) micronutrients to determine if psyllium or PGX® supplementation altered micronutrient status of overweight and obese after 3 months of treatment.’

Chapter 5: will review and discuss the study findings and what the recommendations are for future research.

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CHAPTER TWO

STUDY PROTOCOL

2.1 Background

Dietary fibre is derived from the edible part of plants, and is resistant to digestion and absorption in the small intestine, but available for fermentation in the large intestine (1). Dietary fibre can be further classified as either soluble or insoluble, depending on viscosity when dissolved in solution (2). Of the two types of dietary fibre, soluble fibre has been shown to have the greatest documented effect on cardiovascular and metabolic risk factors (3). Dietary fibre recommendations for adults in Australia, Canada, Europe and the USA are 25–30 g/day to be consumed from fibre-rich sources such as fruit, vegetables, legumes and whole grains (4). However, a deficit exists between the dietary recommendations and what is being consumed within the population. It is estimated that in many Western Countries, including Australia and Canada, adults only consume approximately 15–25 g of dietary fibre/day (5).

Epidemiological and cohort studies have consistently revealed that higher fibre intakes are correlated with lower body weight, BMI, waist circumference (6, 7), improved plasma lipid profiles (8, 9) and improved glycaemia and insulinaemia (10). High dietary fibre intake is associated with a protective effect against cardiovascular disease, diabetes, some cancers (especially colorectal, breast and stomach cancers), hypertension and obesity (which is itself a significant risk factor for developing these conditions) (11, 12), as well as improve gastrointestinal health, thus preventing constipation and alleviating symptoms of diverticular disease (13). However most people find it difficult to eat the required amounts of fibre through increased intake of fruit, vegetables and cereals, as shown by the Continuing Survey of Food Intakes by Individuals 1994–96, 98 (14, 15). Therefore, fibre supplements may provide a cost effective and easy alternative method for increasing the fibre content of a diet without the need for other major dietary modifications.

Although there are many demonstrated health benefits with consuming a fibre rich diet, high intakes of certain fibre sources have been linked to deficiencies of calcium, iron, trace metals, and certain vitamins due to its role in metabolism (16, 17). Many of these micronutrients are essential for normal metabolic functioning via the regulation of key pathways. For example, vitamin A is important for epithelial cell

differentiation, immune function and antioxidant activity (18) and vitamin B_{12} is critical for red blood cell formation and nerve cell formation (9). Folate is integral in red blood cell formation (19), vitamin C is important for antioxidant and anti-atherogenic activity (20) as well as endothelial integrity (21). Vitamin E provides antioxidant activity and protection of pancreatic β-cells (22) whereas calcium and vitamin D aid in bone mineral homeostasis (23) and protection against glucose intolerance and type 2 diabetes (24, 25). Iodine is involved in the production of thyroid hormones, which are crucial for all aspects of human metabolism (26, 27). Iron assists in the formation of red blood cell haemoglobin used to transport oxygen to the cells (9, 27), magnesium contributes to ionic regulation and modulation of glucose transport across membranes (28), potassium is important for cellular function, fluid and electrolyte balance and blood pressure maintenance (9), and zinc is crucial for enzymatic activity, protein and DNA synthesis, as well as cell-mediated immunity (29). The impact of a high fibre diet on the micronutrient status of overweight and obese individuals therefore has the possibility for wide ranging implications on almost all cellular activity (27).

The two types of fibre discussed in the present study were examined as part of a larger long term randomised controlled clinical weight management trial, conducted by Associate Professor Sebely Pal of the faculty of Health Sciences at Curtin University, and are Psyllium fibre and PolyGlycopleX® (PGX®) fibre. Psyllium seed husk is a viscous, water-soluble gel-forming mucilage from the *Plantago ovata* plant and has advantages over other types of soluble fibre because it is less readily fermented and therefore causes less abdominal bloating (30). Psyllium has a long and established record as a blood cholesterol lowering agent and bowel regulator (31). In addition, psyllium has been shown to be an effective supplement, in adjunct to dietary intervention, in the control of body weight and body composition, cholesterol, triglycerides, glucose and insulin levels (32, 33).

PolyGlycopleX® is a highly viscous, non-starch polysaccharide blended from three natural fibre components: konjac (glucomannan), sodium alginate and xanthan gum. These three fibre components form a highly fermentable complex with a developing, high viscosity and water-holding capacity (34). PGX® is three times more viscous than guar gum and approximately seven times as viscous as psyllium (34). PolyGlycopleX® has been shown to have a lipid lowering effect in healthy participants (35) as well as in overweight and obese adults (36). In addition, recent

human studies have shown that PGX® is highly effective in reducing postprandial glycaemia, lowering the glycaemic index of food (37) and modifying satiety hormones in healthy adults (33). Randomised controlled clinical trials are required to verify whether PGX® can be used for long-term weight loss or weight control and whether this novel fibre type is better than other soluble fibres, such as psyllium, currently available in the market.

METHODS

2.2 Participants

Overweight and obese individuals with a body mass index (BMI) between 25-40 kg/m^2 and aged between 18 and 65 years, were recruited from the community in Perth, Australia via advertisements in newspapers, flyers posted around the University and community noticeboards, as well as radio advertising on Curtin FM. Recruitment efforts for the study produced 472 volunteers, who were screened by telephone or online using a Qualtrics Survey (Appendix 2 Screening checklist) for eligibility. Of those screened, 159 were found eligible and 127 complete datasets were used for the current study. Volunteers who met the criteria attended an orientation session at Curtin University, at which time the details of the study were also explained. Exclusion criteria included smoking, lipid lowering medication, use of steroids and other agents that may influence lipid metabolism, use of warfarin, diabetes mellitus, hypo and hyperthyroidism, cardiovascular events within the last 6 months, psychological unsuitability, major systemic diseases, gastrointestinal problems, proteinuria, liver, renal failure, weight fluctuations over the past 6 months, vegetarianism or veganism and participation in any other clinical trials within the last 6 months.

Baseline and 12-week micronutrient data for the current study was extracted from a complete dataset for a 12-month randomised controlled trial. This year-long study examining the effects of two different types of fibre supplement on a range of biochemical outcomes in adults with overweight and obesity, and conducted in 2012 at Curtin University in Western Australia (38, 39). Ethics approval was gained for this study as a part of the 2012 study from the Curtin University Human Research Ethics Committee (HREC) on 23 November 2011, approval number: HR41/2011 (Appendix 1). All components of the present study strictly adhered to the HREC guidelines. All participants provided signed, written informed consent, and were

made aware that their participation was voluntary and that they could withdraw from the study at any time. Ongoing privacy, security and protection of the identity and information of participants was ensured for all future data collected as part of the present study. In addition, the larger clinical trial was registered with the Australian New Zealand Clinical Trial Registry on 20 April 2011, registration number: ACTRN12611000415909.

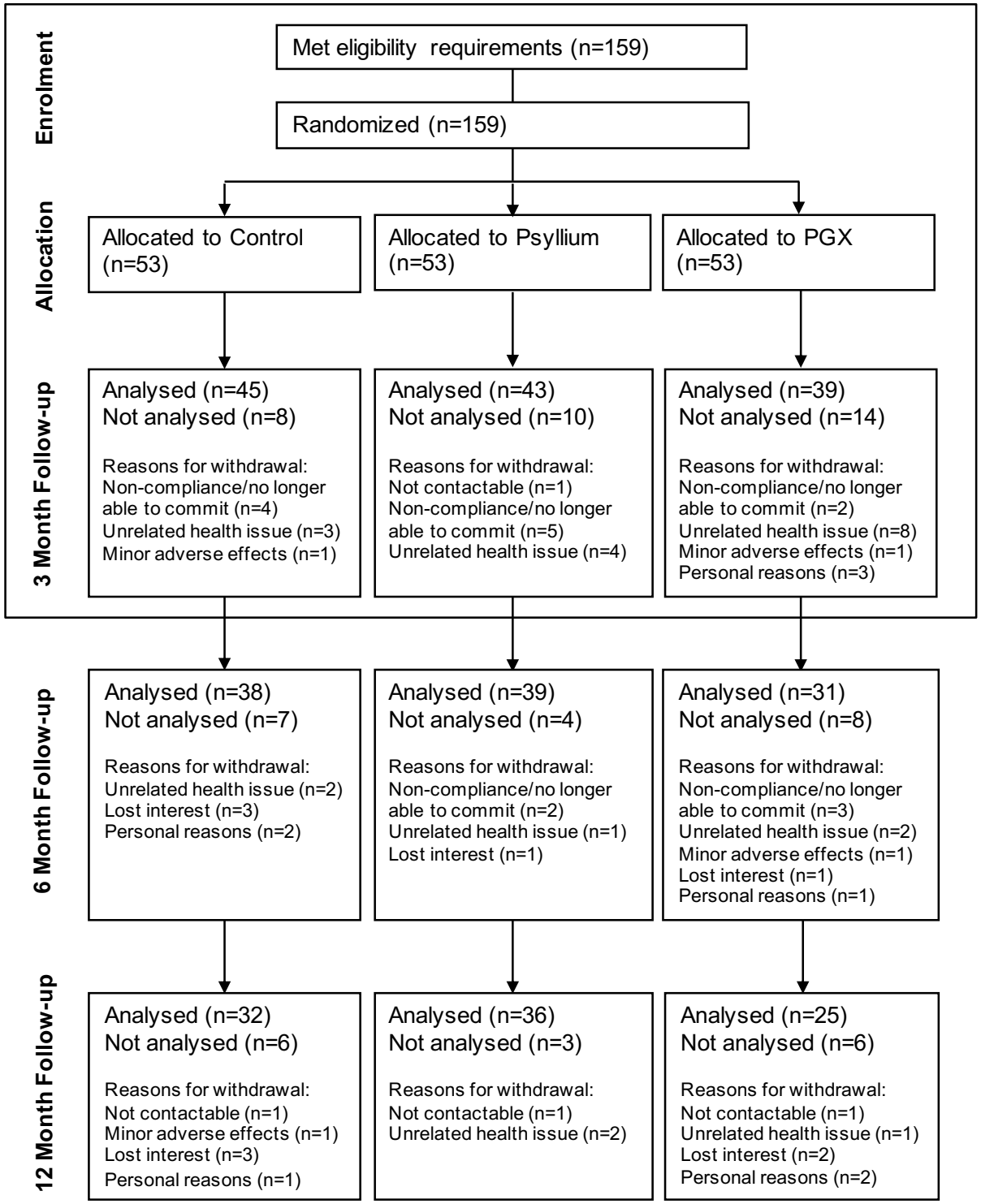
2.2.1 Study design

The study design for the 2012 study, entitled: Comparison of two different fibre supplements on body weight, body composition, metabolic and cardiovascular risk factors in overweight and obese individuals, is as follows:

The study was randomised, controlled double blind, parallel design study over a 12-week period. Group allocation was randomised by the supplement supplier, who had no involvement in the trial, and double blinded to reduce bias or interference from the participants and the research assistants. The trial sponsors used the website (http://www.randomization.com) which randomized the participants to one of three groups (three randomly permuted blocks) and produced a three digit code printed on the identical looking sachets, which became the participants ID. The three groups included a control group, who consumed a placebo (which consisted of rice flour); the psyllium fibre supplement group; or the PGX® fibre supplement group. All participants consumed the supplement with their usual diet and all study requirements and instructions were identical for each group. The rice flour provided an appropriate placebo due to its low energy and fibre content and similarity in texture and appearance to the psyllium supplement. Participants were instructed to consume 1 supplement sachet containing 5 g of the psyllium, PGX® fibre or placebo, mixed with a minimum of 250 mL water, 5 - 10 minutes before breakfast, lunch and dinner every day for 52 weeks. Extra water was taken ad libitum during or after the meal if desired and participants were encouraged to do so. Participants were asked to record any other vitamin, mineral or other supplement for the duration of the study. All identifiable information from participants was coded and kept strictly confidential. Those who missed more than 14 days of supplement consumption were discontinued from the trial.

Participants attended a briefing session, where they were instructed on how to consume the fibre supplements, complete the questionnaires and other paperwork and comply with the study protocol. Dietary intake over the course of the trial was monitored through the completion of 3-day food diaries at each long clinical visit (Week 0, 12, 26 and week 52), and participants were given detailed instructions on how to complete food diaries to a high standard. All participants in the control and the fibre supplement groups were asked to maintain their usual diet for the duration of the study. To monitor compliance, all participants were required to complete a diary to record their supplement consumption and asked to return the empty and unused sachets of the supplements at their clinical visits. These sachets were then counted and recorded. The trial was conducted in compliance with the study protocol. All participants were asked to report any adverse events to the investigators immediately. Medical support was to be provided for any serious adverse events resulting from this study. In addition, such events were to be recorded by the Investigator and reported to HREC. Participants would then be given the option to withdraw from the study and all data and blood samples for this participant would be destroyed after withdrawal. However no such events occurred as a result of participating in this study.

Figure 2.1 Complete participant flow diagram for 2012 study



***The current study examined baseline and 3-month data collected during a 12-month study, as illustrated above.**

ASSESSMENTS

2.3 Micronutrients Blood Analysis

Blood samples were collected at four intervals as part of the aforementioned clinical trial (eg at weeks 0, 12, 26 and 52). For the purposes of this present study, samples collected at weeks 0 and 12 only were analysed. Participants attended Curtin University, after an overnight fast of 10-12 hours, where blood samples were drawn by venepuncture. Samples were collected into lithium heparin or serum separator tubes (5 ml) for antioxidant/vitamins A, C, D, B_{12}, B_9 and E and minerals (calcium, iron, iodine, magnesium, potassium, sodium and zinc) for analysis. The blood collection, preparation, storage and assays were conducted on site by a research assistant trained in venepuncture at the School of Public Health laboratory at Curtin University. Blood samples were then centrifuged at 2,500 rpm at 4ºC for 10 minutes using an Eppendorf centrifuge and prepared for storage at -80ºC. Micronutrients were to be analysed systematically once all the participants had completed the 52-week study. Antioxidant vitamins A, E, D, and total antioxidant capacity as well as vitamin B 6, and 12 were measured using Enzyme-Linked Immunoabsorbant Assay kits (ELISA). ELISA is a method which combines antibody binding with enzymatic detection to identify molecules of interest; the result is a colour change that is measured by spectrophotometry at a particular wavelength (40). Folate was measured by competitive binding using ELISA. All trace metals (calcium, magnesium, iron, zinc and potassium) were analysed by Flame Atomic Absorption Spectroscopy (FAAS). Atomic absorptiometry measures the concentration of gas-phase atoms or ions as a solution via the absorption of light after the solution is vaporized in a flame or graphite furnace (41).

2.3.1 3-day Food Diaries

There are advantages and disadvantages to any dietary assessment method as they rely on participant compliance, accurate recall and recording of food consumption. Food diary data collected over a longer period of time (7-14 days), is thought to be more reflective of usual nutrient intakes. However studies have shown that increased length of food diaries reduces the accuracy of results, as participants can become increasingly less compliant over time (36, 37). Given that there was a high participant burden in the 2012 study with the number of clinical visits clinical assessments, and paperwork to be completed over 12 months; 3-day food diaries

were chosen to minimise participant burden whilst still being reflective of normal dietary intake. The 3-day food diaries are seen as a widely accepted and reproducible method in nutritional research, and include 2 weekday and one weekend day's intake (36). Food diaries were completed at 4 time intervals over the 2012 12-month study, at week 0, 12, 24 and 52. For the purpose of this thesis baseline to 3-month food diary data was analysed. Multiple food diaries completed over the duration of the study serves to increase reflectiveness of 'normal intake'. Participants were also instructed at induction session on how to complete food diaries to a high standard.

2.3.2 Outcome measures

The present study was designed to assess participants' nutritional status at baseline and week 12 (ie before and during the study) in relation to: vitamins A, C, D, B_{12}, B_9 and E and minerals calcium, iron, iodine, magnesium, potassium, sodium and zinc. Other outcome measures that formed part of the larger study, such as weight/BMI and metabolic syndrome risk factors, have been analysed and discussed elsewhere (38, 42).

2.3.3 Sample size calculation

According to Cohen's effect size conventions for an ANCOVA F test, a minimum sample size of 37 subjects per group is estimated to detect a medium effect size (30%) in micronutrient levels between groups at 5% significance level with an 80% study power (43). Considering a 10% drop-out rate, a collected sample of 126 participants from a large study are sufficient for this study with an assignment of 42 participants per group (total 126) (44). For this study 53 participants were recruited for each group (control, psyllium and PGX) for a total of 159 participants.

2.3.4 Statistical analysis

Descriptive statistics were obtained for variables of interest, including baseline outcome measures (presented in Chapter 3) and all outcome measures by groups (the control group who consumed a placebo (rice flour); the Psyllium fibre supplement group; and the PolyGlycopleX® (PGX®) fibre supplement group: presented in Chapter 4). Data was expressed as mean ±SEM. Normality was assessed prior to conducting the following analyses. Between-groups difference in micronutrient

levels was examined using one-way ANOVA and within-group difference (week 0 (baseline) vs week 12) was examined using a paired samples t test. A one-way analysis of covariance (ANCOVA) (carried out by General Linear Models in SPSS) was used to explore the effect of higher daily doses of fibre on the micronutrient levels, adjusted for the baseline micronutrient status at week 0 (baseline) and also the dietary effects during the study period as covariates. If an overall significant between-groups difference was present, post hoc comparisons between the groups will be made using a Least Significant Difference (LSD) method. Statistical significance was considered at $p < 0.05$. Statistical analysis was undertaken using IBM SPSS 21 for Windows (SPSS Inc., Chicago, IL).

2.4 Significance

In the last three to four decades, a great deal of research has brought new insights into the health promoting properties of dietary fibre. Increased dietary fibre in supplement form is now becoming known for its role in satiety, reducing metabolic risk factors and supporting weight loss. However, the long-term effects of this dietary intervention method need to be examined, as earlier studies had shown fibre to act as an inhibitor of micronutrient absorption in the gut, for example calcium, iron, trace metals, and certain vitamins, by impairing their bioavailability. Therefore, the results of this research will aim to provide some clarification of the effect high intakes of certain fibre sources have on the micronutrient status of individuals with overweight and obesity. This is important as impaired micronutrient status can negatively impact health, and may compound the already detrimental effects of overweight and obesity. Adding this information to the body of knowledge may help inform weight management treatments in future.

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CHAPTER THREE

PAPER ONE

McKay, J., Ho, S., Jane, M. et al. Overweight & obese Australian adults and micronutrient deficiency. BMC Nutr 6, 12 (2020). https://doi.org/10.1186/s40795-020-00336-9

3.1 ABSTRACT

Background: Micronutrients have been implicated as an important factor in regulating various metabolic processes and thus playing a role in the aetiology of obesity. Many studies have been conducted worldwide that clearly show a direct link between obesity and micronutrient deficiencies. The aim of this study was to assess the nutritional status of overweight and obese Australian adults to identify any negative associations between BMI and serum micronutrient levels.

Methods: Baseline serum micronutrient data of overweight and obese individuals with a body mass index (BMI) between 25-40 kg/m^2 and aged between 18 and 65 years was compared to the clinical micronutrient reference ranges for associations between BMI and micronutrient status.

Results: There were significant negative associations between BMI and serum vitamin D ($p=0.044$), folate ($p=0.025$), magnesium ($p=0.010$) and potassium ($p=0.023$).

Conclusions: Overweight and obesity appears to impact on the bioavailability and utilisation of micronutrients with absorption, excretion, storage/distribution (fat sequestering, tissue dispersion), metabolism (catabolic losses, possibly oxidative), increased physiologic requirements, and lower absolute total dietary intake being the current theory for observed differences. While vitamins D, folate, magnesium and potassium showed a negative relationship to BMI, there appeared to be no association between BMI and the remaining micronutrients. This may be explained by the fortification of certain processed foods, or the possibility of overweight and obese people eating more to satisfy their nutritional requirements.

Keywords: Obesity, micronutrients, nutrient reference values (NRVs), absorption, metabolism, bioavailability, body mass index (BMI), vitamins, minerals, deficiency.

3.2 BACKGROUND

In most cases, overweight or obesity is due to a positive energy balance stemming from excessive energy intake - relative to energy expenditure - from diets that are typically nutritionally poor (1). Excess body weight has been shown to alter the absorption, distribution, metabolism, and/or excretion of micronutrients. (2). For example, vitamin D from cutaneous and dietary sources has been shown to have decreased bioavailability in obese persons and is potentially commandeered by adipose tissue (3). Thiamine metabolism is also impacted in obese persons, leading to a decrease in cellular absorption and an increase in intracellular conservation (4).

Micronutrient interactions within the food matrix can impact absorption and bioavailability by a number of mechanisms. Minerals with chemical similarities can compete for transport proteins or other uptake mechanisms, as well as for chelating organic substances, facilitating or hindering absorption (5). The overall impact of these interactions will be determined by the relative concentrations of the nutrients. At normal dietary concentrations the absorption of most micronutrients is active or saturable, while at higher intakes passive diffusion can take place (2, 5). A poor nutritional status affects mucosal integrity and can thereby affect absorption of other nutrients (2).

Nutrient reference values as provided by the National Health and Medical Research Centre (NHMRC) are ‘Recommended Dietary Intake’ (RDI) or Adequate Intake (AI), which includes the amounts of specific nutrients required on average each day for sustenance or avoidance of deficiency states (6). Further nutritional advice is also provided in the form of national ‘Dietary Guidelines’. These guidelines provide culturally-relevant food and dietary patterns that will not only achieve nutritional balance, but also reduce the risk of nutrition related deficiency and chronic disease.

When the diet of obese individuals is nutrient poor, the question arises as to whether micronutrient supply from consumed foods is sufficient to provide for biochemical and physiological demands. Especially when the bioavailability and absorption of micronutrients, is impacted by obesity itself. Current research is suggesting that the higher the BMI the more significant the deficiency in certain micronutrients (1, 2, 7).

On the other hand, consumption of nutritionally poor foods may further drive the physiological desire to consume in excess, in order to attain nutritional sufficiency.

Few studies have examined the nutritional status of overweight and obese adults. One such study suggested vitamin D, chromium, biotin, thiamine and vitamin C levels are significantly lower in persons who are obese and that insufficiency in these micronutrients has the ability to impact glucose metabolism and cause insulin resistance (8). Biochemical markers of nutritional status in overweight or obese populations have been previously studied, however these studies have often been limited by small sample sizes, or the examination of only a small number of micronutrients (9).

The current study examined baseline blood micronutrient levels of a group of overweight and obese Australian adults with the most up-to-date serum nutrient reference ranges for Australian adults. The aim of this study was to assess the nutritional status of overweight and obese Australian adults prior to the commencement of a high fibre weight loss clinical trial. Furthermore, to ascertain the extent to which BMI negatively correlates with serum micronutrient levels. Vitamins A, B_{12}, C, D, E and B_9 as well as the minerals, iron, iodine, calcium, potassium, sodium, magnesium and zinc were evaluated.

Hypothesis:

As a negative correlation between micronutrient status and overweight and obesity has been noted in previous studies. It is hypothesised that, in the current study sample, as BMI increases micronutrients levels will decrease.

METHODS

3.3 Participants

Overweight and obese individuals (n = 127) with a BMI between 25-40 kg/m^2 and aged between 18 and 65 years, were recruited from the community in Perth, Australia via advertisements in newspapers, flyers posted around the University and community noticeboards, as well as radio advertising on Curtin FM. Potential participants were screened by telephone or online using a Qualtrics Survey and attended an orientation session at Curtin University to assess suitability for the study, at which time the details of the study were also explained. Exclusion criteria included

smoking, lipid lowering medication, use of steroids and other agents that may influence lipid metabolism, diuretics, use of warfarin, diabetes mellitus, hypo and hyperthyroidism, cardiovascular events within the last 6 months, psychological unsuitability, major systemic diseases, gastrointestinal problems, proteinuria, liver, renal failure, weight fluctuations over the past 6 months, vegetarianism or veganism and participation in any other clinical trials within the last 6 months.

3.3.1 Study design

The baseline data analysed in the current study was extracted from a larger intervention study conducted in 2012, entitled: Comparison of two different fibre supplements on body weight, body composition, metabolic and cardiovascular risk factors in overweight and obese individuals. The 2012 study was randomised, controlled double blind, parallel design study over a 12-month period. Group allocation was randomised by the supplement supplier, who had no involvement in the trial, and double blinded to reduce bias or interference from the participants and the research assistants. Participants attended a briefing session on how to consume the supplements, complete the paperwork and comply with the study protocol. Dietary intake over the course of the trial was monitored through the completion of 3-day food diaries at each clinical visit (week 0, 12, 26 and 52). Participants in all groups were asked to maintain their usual diet for the duration of the study. For the purposes of this study only serum and food diary data obtained from week 0 was used for comparison.

ASSESSMENTS

3.4 Micronutrients Blood Analysis

For the purposes of this present study, assays were conducted on baseline blood samples from 127 participants enrolled in the 2012 study referred to previously (10, 11). Volunteers attended Curtin University, after an overnight fast of 10-12 hours, where blood samples were drawn by venepuncture. Samples were collected into lithium heparin or serum separator tubes (5 ml) for antioxidant/vitamins A, B_{12}, B_9, C, D and E and minerals (calcium, iron, iodine, magnesium, potassium, sodium and zinc) for analysis. All blood samples were collected by a trained phlebotomist. Blood samples were then centrifuged at 2,500 rpm at 4ºC for 10 minutes using an Eppendorf centrifuge and prepared for storage at -80ºC. Micronutrients were

analysed systematically, after all the participants had completed the 52-week study. Vitamins A, E, D, as well as vitamin B_9, and B_{12} and thyroglobulin as a measure of Iodine status were measured using Enzyme-Linked Immunosorbent Assay kits (ELISA). ELISA combines antibody binding with enzymatic detection to identify molecules of interest; the result is a colour change that is measured by spectrophotometry at a particular wavelength (12). Vitamin C was measured by colorimetric assay. All trace metals (calcium, magnesium, iron, zinc, sodium and potassium) were analysed by Flame Atomic Absorption Spectroscopy (FAAS). Atomic absorptiometry measures the concentration of gas-phase atoms or ions as a solution via the absorption of light after the solution is vaporized in a flame or graphite furnace (13).

3.4.1 Outcome measures

This study was designed to assess the nutritional status of overweight and obese participants in relation to: vitamins A, B_{12}, B_9, C, D E, folate and minerals calcium, iron, iodine, magnesium, potassium, sodium and zinc at baseline. Other outcome measures that formed part of the 2012 study, such as weight/BMI and metabolic syndrome risk factors, have been analysed and discussed elsewhere (11, 14).

3.4.2 Statistical Analysis

To determine the nutritional status of this sample (n=127) of overweight/obese individuals, baseline micronutrients were observed with the clinical reference intervals (serum) sourced from the Stedman's Medical Dictionary (established by the Harmonisation Committee of the Australasian Association of Clinical Biochemists (AACB)) or, where there was an absence of data, The Merck Manual (2, 15). The reference range for thyroglobulin (as a marker of iodine status) however was taken from the assay manual as neither Stedman's Medical Dictionary nor the Merck Manual had useful values for this micronutrient. The food diary micronutrient data were compared to the National Health and Medical Research Council (NHMRC) Nutrient Reference Values, which are the recommended dietary intake to maintain good health (16).

For baseline analysis of serum micronutrients, one sample t-tests were conducted. in order to determine the nutritional status of the study sample prior to the intervention.

Baseline serum micronutrients were compared to the Clinical Reference Values (CRV) for Australian adults. Therefore, as the CRVs are standardised values used to determine nutritional status, and baseline values were collected prior to administration of any treatment of the entire study sample (n=126), a one-sample t-test was used. Review of the literature in Chapter 1 and the 'Background section' for this chapter has noted that overweight and obesity is negatively correlated with micronutrient levels. A one-tailed test was used to determine the extent the observed mean for each micronutrient differed from the CRVs in one direction only.

The lower limit was obtained from the clinical reference range for each micronutrient used as the test value, with the exception of thyroglobulin (an indirect measurement of iodine status), where the upper limit was used (17). The baseline data did not meet the assumption of normality for simple linear regressions (or one-sided Pearson's bivariate correlation). Furthermore, a negative correlation between micronutrient levels and overweight and obesity had been noted in the literature previously, so instead a one-side non-parametric bivariate correlation (Spearman's) between BMI and each micronutrient was conducted to explore this correlation further. Statistical significance was considered at $p<0.05$. All statistical analysis was conducted using SPSS 23.0 (IBM® SPSS® Statistics, New York, NY).

RESULTS

3.5 Baseline characteristics

Blood samples, for the 127 overweight and obese participants, were analysed for baseline micronutrient levels. The group results showed that the average age of the study participant was 49.3 ± 1.0 years, the average weight was 94.0 ± 1.5 kg and average BMI was 32.3 ± 0.4 kg/m^2. The gender of the study group was mostly female with 73 women versus 54 men. Of the 127 participants 33.1% were classified as overweight and 66.9% fell within the obese category according to BMI.

3.5.1 Nutritional assessment

Mean baseline serum and dietary intake data is outlined in Table 1. Dietary intake data was analysed using FoodWorks nutritional analysis software. For the purposes of this study iodine levels from blood samples were measured as serum thyroglobulin, as it is a more accurate biomarker for iodine deficiency (17). Values

were expressed as Mean ± SEM in their units of measurement for serum. Table 2 compares the Mean ± SEM serum values with the clinical reference intervals with values being either less than (<), within range or greater than (>) clinical reference intervals (15). Table 3 compares the mean dietary intake data analysed using FoodWorks nutritional software against the Nutrient Reference Values (NRV) for Australia and New Zealand (16). Data from FoodWorks is not available for B_{12} and direct comparison between the NRVs and vitamin A, measured as retinol equivalents (RE) was not possible either as FoodWorks breaks vitamin A into retinol and beta carotene (refer to table 4). Mean ± SEM serum values for micronutrients were also correlated with BMI (Table 4) and significant associations can be seen in Figure 1 a), b), c) and d). The relationship between self-recorded dietary intake and BMI was also examined as shown in Table 5, and there were no significant differences $p>0.05$ found. As data analysis for vitamin B12 cannot be performed in FoodWorks, this micronutrient is not in Table 5.

3.5.2 Correlations

Baseline serum and dietary micronutrient concentrations of the participants, are shown in Table 1). Mean and median values of this population are shown, however for the purposes of statistical analysis, median values were used. The data was not normally distributed due to outliers so median values were seen as the most accurate representation and more likely to be applicable to the wider community than mean values.

The baseline serum values were compared with the clinical reference intervals for nutrients in Australia (AACB) (18)– shown in table 2). Serum values were either 'lower than the recommended range (<)' indicating a deficit of that particular nutrient, 'within range' indicating sufficiency or 'greater than the recommended range (>)' indicating excess. The results (Table 2) showed that 62.7% of the participants in the study were within the healthy reference range for dietary vitamin E (5-20 ug/mL), with a mean value of 7.79 ± 0.5 µg/mL. For vitamin B_{12} 57.1% of the sample population in the study were within range where as 41.3% were in excess of the reference range with mean levels at 722.9 ± 41.3 pg/mL. Vitamin C showed that 96.1% of the sample were in excess of the reference range whereas 100% of subjects did not meet the clinical reference interval for vitamin A; results were considerably less that the reference range of 28-86 ug/dL with the mean sample value

only 5.04 ± 0.2 ug/dL. The majority (89%) did not reach required levels for vitamin D, deficiency is described at <20-24 ng/mL and the sample mean was 10.9 ± 0.6 ng/mL. Only 28% were within the reference range for folate, with deficiency <3 ug/L, the sample mean was 2.5 ± 0.2 ug/L. However, thyroglobulin levels were mostly (84.9%) within range 2-50 ng/ml with a mean of 8.8 ± 1.3 ng/mL. 100% of the sample did not meet the NRV for potassium with deficiency at <3.5 mEq/L; and the sample mean: 2.5 ± 0.02 mmol/L. Sample population serum values for sodium (Mean 118.8 ± 0.9 mmol/L), zinc (Mean 27.9 ± 1.2 µg/dL) and calcium (3.4 ± 0.1 mg/dL) levels were all lower than the clinical reference interval. The majority of participants were within the clinical reference interval for iron with a reference of 30 to 300 ng/mL for serum ferritin and mean values for men 114.4 ± 4.8 µg/dL and women 95.2 ± 4.4 µg/dL respectively. Serum magnesium levels were significantly lower than the clinical reference interval with a mean value of 0.7 ± 0.01 mg/dL compared to 1.8-2.6 mg/dL.

Table 3.3) shows micronutrient status of participants at baseline using self-reported mean daily dietary intake data compared to NRVs for Australia and New Zealand (NHMRC). Table 3.3) indicates that recommended nutritional intake from the diet is being met for some micronutrients (vitamin E, D, B_9, iodine, potassium, calcium and iron for 19-50 year old females), but not for others (sodium, vitamin C, magnesium, zinc and β-carotene). Vitamin E is measured as adequate intake (AI) per day for 19-70 years old's, and for men the reference is 10 mg while for women its 7mg per day. The study participants were under the reference value for men (8.1 mg) and slightly over the reference value for women (7.9 mg). The RDI for vitamin C is 45 mg daily for 19-70 year old's; the study participants well exceeded this RDI at 76.9 mg. For vitamin A, retinol equivalents (RE) are the reference used. Retinol (µg) RDI is 900 RE and 700 RE for β-carotene (µg). Study participants were under the recommendations for retinol with a median value of 322.6 µg and over the RDI for beta carotene at 2134.2 µg. For vitamin D, both men and women were under the AI for both age groups, with dietary intake data showing only 3.3 µg for 19-50 years compared to the recommended 5 µg required daily and 2.8 µg for 51-70 years old compared to 10 µg required. The mean dietary folate intake was just under the 400 µg recommended daily intake value at 332.4 µg; similarly the mean iodine intake was 120.2 µg, which is below the RDI of 150 µg. For potassium, males were below

the AI of 3800 mg daily with a median value of 3207.4 mg, whereas females exceeded AI of 2800 mg with 3053.1 mg. Dietary intake for sodium well surpassed the AI of 460-920 mg daily with 2603.2 mg recorded. Males met the RDI for iron of 8 mg per day. Females of 19-50 years of age were under the recommended 18 mg daily with 11.2 mg, but exceeded the RDI for females aged 51-70 years. The zinc RDI for 19-70 years old was met for females but slightly under recommendations for males at 13.6 mg instead of 14 mg. Both males and females did not meet the RDI for calcium, however females were further below the RDI than males. For the 19-50 year age group, data for females showed a daily intake of 869.4 mg/day compared with the 1000 mg recommended. The 51-70 year age group showed a greater deficit with a mean daily intake of 797.6 mg compared to 1300 mg RDI. The magnesium RDI was met for males in the 19-30 year age category but below the RDI for females at 301.6 mg instead of 310 mg. However the reverse was true for the 31-70 year age category, with females exceeding the RDI and males only achieving a median value of 385.2 mg instead of the recommended 420 mg.

Table 3.4) shows the correlation between BMI and serum micronutrients for participants at baseline. Significant associations (Spearman's rho) were found for vitamin D (r_s=-0.152, p=0.044), folate (r_s=-0.176, p=0.025), potassium (r_s=-0.177, p=0.023), and magnesium (r_s=-0.206, p=0.010). Vitamin D, folate, magnesium and potassium all showed that as BMI increased the serum concentration for these micronutrients decreased (refer to figure 1) a, b c) and d). The line of best fit in figure 1 a), b) and c) all show a downward trend which correlates with the negative r-values obtained for these micronutrients. However, the line of best fit for folate appears to show a neutral association with increased BMI, even though the r-value is negative (Figure 1d). The line of best fit should show a downward trend as BMI increased similar to the other micronutrients, but the flat appearance of the line may be due to possible BMI outliers skewing the line of best fit making it appear flat. Correlations for all other serum micronutrients with BMI were not considered statistically significant at $p<0.05$.

Table 3.5) shows the relationship between self-recorded dietary intake data and BMI, which showed no significant associations at $p<0.05$.

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Table 3.1) Baseline serum and dietary intake micronutrient levels of overweight and obese Australians*

	Mean ± SEM	Median	*n*
Age (years)	49.3 ± 1.0	50	127
Weight (kg)	94.0 ± 1.5	89.9	127
BMI (kg/m^2)	32.3 ± 0.4	31.8	127
SERUM*			
Vitamin E (µg/mL)	7.79 ± 0.5	6.5	126
Vitamin B12 (pg/mL)	722.9 ± 41.3	707.8	126
Vitamin C (mg/dL)	3.7 ± 1.4	3.5	127
Vitamin A (µg/dL)	5.04 ± 0.2	5.6	127
Vitamin D (ng/mL)	10.9 ± 0.6	9.5	127
Folate (ng/mL)	2.5 ± 0.2	1.7	125
Thyroglobulin (ng/mL)	8.8 ± 1.3	5.9	119
Potassium (mmol/L)	2.5 ± 0.02	2.5	127
Sodium (mmol/L)	118.8 ± 0.9	117.6	127
Total Iron (µg/dL)	103.4 ± 3.3	100	127
Male	114.4 ± 4.8	110	54
Female	95.2 ± 4.4	90	73
Zinc (µg/dL)	27.9 ± 1.2	25	127
Calcium (mg/dL)	3.4 ± 0.1	3.3	127
Magnesium (mg/dL)	0.7 ± 0.01	0.7	127
DIETARY INTAKE**			
Vitamin E (mg)	8.9 ± 0.4	7.9	127
Vitamin C (mg)	103.3 ± 6.9	76.9	127
Retinol (µg)	369.0 ± 23.8	322.6	127
Beta carotene (µg)	2681.5 ± 197.8	2134.2	127
Vitamin D (µg)	3.7 ± 0.2	3	127
Folate (µg)	373.2 ± 17.0	332.4	127
Iodine (µg)	128.4 ± 4.6	120.2	127
Potassium (mg)	3095.3 ± 73.1	3081.2	127
Sodium (mg)	2752.4 ± 100.0	2603.2	127
Iron (mg)	12.4 ± 0.4	11.6	127
Zinc (mg)	13.1 ± 0.5	12.3	127
Calcium (mg)	897.5 ± 34.6	869.1	127
Magnesium (mg)	373.4 ± 11.4	358	127

* Serum measurement units were converted to be consistent with the clinical reference values. **Values derived from self-reported data.

Table 3.2) Baseline serum values compared with the clinical reference intervals for nutrients in Australia

Outcome	Reference	*n*	%
BMI (kg/m$^{2)}$	>25 overweight	42	33.1
	>30 obese	85	66.9
Vitamin E (µg/mL)	<5	39	31.0
	Within range	79	62.7
	>18	8	6.3
Vitamin B12 (pg/mL)	<110	2	1.6
	Within range	72	57.1
	>800	52	41.3
Vitamin C (mg/dL)	<0.4	0	0.0
	Within range	5	3.9
	>1.5	122	96.1
Vitamin A (µg/dL)	<30	127	100.0
	Within range	0	0.0
	>80	0	0.0
Vitamin D (ng/mL)	<20	113	89.0
	(No reference)	14	11.0
Folate (ng/mL)	<3	90	72.0
	Within range	35	28.0
	>20	0	0.0
Thyroglobulin (ng/mL)	<2	16	13.4
	Within range	101	84.9
	>50	2	1.7
Potassium (mmol/L)	<3.5	127	100.0
	Within range	0	0.0
	>5.1	0	0.0
Sodium (mmol/L)	<136	120	94.5
	Within range	3	2.4
	>145	4	3.1
Iron (µg/dL) - Male	<65	3	5.6
	Within range	48	8.9
	>175	3	5.6
Iron (µg/dL) - Female	<50	4	5.5
	Within range	68	93.2
	>170	1	1.4
Zinc (µg/dL)	<70	126	99.2
	Within range	0	0.0
	>120	1	0.0
Calcium (mg/dL)	<4.64	116	91.3
	Within range	4	3.1
	>5.28	7	5.5
Magnesium (mg/dL)	<1.6	127	100.0
	Within range	0	0.0
	>2.6	0	0.0

*Measurement units were converted to be consistent with the clinical reference values. "<" may indicate deficiency, ">" may indicate excess, except for thyroglobulin, where a higher value may indicate iodine deficiency.

315 **Table 3.3)** Analysis of dietary micronutrients using 3-day weighed food diary at
316 baseline compared to nutrient reference values for Australia

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Outcome	Gender	Age (years)	Reference	Mean ± SEM	Median	n
Vitamin E (mg)	Male	19-70	10 (AI)	8.5 ± 0.6	8.1	54
	Female	19-70	7 (AI)	9.1 ± 0.6	7.9	72
Vitamin C (mg)	M/F	19-70	45 (RDI)	103.3 ± 6.9	76.9	126
Retinol (µg)*	Male	19-70	900 RE* (RDI)	369.0 ± 23.8	322.6	126
Beta carotene (µg)*	Female	19-70	700 RE* (RDI)	2681.5 ± 197.8	2134.2	126
Vitamin D (µg)	M/F	19-50	5 (AI)	3.6 ± 0.3	3.3	63
	M/F	51-70	10 (AI)	3.7 ± 0.7	2.8	63
Folate (µg)	M/F	19-70	400 (RDI)	373.2 ± 17.0	332.4	126
Iodine (µg)	M/F	19-70	150 (RDI)	128.4 ± 4.6	120.2	126
Potassium (mg)	Male	19-70	3,800 (AI)	3156.3 ± 116.3	3207.4	63
	Female	19-70	2,800 (AI)	3034.2 ± 88.8	3053.1	63
Sodium (mg)	M/F	19-70	460-920 (AI)	2752.4 ± 100.0	2603.2	126
Iron (mg)	Male	19-70	8 (RDI)	13.7 ± 0.7	13.0	54
	Female	19-50	18 (RDI)	11.4 ± 0.6	11.2	37
	Female	51-70	8 (RDI)	11.5 ± 0.7	10.6	35
Zinc (mg)	Male	19-70	14 (RDI)	14.3 ± 0.8	13.6	54
	Female	19-70	8 (RDI)	12.2 ± 0.6	11.2	72
Calcium (mg)	Male	19-70	1000 (RDI)	967.0 ± 64.6	896.7	54
	Female	19-50	1000 (RDI)	861.2 ± 52.9	869.4	37
	Female	51-70	1300 (RDI)	828.6 ± 48.0	797.6	35
Magnesium (mg)	Male	19-30	400 (RDI)	414.2 ± 46.0	444.8	3
	Male	31-70	420 (RDI)	395.4 ± 17.0	385.2	51
	Female	19-30	310 (RDI)	310 ± 58.5	301.6	6
	Female	31-70	320 (RDI)	360.2 ± 16.1	348.8	66

AI: Adequate Intake; RDI: Recommended Dietary Intake; RE: Retinol Equivalents. Use of AI and RDI, as well as age and gender values, is based on currently available data. Incomplete data from FoodWorks nutritional analysis software means that direct comparisons between Retinol Equivalents and dietary intake couldn't be made. *1 µg Retinol Equivalent is equivalent to: 1 µg of all-trans retinol; 6 µg all-trans ß-carotene; or 12 µg of a-carotene, ß-cryptoxanthin and other provitamin A carotenoids (16).

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Table 3.4) Correlation between BMI and serum micronutrients of participants in the study

	n	Correlation Coefficient	*Sig. (1-tailed)
Vitamin E	126	-0.019	0.418
Vitamin B12	126	-0.093	0.150
Vitamin C	127	-0.133	0.068
Vitamin A	127	0.078	0.190
Vitamin D	127	-0.152*	0.044*
Folate	125	-0.176*	0.025*
Iodine	119	-0.067	0.236
Potassium	127	-0.177*	0.023*
Sodium	127	0.092	0.151
Iron	127	-0.122	0.086
Zinc	127	-0.034	0.352
Calcium	127	-0.087	0.166
Magnesium	127	-0.206*	0.010*

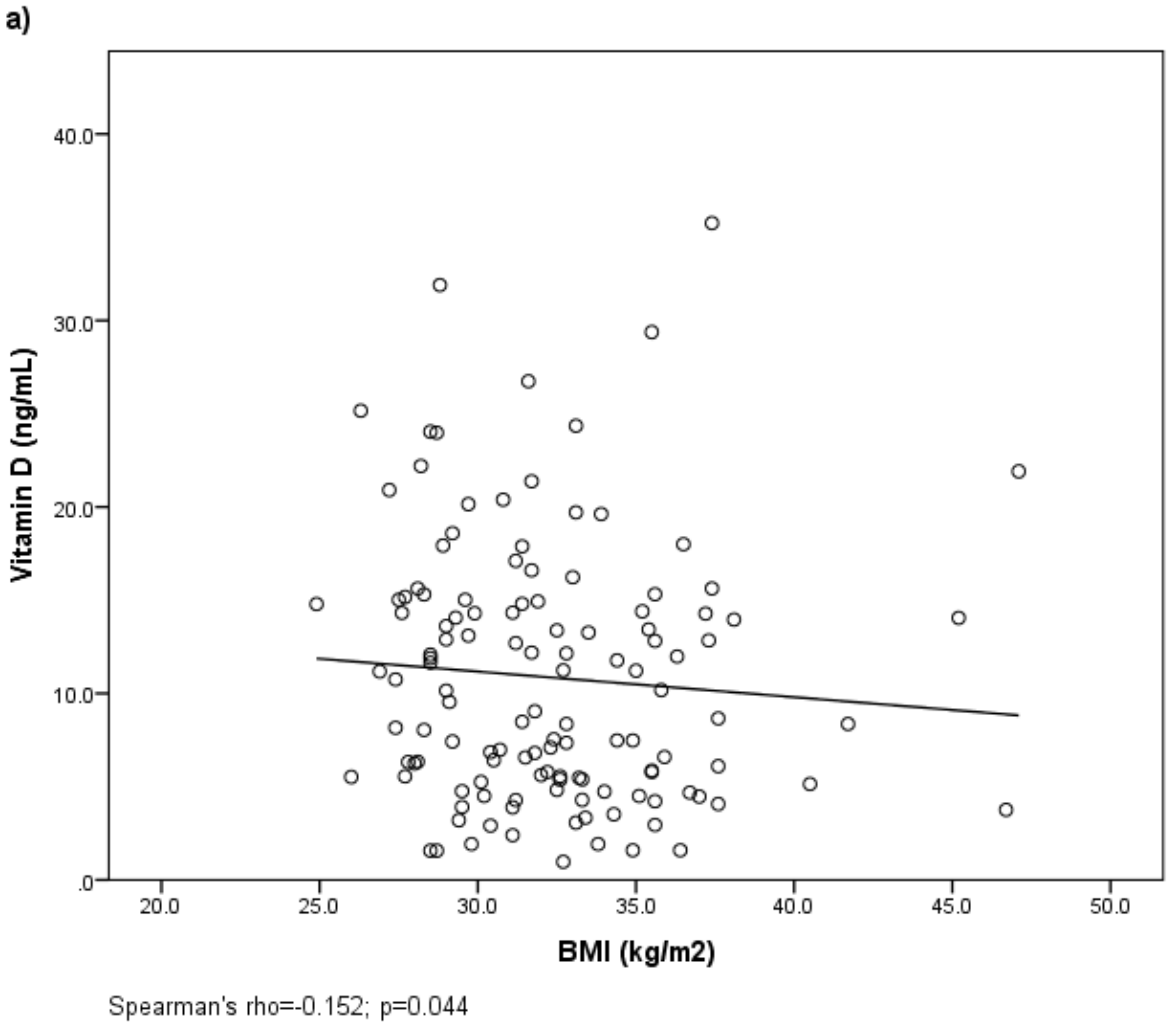
*Indicates significance at $p<0.05$

Table 3.5) Relationship between dietary intake and BMI

	n	Correlation Coefficient	Sig. (1-tailed)
Vitamin E	126	0.018	0.419
Vitamin B12	N/A	N/A	N/A
Vitamin C	126	-0.049	0.294
Retinol	126	-0.110	0.111
Beta Carotene	126	0.024	0.396
Vitamin D	126	0.038	0.335
Folate	126	-0.005	0.478
Iodine	126	0.119	0.092
Potassium	126	0.035	0.350
Sodium	126	-0.118	0.093
Iron	126	0.048	0.297
Zinc	126	-0.023	0.400
Calcium	126	0.014	0.436
Magnesium	126	0.096	0.142

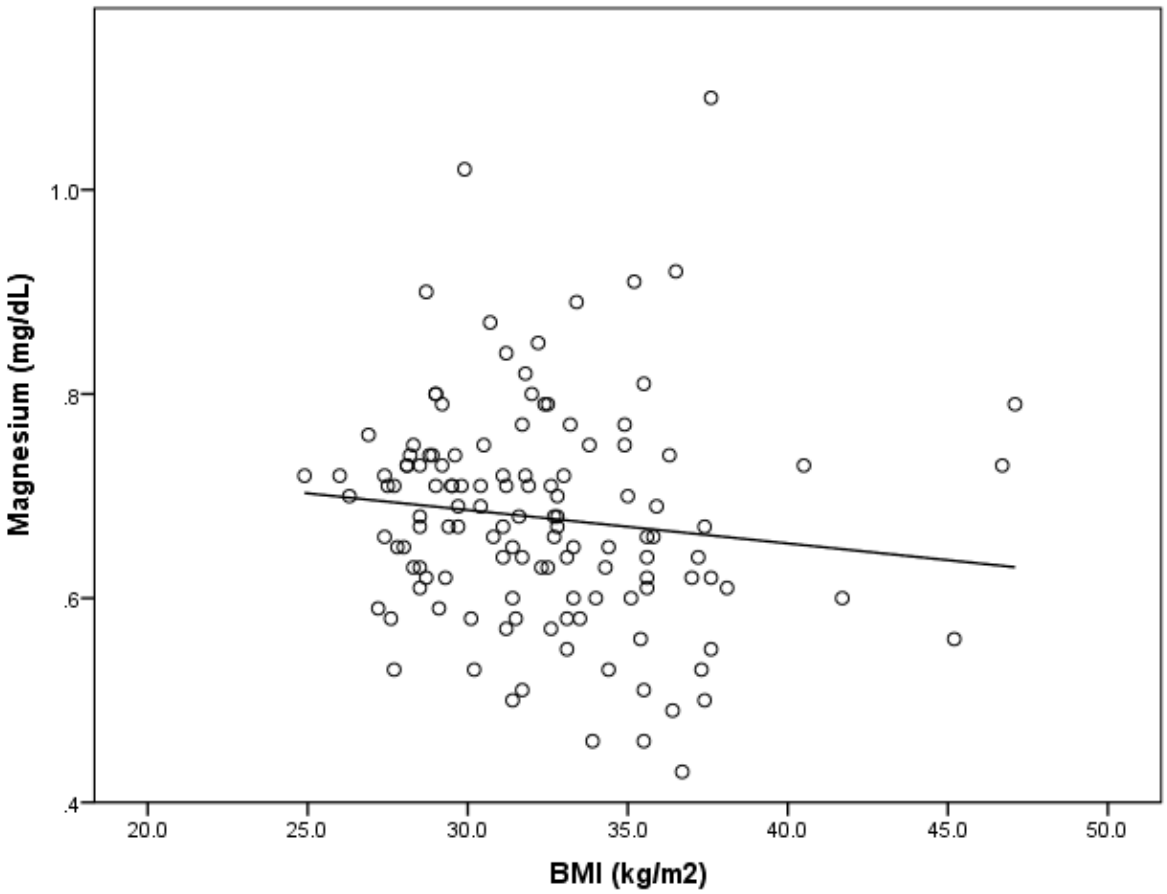
*There were no significant between group differences $p<0.05$. Data analysis for vitamin B_{12} cannot be performed in FoodWorks.

330 **Figure 1)** Significant associations between BMI and **a)** Vitamin D, **b)** Magnesium,
331 **c)** Potassium, and **d)** Folate. All *p*-values are one-sided.



332

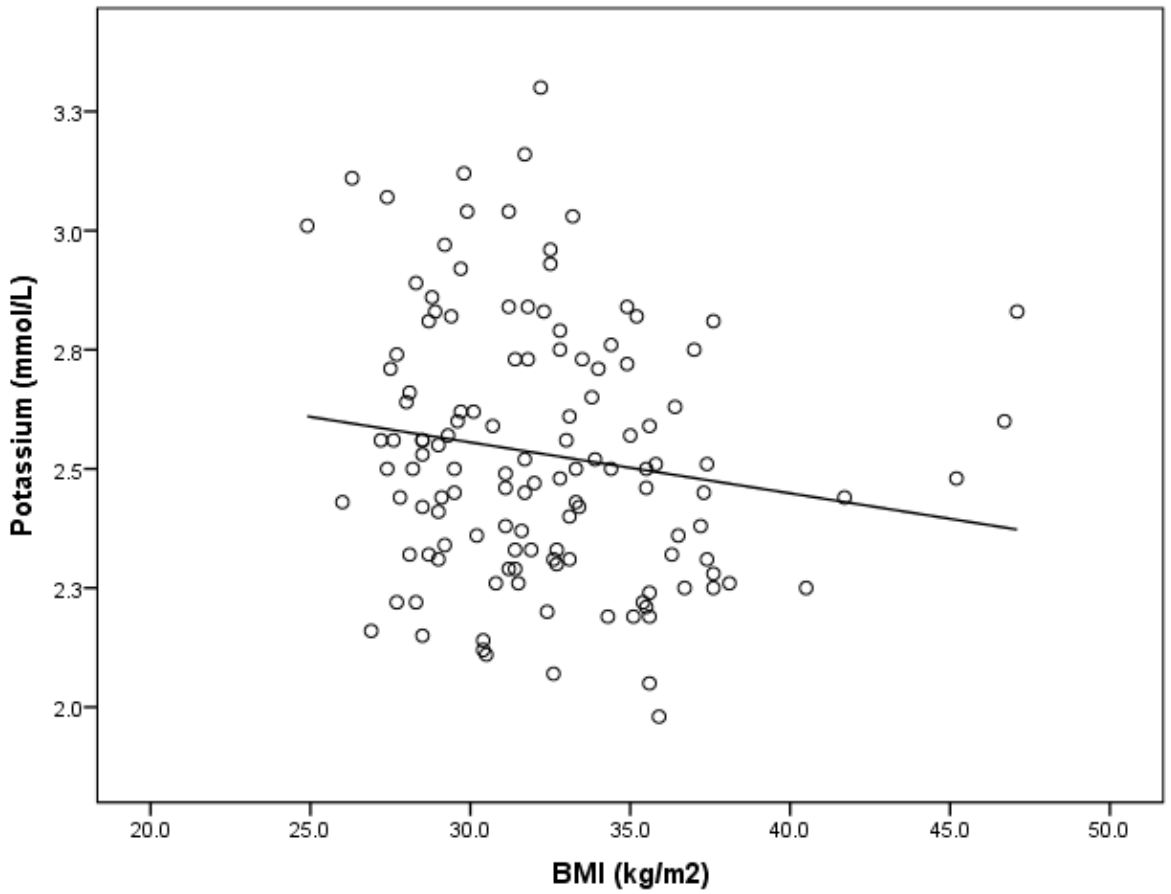
b)



Spearman's rho=-0.206; p=0.010

333

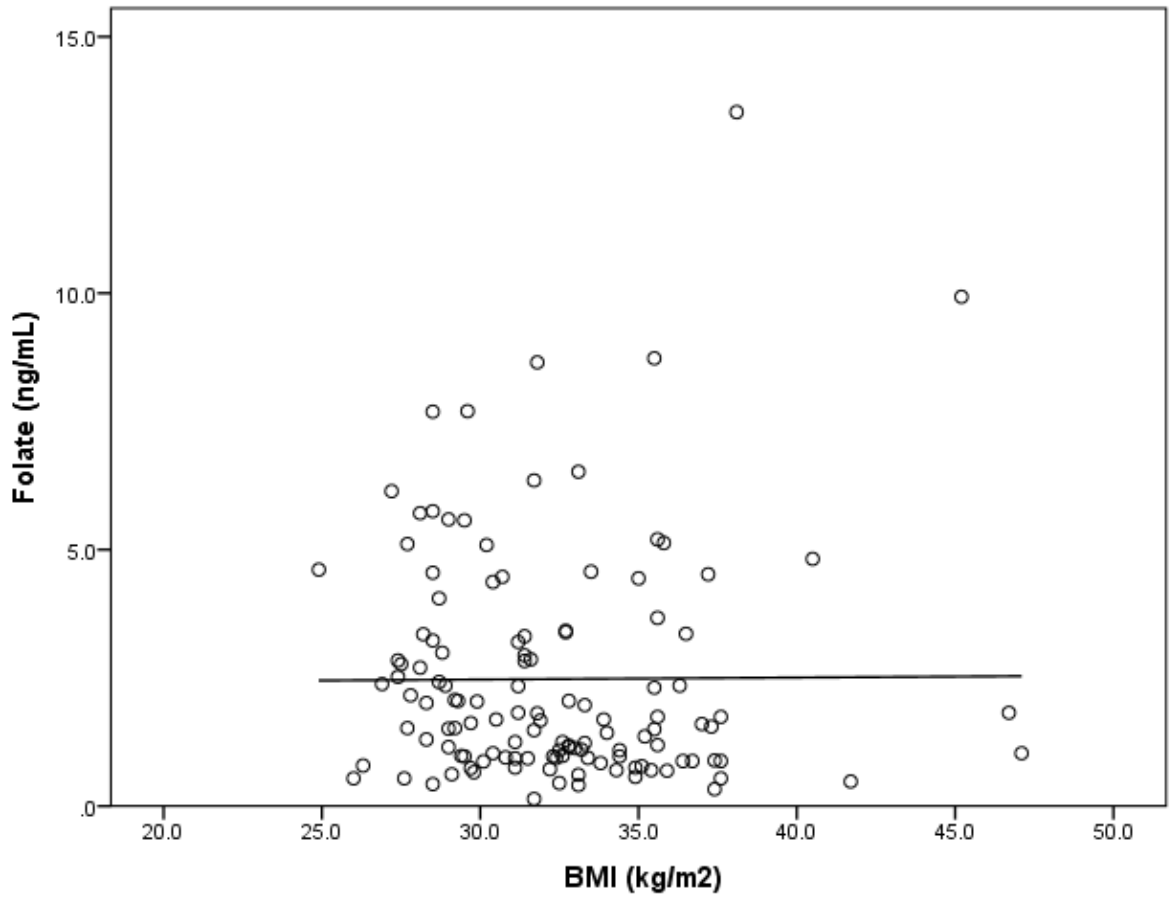
c)



Spearman's rho=-0.177; p=0.023

334

d)



Spearman's rho=-0176; p=0.025

3.6 DISCUSSION

Overweight and obesity is one of the predominant health issues in todays' global society, having superseded malnutrition in recent years (19, 20). The diet of an overweight or obese individual is typically energy dense and nutrient poor, thus low micronutrient levels may result from inadequate dietary intake and/or alterations in nutrient absorption or metabolism over time (19). Baseline serum micronutrient results for study participants indicated that vitamin D, magnesium, potassium and folate status appeared to be affected by BMI. When serum and dietary intake data was compared to the clinical reference intervals and the Nutrient Reference Values for Australia and New Zealand, many micronutrients were also outside normal ranges or recommendations.

This study found a negative correlation (Spearman's rho r_s=-0.152, p=0.044) between serum vitamin D status and body mass index (refer to table 3.4 and figure 1a), with the majority (89%) not reaching the required levels for Vitamin D which is 5 µg/day for 19-50 year old Australian adults (refer to table 3.4). Vitamin D deficiency is noted in serum levels less than 50 nmol/L, with clinical reference ranges from 60-160 nmol/L (21). Most vitamin D is obtained from sun exposure, however dietary intake of vitamin D containing foods is still important (22). Oily fish such as salmon and mackerel, eggs, mushrooms and fortified foods are among the highest vitamin D containing foods (6, 21). In Australia, there is mandatory fortification of edible oils and spreads, with a minimum of 220 IU vitamin D per 100 g of food. Voluntary fortification to breakfast cereals, skim milk and powdered milk, yoghurts and cheese also occurs depending on brand, and also contributes to vitamin D intake but is not a major source (23). Baseline dietary intake data from study participants was compared to the NRV's for Australia and New Zealand (Refer to table 3.3) and it was found that vitamin D obtained from dietary sources was below the daily recommended amount. For the 19-50 year age group 5 µg is the AI per day, while the mean showed only 3.6 ± 0.3 µg was obtained from dietary sources. For 51-70 year old's the AI for vitamin D increases to 10 µg per day, however self-recorded intake showed a significant deficit at only 3.7 ± 0.7 µg per day. This could help explain why serum vitamin D levels were below the clinical reference intervals (refer to table 3.2) with 89% of participants having serum levels less than 20 ng/mL

Vitamin D has also been shown to have decreased bioavailability from cutaneous and dietary sources in overweight and obese populations, as it is potentially sequestered by adipose tissue (24). This may explain significant negative association between BMI and serum vitamin D levels during this study. A study by Sadiya et al. (2016) demonstrated that large amounts of vitamin D3 is stored in adipose tissue after vitamin D3 supplementation, and suggests that overweight and obese participants may store more vitamin D than healthy weight participants because they have larger amounts of adipose tissue (25). An Australian study by Gill, et al. (2014) found a direct correlation between BMI and vitamin D status, indicating that those with a BMI >25 had lower serum vitamin D than those with a BMI <25. Secondly, those who undertook regular physical activity had higher serum vitamin D than those who were inactive (21). Seasonal changes in vitamin D levels are also prevalent with lower serum vitamin D3 levels (≤ 50 nmol/L) found in winter and spring (26). Due to the large sample size and the rolling roster of clinical appointments, some participants baseline values were obtained during summer, autumn and winter, which may have resulted in some variability in the findings (26). While seasonal variations are important, it could be argued behavioural changes are more significant. In summer, although participants are more inclined to have more skin exposed, they are also more likely to wear sunscreen and a hat due to the harsh Australian sun and the associated increase in skin cancer risk (27). A large study by Daly et al. (2012) examined serum vitamin D in 11,247 samples collected Australia wide; results showed that vitamin D deficiency (<50 nmol/L) to be common in adults aged 25 years and over (31% of total sample), with women, those with obesity, the elderly, those from a non-European background and those with insufficient physical activity levels being most at risk of low serum vitamin D (27).

Table 3.4) shows that serum magnesium levels were also lower in those with higher BMIs (see figure 1b). This finding is further supported by all participants being outside normal serum parameters of the clinical reference intervals (Refer to table 3.2). Typically green leafy vegetables, nuts, seeds, legumes and whole grains, are good sources of magnesium (6). The RDI for Australians 19-30 years of age is 400 mg per day for men and 310 mg per day for women, and for those 31-70 years of age its 420 mg per day for men and 320 mg per day for women (Table 3.3). When using the self-reported dietary intake data and comparing the mean values to the NRVs,

dietary intake of magnesium was being met. However this did not translate to serum magnesium with all participants being below the clinical reference intervals (Table 3.2). It is estimated only 30% to 40% of dietary magnesium is absorbed by the body, so regular intake is essential (28). Previous studies have linked obesity, and obesity-related metabolic risk factors such as glucose intolerance, cardiovascular disease, dyslipidaemia and insulin resistance, with low serum magnesium (29). Low dietary intake of magnesium, impairs intestinal absorption and promotes higher circulating inflammatory markers in overweight and obese individuals (30). Intestinal inflammation is known to impair micronutrient absorption (31). There is also a correlation between lower serum magnesium and low vitamin D levels in overweight and obese individuals. Although a magnesium-regulating hormone or factor has yet to be described, the effect of vitamin D on serum magnesium concentration has been confirmed in some studies (32). A study by Farhanghi, et al. (2009) showed that low baseline concentrations of serum magnesium in obese participants can induce higher renal magnesium retention and higher magnesium absorption in the gut following vitamin D supplementation (30). The injection of the metabolite of 1,25 (OH) 2 vitamin D (equivalent to 600000 IU) given in this study significantly increased serum magnesium concentrations in obese participants with baseline serum concentrations lower than 1.5 meq/l (30). While this may explain lower magnesium and vitamin D concentrations in those with higher BMI in the current study, measuring total body magnesium status accurately can be a challenge as serum magnesium represents approximately 1% of total body Mg, which may reflect renal handling rather than dietary intake (28).

Significantly lower baseline potassium concentrations in participants with a higher BMI was also found (Table 3.4 and Figure 1c). Potassium is an intracellular cationic electrolyte, necessary for normal cellular function (33). Potassium is not stored in the human body, but excreted by the kidneys, therefore a regular dietary supply is required (34). Potassium plays a critical role in insulin secretion, hypertension and carbohydrate metabolism, glycogen storage and glucose homeostasis (34, 35). Several studies have shown that potassium and central adiposity are inversely related. Higher serum potassium is directly correlated with lower central adiposity, which is a risk factor of metabolic syndrome (MS) (34). High intakes of potassium has been shown to have a protective effect over developing overweight or obesity

and MS (33). Fruit and vegetables are a major source of potassium, as well as macronutrients, other micronutrients and dietary fibre. Under normal physiological conditions, potassium is very well absorbed by the body with about 90% absorption from dietary sources (16). Current evidence suggests that overweight and obesity reduces sodium-potassium channel sensitivity, however the exact mechanisms are unclear (33). Dietary intake data in table 3.3 showed that males were slightly below the AI for potassium at 3800 mg per day, whereas females exceeded the AI of 2800 mg per day. However the serum potassium levels for all study participants as compared to the clinical reference intervals (Table 3.2) was below the acceptable range of 3.5-5.1 mmol/L. Assessing potassium levels via serum is not the best indication of potassium status because most potassium in the body is stored inside cells. Although serum levels can provide some indication of potassium status, they are a poor reflection of tissue potassium stores (6, 33).

Baseline serum folate levels also correlated negatively with BMI (Refer to table 3.4 and figure 1d). The form of folate used in supplements and food fortification is folic acid. Folate and folic acid are found in dark green leafy vegetables, legumes, fortified cereals and foods and has a bioavailability of 50-85% depending on the food and form consumed (36). Serum folate levels as shown in Table 3.2), indicated that 72% of participants were below the clinical reference interval and 28% were within normal reference range for serum. Dietary intake data (Table 3.3) showed that males and females were just under the RDI for folate of 400 µg per day at 373.2 ± 17.0 µg. Recent studies on folic acid fortification have revealed that individuals with obesity present low fasting serum but high erythrocyte folate concentrations, as well as high levels of serum folate oxidation products (37). It has been shown that high erythrocyte folate status can reflect long-term excess folic acid intake; increased folate oxidation products are correlated with increased folate degradation as obesity can result in increased cytochrome P450 2E1 activity. Cytochrome P450 2E1 is a monooxygenase enzyme that can use folic acid as a substrate (37). Thus more folate is being metabolised in individuals with overweight and obesity and may explain the results of the present study (refer to table 3.4).

Calcium intake of the majority of participants was also below the reference value recommendation (Table 3.3). In addition, serum calcium levels were well below the

clinical reference interval for 91.3% of participants (See table 3.2), however within this study there was no correlation between calcium and BMI. Calcium intake recommendations from food sources such as dairy products, nuts, green leafy vegetables, and fortified foods and milks, for Australian adults is 1,000 mg/day for adults between 19 and 50 years of age and 1,300 mg/day for older groups (16). According to the Australian Health Survey 2011-2012, over half of the Australian population aged two years and over had inadequate usual intakes of calcium (38). Low calcium intake is considered a risk factor for certain disorders, including osteoporosis, hypertension, cancer, insulin resistance, and the metabolic syndrome (39). Interestingly, low dietary calcium intake was listed among the risk factors significantly associated with overweight and obesity in a number of published studies (40, 41) and there appears to be a direct link between high dietary calcium levels and increased faecal fat excretion. The proposed mechanism by which calcium may contribute to a negative energy balance is the formation of insoluble calcium/fatty-acid soaps, which pass unabsorbed through the intestinal tract and are excreted in the faeces (42, 43), appetite control as demonstrated from intervention studies involving dairy calcium supplementation (43) and cellular mechanisms such as the mobilisation and oxidation of fats (44). Low dietary calcium can lead to an elevated cytosolic calcium and free ionic calcium in the cytosol plays a significant part in metabolic disorders related to insulin resistance and obesity (44).

Serum sodium levels were also significantly lower than the recommended clinical reference interval (Table 3.2), however exceeded the NRVs in the 3-day dietary intake data (Table 3.3). Sodium levels within the body are maintained within a narrow range of 135 to 145 mEq/L, and the mechanisms which maintain the plasma sodium concentration in a narrow range are thirst and antidiuretic hormone (arginine vasopressin) release (45). Hyponatremia (low sodium) indicates hypotonicity - water excess for the amount of sodium present (46). Participants were instructed to fast prior to clinical visits, however were still allowed to drink water to maintain hydration for ease of venepuncture. No fasting obviously occurred during recording of the 3-day food diaries completed by participants, so this may explain why self-recorded dietary intake versus serum sodium was so different. The low serum sodium levels obtained from study participants at baseline may be due to simple dilution of serum prior to blood samples being taken, especially as the dietary intake data shows

that participants were exceeding the NRV for sodium. Fasted serum potassium was also low, and sodium and potassium correlate physiologically via the sodium potassium pump. There may have been some temporary interplay between the diluted serum sodium and increased urinary potassium and sodium losses via reduced renal recycling in the loop of Henle. However this dilution theory is not supported by the available literature and the sodium result may be due to a measurement error.

Micronutrients (and macronutrients) have been implicated as an important factor in regulating various metabolic processes and thus playing a role in the aetiology of obesity. Many studies are being conducted worldwide that clearly show a direct link between obesity and micronutrient deficiencies (24, 47). Deficiencies of various micronutrients, such as fat-soluble vitamins, B complex, vitamin C and ions such as calcium and magnesium, have been associated with increased BMI as overweight or obese is synonymous with an energy dense, nutrient poor diet (8). Current research shows that micronutrients play a crucial role in bioavailability and absorption of nutrients in the gastrointestinal tract, as well as regulate the hunger/satiety hormones (8). If dietary insufficiency is ongoing then deficiency states may present, however this is potentially offset by food fortification of vitamins and minerals in a variety of processed foods and beverages.

In an ideal world, micronutrient requirements would be met by a varied diet high in fruit, vegetables and wholegrains. However several studies have shown that people are simply not consuming the amounts required to achieve and maintain nutritional sufficiency (38). In many cases, micronutrient food fortification, which involves adding specific nutrients to flour, cereals, processed or ready to eat foods, infant formula fortification, and vitamin-enriched drinks, has been used to treat and prevent nutritional deficiency diseases in populations at risk (48). Many industrialized countries have used fortification to prevent deficiencies of vitamins A and D, several B vitamins (thiamine, riboflavin and niacin), iodine and iron (48). In addition, vitamins and minerals may be obtained easily from artificial sources, such as nutritional supplements (49). Key findings from the 2011-2012 Australian health Survey ‘Usual Nutrient Intake’ data showed that 73% of females and 50% of males aged two years and over did not meet their calcium requirements; 17% of males and 14% of females had inadequate usual intakes of vitamin A. One in eleven adult

females (aged 19 and over) did not meet the requirement for folate, however almost all males met the dietary requirement through intake; insufficient B_{12} intake accounted for between 5-8% of females dependent on age and less than one percent of males; 40% of 14-18 year old females and 38% of 19-50 year-old females had inadequate iron intakes compared to only 3% of males; less than 5% of the population did not meet their dietary needs for vitamin C and vitamin E; and, from age 14 males have a much higher requirement of zinc than females due to its key role in the male reproductive system (50). According to the Australian Health Survey results 37% of men and one in ten women (9%) had inadequate usual zinc intakes (51). One in every three people aged two years and over (37% of males and 34% of females) did not meet the requirements for magnesium, however 76% of males and 42% of females aged two years and over exceeded the UL for sodium (51).

3.6.1 Implications of associations

The exact mechanisms that explain the relationship between folate metabolism and obesity are still being established. Previous studies have reported people with higher BMI have high erythrocyte folate concentrations, as well as high levels of circulating serum folate oxidation products, but can also have low fasting serum levels (52). Overweight and obesity are associated with epigenetic changes such as abnormal DNA methylation patterns for genes involved in metabolic regulation (52). Significant associations between serum folate, DNA methylation, BMI, and body fat percentage have been reported (53). This supports the results obtained in this study as a negative correlation between BMI and baseline fasted serum folate levels was found. The current hypothesis is that higher folate intake could act as a protective factor against obesity by epigenetic mechanisms, thus a poor folate status could contribute adversely to an individual's weight (52). A prolonged deficiency in folate can lead to folate deficiency anaemia (megaloblastic) and pancytopenia. Glossitis, angular stomatitis and oral ulcers as well as neuropsychiatric manifestations such as depression, insomnia, and psychosis are also known to occur with folic acid deficiency (36). Neural tube defects are also a concern for the foetus of a pregnant woman with an insufficient folate intake (36).

A negative association with BMI for vitamin D, potassium and magnesium, implies people with higher BMI are at risk of deficiency and deficiency related conditions.

585 Vitamin D, potassium and magnesium all play essential roles in bone health and
586 calcium storage, resorption and regulation (3, 25). A study by Sadiya et al
587 demonstrated that large amounts of vitamin D3 is stored in adipose tissue and
588 suggests that overweight and obese participants may store more vitamin D than
589 healthy weight participants because they have larger amounts of adipose tissue (25).
590 There is also a correlation between lower serum magnesium and low vitamin D
591 levels in overweight and obese individuals. Vitamin D plays a role in renal
592 magnesium retention, thus low Vitamin D would contribute to low magnesium levels
593 (28, 30). Current evidence suggests that overweight and obesity alters potassium
594 channel function, however this mechanism is not currently well understood (54).
595 However a relationship between low potassium and central adiposity has been
596 established (34). Assessing potassium and magnesium levels via serum is not the
597 most accurate measure of micronutrient status as most potassium in the body is
598 stored inside cells and serum magnesium represents approximately 1% of total body
599 Mg, however serum magnesium and potassium results do still provide reliable
600 information on overall micronutrient status. Prolonged deficiencies of these
601 micronutrients can lead to osteoporosis and poor bone health for vitamin D (27). For
602 magnesium and potassium deficiency symptoms are similar and include, fatigue,
603 numbness, tingling, cramps, seizures, personality changes, abnormal heart rhythms,
604 and coronary spasms can occur (55, 56). Severe deficiency can also result in
605 hypocalcemia because mineral homeostasis is disrupted (57).

606

607 **3.6.2 Strengths and limitations**

608 This study is unique in the fact that not many studies have examined the nutritional
609 status of overweight and obese individuals, as most focus on over-nutrition in terms
610 of macronutrients, and interventions to treat this disease. The baseline figures suggest
611 show that serum micronutrient levels for vitamin D, calcium, folate, potassium,
612 sodium, magnesium and vitamin A were all below the NRV recommendation. The
613 study participants who fall below the clinical reference intervals for these
614 micronutrients may be said to have ‘high calorie malnutrition’, which is an emerging
615 theory in obesity research. In addition, it has been suggested that overweight and
616 obese individuals, who consume a large amount of highly processed food within the
617 standard ‘Western Diet’, have a physiological drive to eat excessive amounts due to
618 the poor nutritional content of these foods, and the body’s innate need to achieve

619 micronutrient sufficiency (58). This study provides some useful data on another
620 aspect of the overall health of this population subgroup.

621

622 Possible limitations of the current study may include that the blood samples and
623 dietary intake data from 3-day food diaries were only collected at baseline whereas
624 multiple time periods throughout the year may have been of more significance. Many
625 deficiency states happen over a period of time depending on the micronutrient, so
626 analysis of a 'snap shot' on the time scale may not be the most representative of
627 micronutrient levels. Some also argue serum measurements aren't the most accurate
628 assessment of nutritional status for every micronutrient. For example, bone mineral
629 density is potentially a more accurate measure than of calcium status than serum
630 calcium levels, as serum calcium is so tightly regulated within the blood.
631 Consequently if serum calcium levels fluctuate this is not necessarily an indication of
632 status, but rather something is affecting bone calcium release or renal processing and
633 reabsorption (59). Also in this study, iodine status is measured by assessing
634 thyroglobulin levels in the blood. Higher thyroglobulin levels suggest that the thyroid
635 is working harder to compensate for low iodine levels and may be an indication
636 of iodine deficiency (17). However some experts suggest urinary iodine tests are a
637 more accurate measure of status (60). For the purposes of the confines of this study,
638 serum measurement of micronutrients was the best method available at the time.
639 Misreporting by participants also had the potential to introduce confounders for this
640 study. Including under reporting or not enough detail recorded by participants of
641 food diaries, consumption of multivitamins that weren't recorded, starting
642 medications that may interfered with the data.

643

644 **3.6.3 Significance**

645 The baseline micro-nutritional status of individuals with overweight and obesity has
646 not often been examined. To date, studies examining the effects of overweight and
647 obesity on the micronutrient status of an individual are limited. However, the
648 available research suggests an inverse relationship between obesity and
649 micronutrients; negative health implications for an individual of prolonged
650 deficiency or low status are far reaching. Comparing serum and dietary intake
651 micronutrient levels of overweight and obese Australians against the clinical

reference intervals and nutrient reference values for Australia, respectively, has highlighted the disparity between dietary guidelines and micronutrient levels in the study sample.

3.7 Conclusion

There is limited research examining micronutrient status in individuals with overweight and obesity, and it is probable that different mechanisms explain nutrient-specific increases or decreases among the overweight and obese. Several hypotheses have been proposed to explain the variability of serum micronutrient levels in this subset of the population. These hypotheses include how a vitamin or mineral is absorbed, excreted, stored/distributed, whether its sequestered by fat or dispersed in tissue, or lost to metabolic processes (either catabolic loses or oxidative), as well as increased physiologic requirements, and lower absolute total dietary intake (4, 9). On the other hand, overeating to compensate for a nutritionally poor diet and food fortification combined, may explain why serum micronutrient levels are not more severely affected in the overweight and obese participants in this study.

This study aimed to identify potential relationships between BMI and baseline micronutrient status, and found that participants within this sample with a higher BMI tended to have lower serum vitamin D, folate, magnesium and potassium levels. Serum micronutrient levels compared to the clinical reference intervals and dietary intake data compared to the NRVs also supported the theory that diet quality in this subset of the population is poor. More research is needed to further clarify the associated health implications of micronutrient deficiency or surplus in overweight and obese individuals.

680

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CHAPTER FOUR

PAPER TWO

McKay, J., Ho, S., Jane, M., S, Pal. Micronutrient status of individuals with overweight and obesity following 3 months' supplementation with PolyGlycopleX (PGX®) or psyllium. (Submitted to Journal of Human Nutrition and Dietetics 06/10/2020).

4.1 ABSTRACT

Background: Safe and effective weight control strategies are needed to curtail the current obesity epidemic worldwide. Increasing dietary fibre has shown positive results with weight loss as well as in the reduction of metabolic syndrome risk factors. However, fibre can act as an inhibitor to the bioavailability of micronutrients in the gastrointestinal tract. While there is a substantial amount of scientific research into psyllium fibre, PolyGlycopleX (PGX®) is a novel fibre and as yet the effects of PGX® on micronutrient status is not well researched.

Aim: To determine whether 3-months' supplementation with 15 g of control (rice flour), psyllium or PGX® fibre daily affects micronutrient status of overweight and obese adults.

Methods: Overweight and obese individuals with a BMI between 25-40 kg/m^2 and aged between 18 and 65 years, but otherwise healthy, were instructed to consume a 5 g sachet of psyllium, PGX® fibre or a control (rice flour) three times a day for 52 weeks as part of a larger long-term study. Blood sample data for the first 3 months were analysed for associations between serum micronutrient levels and psyllium fibre and/or PGX® supplements.

Results: No significant differences were found between treatment groups (including control) and micronutrient status after 3 months, at $p>0.05$.

Conclusions: There were no significant between group differences in serum micronutrient concentrations after a 3-month control (rice flour), psyllium fibre, or PGX® supplementation of 15 g per day. However self-recorded dietary intake data showed significant reductions in macro and micronutrient consumption after 3 months. High fibre supplementation as an adjunct to weight management treatment for overweight and obese individuals is unlikely to compromise their nutritional status over the short term, however further research is recommended to monitor the micronutrient status over a longer period of time or with a higher fibre dosage.

Trial registration: Data for this study was extracted from a clinical trial was registered with the Australian New Zealand Clinical Trial Registry on 20 April 2011, registration number: ACTRN12611000415909.

Keywords: Obesity, dietary fibre, psyllium, PGX®, micronutrients, absorption, bioavailability, deficiency.

4.2 BACKGROUND

Obesity is a disease with a diverse aetiology. Researchers have started referring to obesity as a pandemic, as currently there are in excess of >2billion people worldwide who are classified as overweight or obese (1). In Australia the prevalence of overweight and obesity in adults aged 18 years and over has continued to rise to 63.4% in 2011-12 from 61.2% in 2007-08 and 56.3% in 1995 (2). Compared to the 'normal' weight BMI category (BMI of 18.5 –<25), obesity (BMI of ≥30) is associated with significantly higher all-cause mortality in 25-80 year old adults of both genders (3). Obesity is the result of both genetic and environmental influences; including genetic predisposition, energy imbalance, and environmental and social factors (4). The habitual consumption of more than the required amount of total energy, with food consumed being more processed or of low nutritional density, is said to contribute to excess weight (5). Over time this pattern of eating may lead to a low micronutrient status (6). Micronutrient deficiency can be defined as a prolonged lack of essential vitamins and minerals required by the body for proper growth and development or maintenance of optimal health (7). Overweight or obesity may negatively impact the bioavailability and utilisation of micronutrients by interfering with such processes as absorption, excretion, storage/distribution (eg. fat sequestering, tissue dispersion), or metabolism (eg. catabolic loses, possibly oxidative) (8). Increased physiologic requirements, and/or lower absolute total dietary intake may also impair the micronutrient status of adults with overweight and obesity (9).Therefore evidence suggests that obesity is itself a contributor to malabsorption of vitamins and minerals (10), a problem that can be compounded by the fact that many micronutrients are directly involved in the digestion, absorption, and utilisation of other nutrients from the food matrix (11). In addition, the importance of certain micronutrients as cofactors in glucose metabolic pathways, pancreatic β-cell function and in the insulin signalling cascade, suggests

that deficiency in these micronutrients may play a role in not only obesity, but also the development of lifestyle related diseases such as type 2 diabetes (8).

One food component that is commonly lacking in the diet of overweight or obese individuals is dietary fibre, often due to inadequate intake of fibre rich nutrient dense foods such as fruit and vegetables. The benefits of dietary fibre are well known, however most people find it difficult to eat the required amounts of fibre through increased fruit and vegetable intake, as well as cereals and grains as shown in the Australian Health Survey: Consumption of food groups from the Australian Dietary Guidelines 2011-2012 (12). Present estimations of dietary fibre intake in Australian, Canadian, European and American adults is approximately 15–25 g/day, which is below the current recommendations for adults of 25–30 g/day in these regions (13, 14). Research has consistently shown that those who routinely consume foods high in dietary fibre are less likely to be overweight or obese than those with a low fibre diet, as fibre promotes satiety (15), and can lead to reduced energy intake (16-18).
The effects of a high fibre diet on overall health have been well-researched. Fibre supplements may provide a cost effective and easy alternative for increasing the fibre content of a persons' diet without the need for other major dietary modifications (17, 19). However, high intakes of certain dietary fibre sources have been linked to deficiencies of calcium, iron, trace metals, and vitamin D and E due to its role in the food matrix and metabolism (20). The most common fibre promoted for health benefits within the commercial market is psyllium. Psyllium is widely consumed in Metamucil®, a commercially available fibre product that is marketed as a tool to promote bowel regularity and general wellbeing. Psyllium is less readily fermented in the gut and results in less flatulence and abdominal bloating than other types of soluble fibre, making it well tolerated and accepted (21). Psyllium studies have found beneficial effects on glucose and insulin homeostasis, lipids and lipoprotein, body weight, body composition and appetite (15).

PolyGlycopleX (PGX®) is another soluble fibre supplement that is showing positive health benefits. PGX® is a novel, highly viscous functional non-starch polysaccharide complex, manufactured by a proprietary process (EnviroSimplex®). PolyGlycopleX® is 3–5 times more viscous than any known individual polysaccharide (22) and is a compound of 3 different natural fibres: konjac (glucomannan), sodium alginate and xanthan gum (18).

Recent research has shown that adding 2.5–5 g of PGX® to a meal is highly effective in reducing postprandial glycaemia, lowering the glycaemic index of food (23) and modifying satiety hormones (18).

Although dietary fibre is not in itself a nutrient, it is considered among the six major nutrient groups along with proteins, carbohydrates, fats, vitamins, and minerals, and is a required listing on the nutrient food label in most countries that have labelling requirements (24). Studies looking at fibre supplementation have shown that increasing dietary fibre can convey a multitude of health benefits (15). This is especially true for overweight or obese individuals attempting weight loss, as high dietary fibre supplementation has been correlated with weight, BMI and waist circumference reduction (17, 18); however little is known regarding the effect this strategy has on the micronutrient status in this already vulnerable group. The biochemical effect of therapeutic doses of fibre in supplement form for weight loss need to be evaluated, as a high intermittent dosage may impair absorption of micronutrients in the gut, especially if taken prior to or during a meal (20). This study aims to investigate the effect of 15 g daily supplementation with two fibre types, PGX® and psyllium, on micronutrient status when added to the habitual diet of overweight and obese Australian adults over three months. It was hypothesised that the absorption of some micronutrients may be compromised with the increase in daily fibre supplementation in the food matrix.

METHODS

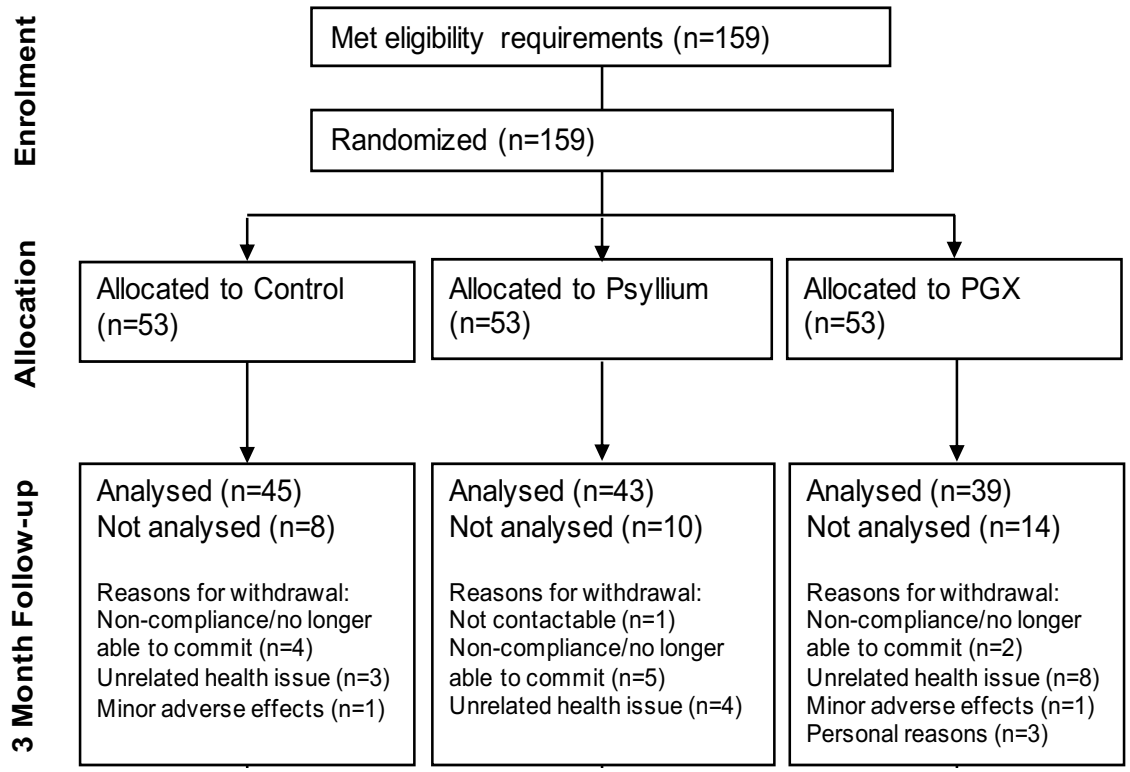
4.3 Participants

Overweight and obese individuals (n=159) aged between 18 and 65 years and with a body mass index (BMI) between 25-40 kg/m^2, were recruited from the community in Perth, Australia. Advertisements for the study were made via newspapers, flyers posted around Curtin University and local noticeboards, as well as radio advertising on Curtin FM. Individuals were screened either by telephone or online via Qualtrics using a questionnaire. Eligible participants attended an orientation session at Curtin University to assess suitability for the study, at which time the study details were also explained. Successful applicants were those who did not smoke, or take any medications that were lipid lowering, use steroids or other agents that may influence lipid metabolism. Those who took blood pressure medications such as warfarin were also excluded as were those with Type 1 or 2 diabetes mellitus, hypo and

hyperthyroidism, or had any cardiovascular events within the last 6 months. The remaining exclusion criteria included, psychological unsuitability, major systemic diseases, gastrointestinal problems, proteinuria, liver, renal failure, any significant weight fluctuations over the past 6 months, vegetarianism or veganism and participation in any other clinical trials within the last 6 months.

All components of the present study strictly adhered to the Human Research Ethics Committee (HREC) guidelines. Ethics approval was gained for this study as a part of the larger study from the Curtin University HREC on 23 November 2011, approval number: HR41/2011 (Appendix 1). All participants were made aware that their participation was voluntary and that they could withdraw from the study at any time. All participants provided signed, written informed consent forms prior to the commencement of the trial. Privacy, security and protection of the identity and information of participants was ensured for all future data collected as part of the present study. In addition, the larger clinical trial was registered with the Australian New Zealand Clinical Trial Registry on 20 April 2011, registration number: ACTRN12611000415909.

Figure 1) Participant Flow Diagram



* PGX: PolyGlycopleX

4.3.1 Study design

Serum micronutrient and dietary intake data was assessed at baseline and at 3 months for this study. The study was a randomised, controlled, double blind, parallel design intervention. The trial sponsors used the website (http://www.randomization.com) to ensured randomization to one of three groups (three randomly permuted blocks); The control group who consumed a placebo (which consisted of rice flour); the psyllium fibre supplement group; or the PGX® fibre supplement group. The supplement packages (control, PGX and psyllium) were identical in appearance and were provided by the manufacturer via single batch. This ensured the research assistants and participants were both blinded to the type of supplement being consumed. Assignment to a group was random and facilitated by research assistants at Curtin University after participant screening. Individual packages were marked by the participant ID and consisted of 5 g doses of PGX, psyllium, or rice flour (control) with the group allocation only known to the trial sponsors to ensure blinding. 'Quantities of rice flour and psyllium were determined by input, with the amounts weighed and checked by the dispensing and blending department while PGX was

analysed according to USP Monograph FCC9 3rd Sup 2015' (25, 26). All identifiable information from participants was coded to ensure privacy. All requirements and instructions for the study were identical for each of the three groups and participants consumed a supplement in addition to their habitual diet. The control (rice flour) had a low energy and fibre content and was similar in texture and appearance to the fibre supplements, so provided an appropriate placebo. Participants were advised to consume 5 g supplement sachet containing either the psyllium, PGX® fibre or placebo, mixed with a minimum of 250 mL water 5 - 10 minutes before breakfast, lunch and dinner (15g total) every day. Participants were asked to record any other supplements taken, such as vitamin, mineral or other for the duration of the study. All identifiable information from participants was coded and kept strictly confidential. Those who missed consecutive days of supplement consumption were discontinued from the trial.

4.3.2 Data collection

Participants attended a briefing session on how to consume the supplements, complete the paperwork and comply with the study protocol. Dietary intake over the course of the trial was monitored through the completion of 3-day food diaries at each clinical visit. Participants in all groups were asked to maintain their usual diet for the duration of the study. To monitor compliance, all participants were required to complete a diary to record their supplement consumption and asked to return the empty and unused sachets of the supplements at their clinical visits. These sachets were then counted and recorded. The trial was conducted in compliance with the study protocol. All participants were asked to report any adverse events to the investigators immediately. Medical support was to be provided for any serious adverse events resulting from this study. In addition, such events were to be recorded by the Investigator and reported to HREC.

ASSESSMENTS

4.4 Micronutrients Blood Analysis

Fasting blood samples drawn by venepuncture were collected at baseline (week 0) and week 12. Samples were collected into lithium heparin or serum separator tubes (5 ml) for antioxidant/vitamins A, C, D B_9, B_{12} and E and minerals (calcium, iron, iodine, folate, magnesium, potassium, sodium and zinc) and then centrifuged at 2,500 rpm at 4ºC for 10 minutes using an Eppendorf centrifuge and prepared for

storage at -80ºC. Vitamins A, E, D, as well as vitamin B_9, and B_{12} and thyroglobulin were measured using Enzyme-Linked Immunosorbent Assay kits (ELISA). ELISA combines antibody binding with enzymatic detection to identify molecules of interest; the result is a colour change that is measured by spectrophotometry at a particular wavelength (9). Vitamin C and total antioxidant capacity was measured by colorimetric assay. All trace metals (calcium, magnesium, iron, zinc, sodium and potassium) were analysed by Flame Atomic Absorption Spectroscopy (FAAS).

4.4.2 Three-Day Food Diaries

Prior to commencement of the study, each participant attended a briefing session, which included instructions for completing the weighted 3-day food diaries. Completed food diaries were collected at the baseline and week 12 clinic appointments. Participants were required to record all food and drink consumed including water for three consecutive days. As much detail as possible was encouraged, including portion sizes, weights and brands. Dietary micronutrient data from the food diaries was extracted and analysed using Food Works Version 7 (Xyris Software, 2012). Participants were required to complete the food diaries as a reference, as it was necessary to make sure that any changes to serum micronutrients were not as a result of changes in dietary intake, as participants were instructed not to alter their 'normal diet'.

4.4.2 Outcome measures

The present study was designed to assess the nutritional status of overweight and obese Australian adults, via comparison of serum micronutrients and food diary analysis at baseline and week 12 in relation to: vitamins A, C, D, E, B_9 and B_{12} and minerals calcium, iron, magnesium, potassium, sodium and zinc. The outcome measures that formed part of the larger 2012 study include weight/BMI and metabolic syndrome risk factors, which have been analysed and discussed elsewhere (18, 26).

4.4.3 Statistical analysis

A sample size of 24 subjects per group is predicted to provide sufficient power (80 %) to detect a 3% difference in weight before and after treatment within a group. Recruiting a total of 50 subjects per group will accommodate for 50% dropouts. Calculations are based on an average mean weight of 80kg and a standard deviation

of 5% within a group on all eligible subjects. Statistical analysis was conducted using analysis of covariance to determine between groups differences in micronutrients for the two intervention groups, compared to the control group at 3 months (using the baseline values as covariate). Post hoc analysis was conducted using the Least Significant Difference method. Statistical significance was considered at $p<0.05$. All analysis was conducted using SPSS 23.0 (IBM® SPSS® Statistics, New York, NY.

RESULTS

4.5 Participants

There were 53 participants in each group (control, psyllium and PGX®) at the start of the study, at the 3-month mark the control group had 45 participants, the psyllium group had 43 participants and the PGX® group had 39 participants. Reasons for truancy were noncompliance/unable to commit, unrelated health issues, minor adverse effects (abdominal bloating or constipation/diarrhea) or personal reasons. There were no serious adverse effects reported.

4.5.1 Baseline characteristics

Baseline values for serum and self-recorded dietary intake data for all groups are shown in Table 4.1. While participants in the psyllium fibre group showed slightly higher serum values at baseline for most micronutrients compared to PGX®, there were no significant between group differences (Control, Psyllium and PGX®) in serum micronutrient values as seen in Table 4.1). Interestingly, self-reported zinc intake from dietary intake data was found to be significantly lower in the PGX® group compared to control (11.5±0.6 mg vs 14.1±0.8, $p=0.030$).

4.5.2 Serum micronutrient values following 3 months fibre supplementation

Table 4.2) illustrates that there was no statistically significant between group (psyllium, PGX and control) differences in serum micronutrient status after three months' fibre supplementation, with the exception of zinc in the PGX group. However, there were differences in serum values for key micronutrients within group (from baseline) for both fibre groups following supplementation. Comparing serum levels in Table 4.2) at baseline and at weeks 12 shows that folate levels for psyllium went from 2650.5 ± 333.2 pg/mL at baseline to 2833.3 ± 476.2 pg/mL at 3 months (table 4.2) which is an increase of 6.9%. For PGX®, baseline levels were 2191.3 ± 333.8 pg/mL and 2417.9 ± 415.0 pg/mL after 3 months, which translates to

a 10.3% increase. Sodium intake was found to be 11.8% higher following fibre supplementation in both fibre groups with baseline psyllium values of 2730.7 ± 40.3 µg/mL compared to 3051.6 ± 29.8 µg/mL at 3 months. PGX® baseline levels for sodium were 2760.8 ± 41.1 µg/mL and increased to 3040.5 ± 26.2 µg/mL (10.5%). Potassium levels however were 22.2% lower after supplementation, with baseline psyllium group levels of 98.6 ± 1.5 µg/mL and 76.7 ± 3.9 µg/mL after 3 months. Similarly, PGX® baseline potassium decreased 28.8% with initial values of 98.5 ± 1.7 µg/mL and decreasing to 70.1 ± 3.8 g/mL at 3 months.

4.5.3 Nutrient intake data following 3 months of fibre supplementation

Self-reported micronutrient intake values from the 3-day food diary for the three groups are shown in Table 4.3. Baseline values for dietary intake data were used as a covariate for between group differences (a) and the control was used as covariate for within group differences (b) (refer to table 4.3). For vitamin C, a significant reduction in dietary intake at 12 weeks was found compared to baseline and compared to control for PGX® at $p<0.05$ with a mean ± SEM of 102.8 ± 10.5 mg (baseline) and 69.5 ± 5.9 mg and a reduction in recorded intake of 30.3 mg. Folate also showed a significant reduction compared to baseline at $p<0.05$ for the control group and the psyllium group. The control group decreased from 414.0 ± 35.6 µg at baseline to 340.3 ± 24.5 µg at week 12 (-71.1 µg). The psyllium group for dietary intake of folate went from 406.4 ± 32.8 µg to 340.7 ± 22.4 µg after 3 months fibre supplementation (-65.6 µg). Sodium was found to be statistically significant at $p<0.05$ in the psyllium group compared to the control at $p<0.05$, with 2615.4 ± 188.0 mg recorded at baseline and decreasing to 2334.8 ± 128.5 mg at 3 months (-280.7 mg). Zinc showed a significant reduction over 3 months fibre supplementation compared to baseline in the psyllium group with 13.6 ± 1.0 mg at baseline and 11.8 ± 0.7 mg at the 12 week mark (-2.0 mg). Dietary intake of magnesium also decreased significantly in the psyllium group by 37.7 mg from baseline (371.9 ± 19.9 mg) to 3 months (334.3 ± 15.8 mg). Overall, there appeared to be a downward trend in dietary intake data of most micronutrients in both the psyllium and PGX® group compared to baseline and compared to the control group after 3 months fibre supplementation. However only the afore mentioned micronutrients were significant at $p<0.05$. For example, iodine levels at 3 months for the PGX® group was lower at 118.1 ± 7.6 µg compared to the control group 138.3 ± 9.2 µg but not significant at $p<0.05$. Calcium

was also lower in the PGX® group at 3 months (754.0 ± 57.3 mg) compared to baseline (834.3 ± 60.6 mg), but again no significance at $p<0.05$ was found.

Table 4.4) refers to the macronutrient intake from two self-recorded 3-day food diaries, completed at baseline (week 0) and at 3 months (week 12). Participants in both the psyllium and PGX® groups recorded reductions in energy intake following 3 months fibre supplementation ($p<0.05$). Energy intake of participants in the psyllium group decreased from 8859.4 ± 318.4 kJ/d to 7272.3 ± 218 kJ/d with a mean change of -1617.3 kJ/d. The PGX® group participants went from 8783.3 ± 261.3 kJ/d to 7556.3 ± 243.1 kJ/d with a mean reduction of -1090.9 kJ/d. Carbohydrate intake of participants also significantly reduced at $p<0.05$ in both fibre groups with the psyllium group going from 212 ± 8.9 g/d at baseline to 181 ± 7.5 g/d at 3 months (-31.8 g/d). In the PGX® group, carbohydrate intake decreased from 209.8 g/d ± 8.6 of carbohydrate to 175.3 ± 8.4 g/d (-31.5 g/d). For fat intake, participants in the psyllium group showed significant change from baseline at $p<0.05$ with 81.2 g/d ± 4.5 at week 0 and 66.9 g/d ± 3.1 at 12 weeks, with a mean change of -15.6 g/d. Dietary protein intake of participants also reduced significantly in the psyllium group ($p<0.05$) with 104 g/d ± 4.7 recorded at baseline and 85.9 g/d ± 3.6 after 3 months fibre supplementation (-17.6 g/d). Overall dietary fibre intake in both psyllium and PGX® groups increased significantly from baseline to 3 months ($p<0.05$), with the psyllium group participants increasing on average 12.7 g/d from 23.9 g/d ± 1.3 to 36.4 g/d ± 1.2 and the PGX® group increasing on average 14.2 g/d from 21.4 g/d ± 1.2 to 36.6 g/d ± 1.3 ($p<0.05$).

Table 4.1) Baseline characteristics of serum and dietary intake data

	CONTROL Mean ± SEM	n	PSYLLIUM Mean ± SEM	n	PGX Mean ± SEM	n
Age (years)	49.8 ± 1.8	45	49.9 ± 1.7	43	47.9 ± 1.9	39
Weight (kg)	94.7 ± 2.5	45	91.2 ± 2.2	43	96.2 ± 2.9	39
BMI (kg/m^2)	32 ± 0.6	45	31.7 ± 0.5	43	33.2 ± 0.7	39
Serum						
Vitamin E (µg/mL)	7.4 ± 0.7	45	8.2 ± 0.9	42	7.8 ± 0.9	39
Vitamin B12 (pg/mL)	731.5 ± 69.7	44	755.4 ± 72.7	43	677.3 ± 73.1	39
Vitamin C (mg/dL)	63.9 ± 4.6	45	65.8 ± 4.0	43	63.6 ± 4.1	39
Vitamin A (ng/mL)	52.4 ± 3.0	45	49.5 ± 2.9	43	48.9 ± 3.1	39
Vitamin D (ng/mL)	10.7 ± 1.1	45	11.9 ± 1.1	43	9.9 ± 1.0	39
Folate (pg/mL)	2558.3 ± 370.6	45	2650.5 ± 333.2	42	2191.3 ± 333.8	38
Thyroglobulin (ng/mL)	10.2 ± 3.4	41	8.4 ± 0.9	41	7.6 ± 1.7	37
Potassium (µg/mL)	99.8 ± 1.7	45	98.6 ± 1.5	43	98.5 ± 1.7	39
Sodium (µg/mL)	2706.4 ± 29.1	45	2730.7 ± 40.3	43	2760.8 ± 41.1	39
Iron (µg/mL)	1.1 ± 0.1	45	1.0 ± 0.1	43	1.0 ± 0.1	39
Zinc (µg/mL)	0.3 ± 0.0	45	0.3 ± 0.0	43	0.3 ± 0.0	39
Calcium (µg/mL)	33.9 ± 1.4	45	35.3 ± 1.5	43	33.5 ± 1.2	39
Magnesium (µg/mL)	6.7 ± 0.2	45	6.9 ± 0.2	43	6.7 ± 0.1	39
Dietary intake						
Vitamin E (mg)	9.1 ± 0.8	44	8.8 ± 0.7	43	8.6 ± 0.7	39
Vitamin C (mg)	109.1 ± 13.2	44	102.9 ± 12.7	43	97.1 ± 9.5	39
Retinol (µg)	365.4 ± 32.2	44	357.5 ± 46.3	43	385.7 ± 45.5	39
Beta carotene (µg)	2840.3 ± 415.4	44	3087.4 ± 346.8	43	2054.8 ± 182.3	39
Vitamin D (µg)	3.8 ± 0.5	44	3.5 ± 0.3	43	3.7 ± 0.4	39
Folate (µg)	398.1 ± 32.8	44	410.4 ± 31.4	43	304.1 ± 17.3	39
Iodine (µg)	132 ± 7.6	44	128.9 ± 7.3	43	123.9 ± 9.3	39
Potassium (mg)	3196.0 ± 118.6	44	3167.2 ± 136.7	43	2902.3 ± 120.9	39
Sodium (mg)	2961.1 ± 174.6	44	2613.8 ± 181.8	43	2669.7 ± 158.1	39
Iron (mg)	13.4 ± 0.8	44	12.1 ± 0.6	43	11.8 ± 0.6	39
Zinc (mg)	14.1 ± 0.8	44	13.6 ± 0.9	43	11.5 ± 0.6*	39
Calcium (mg)	907.1 ± 59.3	44	901.8 ± 48.8	43	882.0 ± 73.1	39
Magnesium (mg)	387.7 ± 20.3	44	373.7 ± 19.0	43	357.0 ± 19.7	39

*Significant compared to Control at $p<0.05$

Table 4.2) Between group differences in serum micronutrients from baseline to 3 months

	CONTROL			PSYLLIUM			PGX		
	Mean ± SEM	**Difference to baseline**	**n**	**Mean ± SEM**	**Difference to baseline**	**n**	**Mean ± SEM**	**Difference to baseline**	**n**
Vitamin E (µg/mL)	6.8 ± 0.6	-0.6	45	8.8 ± 1.0	0.6	43	7.8 ± 0.9	0.0	39
Vitamin B12 (pg/mL)	688.8 ± 65.6	-42.7	44	757.3 ± 72.7	1.9	43	688.9 ± 65.1	11.6	39
Vitamin C (mg/dL)	62.3 ± 3.4	-1.6	45	60.8 ± 3.2	-4.9	43	57.7 ± 2.8	-5.9	38
Vitamin A (ng/mL)	48.7 ± 3.0	-3.7	44	46.9 ± 3.2	-2.6	43	49.5 ± 3.2	0.6	39
Vitamin D (ng/mL)	10.0 ± 1.4	-0.7	45	10.3 ± 1.0	-1.6	43	8.6 ± 0.8	-1.3	39
Folate (pg/mL)	2822.9 ± 518.8	264.7	45	2833.3 ± 476.2	182.8	41	2417.9 ± 415.0	226.6	38
Thyroglobulin (ng/mL)	11.2 ± 3.4	1.0	42	8.5 ± 1.0	0.1	42	7.2 ± 1.2	-0.4	38
Potassium (µg/mL)	71.2 ± 3.5	-28.6	45	76.7 ± 3.9	-21.8	43	70.1 ± 3.8	-28.4	39
Sodium (µg/mL)	3012.4 ± 28.4	306	45	3051.6 ± 29.8	320.9	43	3040.5 ± 26.2	279.6	39
Iron (µg/mL)	0.8 ± 0.0	-0.2	45	0.8 ± 0.0	-0.3	43	0.9 ± 0.1	-0.1	39
Zinc (µg/mL)	0.5 ± 0.0	0.2	45	0.5 ± 0.0	0.2	43	0.5 ± 0.0	0.2	39
Calcium (µg/mL)	43.9 ± 0.7	9.9	45	44.2 ± 0.7	8.9	43	43.2 ± 0.7	9.7	39
Magnesium (µg/mL)	7.2 ± 0.1	0.5	45	7.4 ± 0.1	0.5	43	7.3 ± 0.2	0.5	39

*There were no significant between group differences $p>0.05$.

Table 4.3) Within and between group differences in dietary micronutrient levels from baseline to 3 months

	Baseline	n	12 Weeks	Difference	n
Vitamin E (mg)					
Control	9.5 ± 0.9	39	8.5 ± 0.6	-1.033	40
Psyllium	8.8 ± 0.7	41	7.7 ± 0.5	-1.100	41
PGX	8.6 ± 0.8	33	8.5 ± 0.7	-0.137	32
Vitamin C (mg)					
Control	102.4 ± 9.7	39	115.4 ± 17.1	14.367	40
Psyllium	98.7 ± 12.0	41	104.9 ± 11.5	6.192	41
PGX	102.8 ± 10.5	33	69.5 ± 5.9[a,b]	-30.276	32
Retinol (µg)					
Control	394.8 ± 33.4	39	358.5 ± 25.7	-33.701	40
Psyllium	352.3 ± 48.3	41	3356.0 ± 460.2	-47.459	41
PGX	363.9 ± 44.6	33	2818.2 ± 515.0	1410.918	32
B Carotene (µg)					
Control	2893.6 ± 453.9	39	2971.3 ± 345.9	143.611	40
Psyllium	3044.8 ± 349.2	41	3356.0 ± 460.2	311.177	41
PGX	2058.6 ± 207.9	33	2818.2 ± 515.0	961.498	32
Vitamin D (µg)					
Control	3.9 ± 0.5	39	3.4 ± 0.3	-0.498	40
Psyllium	3.5 ± 0.3	41	3.2 ± 0.3	-0.380	41
PGX	3.7 ± 0.5	33	3.0 ± 0.3	-0.685	32
Folate (µg)					
Control	414.0 ± 35.6	39	340.3 ± 24.5[a]	-71.103	40
Psyllium	406.4 ± 32.8	41	340.7 ± 22.4[a]	-65.642	41
PGX	310.0 ± 19.5	33	320.5 ± 24.7	48.145	32
Iodine (µg)					
Control	135.6 ± 8.2	39	138.3 ± 9.2	2.792	40
Psyllium	125.4 ± 7.1	41	130.1 ± 8.2	4.754	41
PGX	118.8 ± 8.2	33	118.1 ± 7.6	-0.894	32
Potassium (mg)					
Control	3279.3 ± 122.3	39	3238.9 ± 181.4	-44.486	40
Psyllium	3134.1 ± 141.4	41	2905.0 ± 123.5	-229.108	41
PGX	2855.1 ± 134.3	33	2825.7 ± 142.1	36.487	32
Sodium (mg)					
Control	3081.846 ± 185.5	39	2844.8 ± 157.0	-214.018	40
Psyllium	2615.4 ± 188.0	41	2334.8 ± 128.5[b]	-280.569	41
PGX	2639.4 ± 179.1	33	2490.1 ± 150.0	-93.027	32
Iron (mg)					
Control	14.2 ± 0.8	39	12.9 ± 0.7	-1.389	40
Psyllium	12.0 ± 0.6	41	11.1 ± 0.6	-0.904	41
PGX	11.7 ± 0.7	33	11.6 ± 0.8	0.531	32
Zinc (mg)					
Control	14.7 ± 0.8	39	13.9 ± 0.8	-0.900	40
Psyllium	13.6 ± 1.0	41	11.8 ± 0.7[a]	-1.857	41
PGX	11.6 ± 0.7	33	12.7 ± 1.1	1.321	32
Calcium (mg)					
Control	952.3 ± 62.6	39	885.1 ± 56.0	-62.324	40
Psyllium	888.3 ± 50.3	41	806.0 ± 44.2	-82.305	41
PGX	834.3 ± 60.6	33	754.0 ± 57.3	-74.471	32
Magnesium (mg)					
Control	400.8 ± 22.0	39	369.9 ± 19.4	-31.998	40
Psyllium	371.9 ± 19.9	41	334.3 ± 15.8[a]	-37.584	41
PGX	359.4 ± 22.4	33	349.1 ± 23.8	-5.595	32

*Values are expressed as mean ± SEM. [a]significant compared to baseline at $p<0.05$; [b]significant compared to Control at $p<0.05$

Table 4.4) Intake of macronutrients from 3-day self-recorded food diaries from baseline to 3 months.

Variable	Group	Baseline	n	3 months	n	Mean change	*P*
Energy (kJ/d)	Control	8636.4 ± 294.4	44	9013.1 ± 223.6[a]	39	115.5	NS
	Psyllium	8859.4 ± 318.4	43	7272.3 ± 218[b]	41	-1617.3	0.000
	PGX	8783.3 ± 261.3	39	7556.3 ± 243.1[b]	33	-1090.9	0.001
Carbohydrate (g/d)	Control	209.8 ± 8.8	44	212.6 ± 7.7[a]	39	-1.6	NS
	Psyllium	212 ± 8.9	43	181 ± 7.5[b]	41	-31.8	0.002
	PGX	209.8 ± 8.6	39	175.3 ± 8.4[b]	33	-31.5	0.003
Fat (g/d)	Control	80.2 ± 4.2	44	83.8 ± 3.2[a]	39	-0.5	NS
	Psyllium	81.2 ± 4.5	43	66.9 ± 3.1[b]	41	-15.6	0.000
	PGX	82.8 ± 3.3	39	72.2 ± 3.4[b]	33	-9.0	NS
Protein (g/d)	Control	101.7 ± 4	44	109.9 ± 3.7[a]	39	6.4	NS
	Psyllium	104 ± 4.7	43	85.9 ± 3.6[b]	41	-17.6	0.000
	PGX	101.6 ± 3.9	39	94.4 ± 4.1[b]	33	-6.5	NS
Total Fibre (g/d)	Control	22.6 ± 1.2	44	24.2 ±1.2[a]	39	0.8	NS
	Psyllium	23.9 ± 1.3	43	36.4 ± 1.2[b]	41	12.7	0.000
	PGX	21.4 ± 1.2	39	36.6 ± 1.3[b]	33	14.2	0.000

*Values are mean ± SEM with baseline as a covariate. Mean change from baseline. *P* values are within group differences compared to baseline. Different letters in superscript represent differences between groups $p<0.05$.

4.6 DISCUSSION

Research has shown higher intake of dietary fibre may reduce the risk of chronic diseases, including cardiovascular diseases (CVDs), cancer, type 2 diabetes, and obesity (16, 17, 19). The health benefits provided by a higher dietary fibre intake are thought to occur via the following mechanisms: reducing the absorption of glucose and LDL cholesterol; increased satiation and satiety effect which can reduce food intake, and may promote weight loss; gut microbe–induced production of short-chain fatty acids, which have immunomodulatory and anti-inflammatory properties; trapping of bile acids and carcinogenic substances; and increased intake of biologically active compounds, such as phytochemicals and antioxidants (27, 28). However, it is still not well known how a higher fibre intake through supplementation affects micronutrients absorption in the gastrointestinal tract, especially in overweight and obese individuals. Therefore, the aim of this study was to determine whether 3-months' supplementation with psyllium or PGX® fibre affects micronutrient status of overweight and obese adults.

Current literature, although limited suggests that a higher fibre intake can either inhibit or promote bioavailability depending on the nutrient, the absorptive mechanism involved, and the composition of the food matrix as a whole (29). Factors affecting vitamin or mineral bioavailability include how it is absorbed, excreted, stored/distributed; whether its sequestered by fat or dispersed in tissue, metabolic processes (catabolic loses, possibly oxidative), increased physiologic requirements, or lower absolute total dietary intake (30, 31). This may help explain the variability that can occur within serum micronutrient levels. The habitual diet of overweight and obese individual is often seen as energy-dense and nutrient-poor, so maintaining nutritional sufficiency can potentially be compromised. However due to the satiating effects of fibre, if a therapeutic dose is consumed, less total food consumption may occur. Therefore, nutrient intake and nutrient status may be affected in the long term, particularly if malabsorption from the combined effects of obesity on bioavailability and a poor diet are already in effect.

This study examined the micronutrients present in serum samples and self-reported dietary intake data of overweight and obese individuals at baseline and 3 months following consumption of 15g per day of either a control (rice flour), psyllium or PGX® fibre supplement. According to the serum values (table 4.1 and table 4.2), there were no between group differences in micronutrient status compared to control, both at baseline as well as after 3 months of fibre supplementation. Self-reported dietary intake using the 3-day food diaries (table 4.1) showed the baseline value for zinc was significantly lower in the PGX® group compared to the control group ($p<0.05$). As this is a baseline value, it's significance is probably not related to an increase in dietary fibre, and could be explained by simple misreporting or poor zinc status in overweight and obese (32, 33). All other micronutrients for self-reported dietary intake data at baseline, showed no significant differences compared to control, at $p<0.05$.

There was no significant difference between groups in serum micronutrient levels following 3 months fibre supplementation (table 4.2), however there was a notable increase/decrease for some key micronutrients from baseline to 3 months (within groups) (table 4.1 and table 4.2). Folate, sodium and potassium all showed changes from baseline, while not significant at $p<0.05$ it shows the potential for serum levels to be further affected over a longer time period (greater than 3 months). Between

group differences in self-reported dietary nutrient intake after three months of fibre supplementation (table 4.3), showed sodium intake was significantly lower in the psyllium group compared to the control. Between group dietary intake data for vitamin C was also significantly lower in the PGX® group at 3 months compared to the control group at $p<0.05$. Iodine and calcium were also lower in the PGX® group compared to the psyllium group and control group (table 4.3) following 3 months fibre supplementation, but these findings were not statistically significant at $p<0.05$. There appeared to be a downward trend for most dietary micronutrients in both fibre groups at 3 months, compared to the control (table 4.3).

While these results may reflect differences or changes in dietary intake (respectively), it may potentially be the result of inaccurate self-reporting (32). Overweight and obese participants have been shown to under-report dietary intake in self-reported food diaries (34), therefore these results may be due to simple misreporting of 3-day food diaries completed by participants as opposed to any actual changes. Another possibility of reduced nutrient intake data after 3 months dietary fibre supplementation of 15 g per day is due to the satiating effect of dietary fibre. Participants physically consumed less food as they were simply not as hungry, meaning over all, recorded dietary macro and micronutrient intake was reduced. This theory was supported by the significant reductions in energy, fat, carbohydrate and protein and the significant increase in fibre intake per day in the self-recorded dietary macronutrient intake following 3 months fibre supplementation (table 4.4).

The fact that there were no between group differences in the serum micronutrients following three months of PGX® or psyllium fibre supplementation is a positive outcome for the present study. Dietary fibre supplementation is becoming recognised as an adjunct to weight management programs for overweight and obese individuals, and potentially complements many existing weight loss programs. However, as with any new treatment, it is essential to identify any negative effects that a new method or product may have on patients' health, in this instance their micronutrient status. Recent research promotes the safety and efficacy of the novel fibre supplement PGX®, as well as showing positive effects for metabolic syndrome (MS) and associated risk factors such as CVD, blood pressure and blood lipids (18, 26). However, the effect of PGX® or psyllium fibre on micronutrient status, when used as a part of a weight loss treatment in overweight and obese individuals, has not been

well researched to date. The micronutrients examined in this study play an essential role in regulating biochemical pathways associated with metabolism. As obesity is a multifaceted and complex disease, the daily intake of 15 g of supplemented dietary fibre to help weight loss, needed to be ruled out as a possible confounder to attaining a better health outcome for this subpopulation.

4.6.1 Strengths and Limitations

This study is unique in that there has been very limited research examining the micronutrient status of overweight and obese individuals taking high fibre doses to assist with weight loss. While there is still much to learn about how fibre and micronutrients interact within the digestive system, the results of this study add to the body of knowledge in this area.

One of the limitations of this study was the time frame examined (baseline to 3 months only). It is possible that while no significant differences were found in micronutrient status following 3 months of high supplementary fibre (15 g per day), longer term fibre treatment or higher fibre dosages may have produced different results. It would be advisable for any future studies in this area to be conducted over a greater duration than 3 months as well as vary the dosage of dietary fibre supplement. A second limitation in this study was the use of self-reported dietary intakes. With any self- reporting nutritional data, potential bias is introduced as overweight and obese populations have higher incidence of under report their dietary intake (35). While food diaries are a useful tool in the examination of dietary intake in weight management trials, the fluctuations in folate, zinc, magnesium sodium and vitamin C data found in this study may not represent changes to dietary intake, but rather inconsistent food consumption reporting. Food diary data collected over a longer period of time (7-14 days) is thought to be more reflective of usual nutrient intakes. However studies have shown that increased length of food diaries reduces the accuracy of results as participants can become increasingly less compliant over time (36, 37).

Furthermore, the fibre dosage itself needs to be considered. Participants were consuming an extra 15 g of fibre each day in supplement form prior to breakfast, lunch and dinner. If this fibre amount was to increase to attempt to maximize or encourage further weight loss, this may produce more significant effects on serum micronutrient status. A further point to consider for any future studies is whether any

significant results or differences between fibre groups from baseline to 3 months is due to the increase in PGX® or Psyllium fibre supplementation and not due to possible weight loss. High dietary fibre promotes satiety, so participants physically consumed less food and fewer kilojoules. Over a longer time period this possibly may have led to more significant differences in serum micronutrients.

4.6.2 Significance

As high fibre diets have been linked to deficiencies of calcium, iron, trace metals, and certain vitamins in previous studies, it is important to update the body of knowledge in determining the possible health implications of therapeutic doses of fibre. If fibre is to be recommended as a potential adjunct to weight loss treatments for overweight and obese individuals, it is important to identify any possible limiting factors. The results from this research trial provide further evidence as to the physiological and biochemical effects high intakes (15 g daily) of psyllium or PGX® fibre supplementation have on the micronutrient status of an overweight or obese individual.

4.7 Conclusions

Research examining methods to curb the obesity epidemic have focused on the development of safe and effective treatment options to assist with the current weight management strategies. One such option is the use of fibre supplements, which has been shown to improve satiety and reduce metabolic syndrome risk factors in overweight and obese individuals (18, 38). Previous research had suggested that higher fibre consumption may impair the bioavailability of certain micronutrients, such that interference to the food matrix may influence the release, transformation, and subsequent absorption of some nutrients in the digestive tract (8). This in turn may influence energy metabolism potentially contributing to and continuing the cycle of ongoing weight issues. As there were no significant changes in serum micronutrient status following 3 months of 15 g daily PGX® or psyllium fibre intervention, short-term fibre supplementation may be adopted as a useful strategy to assist with weight management without significantly impacting on the micronutrient status of overweight and obese individuals. However self-recorded dietary intake data of macro and micronutrient intake for this study illustrated that there is a downward trend in nutrient intake over 3 months due to the satiating effect of fibre. Thus, research over a longer study period as well as different fibre dosages is needed

before this strategy can be confidently recommended as an ongoing treatment to aid weight loss in overweight and obese individuals over the long-term.

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CHAPTER FIVE

REVIEW AND DISCUSSION

5.1 Summary

Obesity is now a global health problem. There are currently as many people worldwide dying from obesity and its comorbidities as there are from starvation and malnutrition (1). In Australia the prevalence of overweight and obesity in adults aged 18 years and over has continued to rise to 63.4% in 2011-12 from 61.2% in 2007-08 and 56.3% in 1995 (2). Food affects every biochemical and physiological process of the human body. An imbalance or lack of essential macro and micronutrients can have a significant effect on mood, behaviour, energy levels, and intellectual and physical performance (3).

Micronutrients play a critical role in almost every biochemical pathway and therefore play an essential role in sustaining 'normal function'. For example, vitamin E provides antioxidant activity and protection of pancreatic β-cells (4), whereas vitamin A is important for epithelial cell differentiation, immune function and antioxidant activity (5). Vitamin C is important for antioxidant and anti-atherogenic activity (6) as well as endothelial integrity (7). Calcium and vitamin D aid in bone mineral homeostasis (8), and protection against glucose intolerance and type 2 diabetes (9). (Vitamin B12 is critical for red blood cell formation and nerve cell formation (5) and folate is integral in red blood cell formation (5). Iodine is involved in the production of thyroid hormones, which are crucial for all aspects of human metabolism (5) and iron assists in the formation of red blood cell haemoglobin used to transport oxygen to the cells (5). Zinc is crucial for enzymatic activity, protein and DNA synthesis, as well as cell-mediated immunity (10), Magnesium contributes to ionic regulation and modulation of glucose transport across membranes (11) and potassium is important for cellular function, fluid and electrolyte balance and blood pressure maintenance (5). The nutritional status of an individual can influence the development and outcome of chronic disease and overweight and obese populations are at higher risk of disease development due to long-term 'high calorie malnutrition'(1). High calorie malnutrition can be defined as consuming a diet of calorie yielding foods with insufficient non-caloric nutrients such as vitamins and minerals (1, 12).

A diet rich in fibre is beneficial for the maintenance of good health (13). Current recommendations state that adults should aim to consume between 25 g and 30 g of dietary fibre per day from a diet rich in fruit, vegetables, legumes and wholegrains (14, 15). However less than 6% of Australian adults currently meet the required daily intake of fruit and vegetables (2), which indicates that dietary fibre intakes may also be below recommended daily amount. A diet high in fibre not only prevents constipation and regulates overall gastrointestinal health, it has a protective affect against cardiovascular disease (CVD), type 2 diabetes, some cancers (especially colorectal, breast and stomach cancers), hypertension, and obesity, which is itself a significant risk factor for developing these conditions (16). In those that have difficulty meeting their daily requirements, a dietary fibre supplement may be an easy and cost effect measure to increase fibre intake. However, in the past high intakes of fibre have been linked to deficiencies of calcium, iron, trace metals, and certain vitamins due to its role in metabolism and potential binding of nutrients within the food matrix (17, 18).

Psyllium seed husk is a popular and commercially available fibre supplement. Psyllium fibre is a viscous, water-soluble fibre from the Plantago ovata plant and is less readily fermented in the gut, causing less abdominal bloating than other dietary fibres (19). PolyGlycopleX® (PGX®) is a non-starch polysaccharide compound comprising three natural fibre components (glucomannan or konjac, sodium alginate, and xanthan gum) (20). It is a highly fermentable complex approximately seven times more viscous than psyllium due to synergistic complexing of the three fibre sources in the proprietary processing (20).

To date, an abundance of research has examined the effect of fibre supplementation on weight and metabolic syndrome risk factors in overweight and obese individuals (21), however there is limited research on the effect of fibre supplementation on the micronutrient status of this already vulnerable group

This project initially aimed to assess the nutritional status of a cohort of overweight and obese Australian adults enrolled in a weight management trial. Baseline serum samples were compared to the clinical reference intervals for key micronutrients (vitamins A, D E and C and minerals iron, sodium, magnesium, potassium, calcium and folate) (22). This study also aimed to determine the impact of 15 g per day of psyllium or PGX® fibre supplementation over 3 months on the (serum) micronutrient

status in this group. Self-recorded dietary intake data at baseline and 3 months was analysed using nutritional analysis software Foodworks 7 Professional (Xyris Software, Australia). Intake values for macro and micronutrients were then compared to the NRVs for Australia and New Zealand (14).

5.2 Intervention

Overweight and obese individuals with a body mass index (BMI) between 25-40 kg/m^2 and aged between 18 and 65 years, but otherwise healthy, were recruited from the community in Perth, Western Australia. Participants were instructed to consume a 5 g sachet of psyllium, PGX® fibre or a rice flour placebo three times a day for 52 weeks, as part of a 12-month study examining the effects of daily fibre supplementation on metabolic syndrome risk factors in overweight and obese individuals. However, for this current project serum micronutrient and self-recorded dietary intake data from the trial was extracted at baseline and at 3 months and analysed. Fifty-three participants were allocated to each group (control, psyllium and PGX®) at the start of the study, by 3 months the control group had dropped to 45 participants, the psyllium group had 43 participants and the PGX® group had 39 participants. Dropout reasons include noncompliance/unable to commit, unrelated health issues, minor adverse effects or personal reasons.

5.3 CHAPTER 3/PAPER 1: Overweight and obese Australians adults and micronutrient deficiency

Hypothesis:

As a negative correlation between micronutrient status and overweight and obesity has been noted in previous studies. It is hypothesised that, in the current study sample, as BMI increases micronutrients levels will decrease.

CHAPTER OVERVIEW

Micronutrients obtained from food are essential for normal metabolic functioning and a healthy micronutrient status depends on unimpaired absorption in the small intestine. Overweight or obesity may negatively impact the bioavailability and utilisation of micronutrients by interfering with such processes as absorption, excretion, storage/distribution (eg. fat sequestering, tissue dispersion), or metabolism

(eg. catabolic loses, possibly oxidative) (23). Increased physiologic requirements, and/or lower absolute total dietary intake may also impair the micronutrient status of adults with overweight and obesity (24). According to baseline serum micronutrient results (Table 3.1 and 3.2), a large percentage of participants were below the clinical reference intervals for potassium (100%), magnesium (100%), zinc (99.2%), sodium (94.5%), calcium (91.3%), vitamin A (100%), vitamin D (89%) and folate (72%). Poor dietary intake of these micronutrients is one possible reason for low serum levels as well as poor bioavailability and absorption in the gut (25). Dietary intake data gathered from participants using a 3-day food diary further supported this theory as micronutrient levels at baseline from self-recorded dietary intake were lower than the NRV's for vitamin D, retinol, iron (females) and calcium as well (refer to Chapter 3, Table 3.3).

Significance between BMI and baseline serum micronutrients was also established, with table 3.4 and Figures 3.1a, b, c, d showing the correlation between BMI and a decrease or increase in nutrient levels. Significant negative associations were found between BMI and baseline serum vitamin D ($p=0.044$), magnesium ($p=0.010$) and potassium ($p=0.023$), whereas a significant positive association was found between BMI and serum folate ($p=0.025$). Inadequate amounts of these micronutrients may have health implications in the long term as each one of these nutrients is responsible for key pathways or processes within the body. For example low levels of Vitamin D may impact calcium absorption and bone mineral homeostasis (26). Magnesium is a cofactor in hundreds of enzymatic reactions that regulate diverse biochemical reactions in the body (27). Without magnesium, muscle and nerve function, protein synthesis, insulin secretion effecting blood glucose control, and blood pressure regulation would all be effected (11, 28). Potassium plays an important role in regulating fluid and electrolyte levels and the acid-base balance to maintain blood pH levels (29), therefore less than adequate levels of potassium has the potential to affect all cellular function. Low folate may result in low energy and iron levels as folate is essential for red blood cell formation and helping iron function properly within the body (30). Folate is also involved in the production of DNA and RNA, so is essential for periods of growth of cells and tissues such as during infancy, adolescence and pregnancy (31). The remaining serum micronutrients showed no association to BMI at baseline. Further to this, no correlation was found between self-recorded dietary intake and BMI at baseline (Table 3.5).

Fortification of processed foods and beverages may mitigate the effect of nutrient-poor diets on the micronutrient status of this subpopulation. In addition, it is possible that poor micronutrient intake may contribute to the development and/or maintenance of obesity as overweight and obese people may need to eat more to satisfy their nutritional requirements' however further research is needed to clarify and/or support this notion.

5.4 CHAPTER 4/PAPER 2: Between group comparisons in (blood) micronutrients to determine if psyllium or PGX® supplementation altered micronutrient status of overweight and obese after 3 months of treatment.

Hypothesis: It was hypothesised that the absorption of some micronutrients, may be affected due to the increase in daily fibre supplementation intake after 3 months.

CHAPTER OVERVIEW

The role fibre consumption plays in mitigating the effects of weight-related diseases, particularly CVD, metabolic syndrome and type 2 diabetes, is well-researched in the scientific literature. When considering fibre supplementation to support weight management in individuals with overweight and obesity, other factors, such as any potential adverse effects, need to be examined. Previous research dating as far back as 1994 has suggested that the bioavailability and absorption of key micronutrients may be affected by high dietary fibre (32). Being overweight or obese can also impact nutrient absorption in the gut (33), and typically this subpopulation tends to have a nutrient poor diet along with poor micronutrient status (23). Therapeutic doses of fibre taken daily also have the potential for wide-ranging implications on cellular activity, and could affect physiological processes including, food cravings and appetite control depending on the dose amount (1, 34). Therefore, the present study was designed to examine this possibility with respect to a daily psyllium or PGX® fibre supplementation of 15 g in overweight and obese individuals over 3 months.

There were no significant between group differences in the serum micronutrient results at baseline or after a 3-month psyllium or PGX® fibre supplementation intervention, compared to the control group (Table 4.1 and 4.2). However between and within group differences in self-recorded dietary intake data following fibre supplementation were significant (Table 4.3 and 4.4). Vitamin C, folate, sodium, zinc

and magnesium all significantly reduced from baseline to 3 months as well as an overall downward trend for the other micronutrients. Macronutrient intake for participants also significantly reduced in both fibre groups (table 4.4). Energy, fat, protein and carbohydrate intake all decreased following 15 g fibre consumption per day, and as expected fibre intake significantly increased. In other words, even though participants' serum micronutrient status was not affected over 3 months by increasing psyllium or PGX® fibre intake by 15 g per day, dietary intake of nutrients significantly decreased. This may be due to the satiating effect of fibre, meaning participants physically consumed less food as they weren't as hungry (35). This may have had a non-effect on serum micronutrient levels in the short term, however micronutrient status may be impacted by longer-term (greater than 3 months) high fibre supplementation. Therefore further research examining the micronutrient status of overweight and obese populations taking fibre supplements to assist with weight loss for more than 3 months is needed.

5.5 Strengths of study

To date few studies have examined the baseline nutritional status of overweight and obese individuals, a subpopulation that tends to consume energy dense, nutrient poor diets. However, the limited literature currently available has shown that obesity impacts the bioavailability and absorption of some micronutrients, a factor that may be further compounded by the low levels of micronutrients in the diet of individuals with obesity.

The majority of studies available for this subpopulation have focused more on over-nutrition in terms of excess energy intake versus energy expenditure, health implications of 'over-nutrition', and the effects of interventions to treat this issue (36). The results of this study show, 'high-calorie malnutrition' in overweight and obesity does occur, with baseline serum levels in chapter 3 showing insufficiency when compared to the clinical reference intervals (22). Lower than recommended baseline serum levels were found for vitamin D, vitamin E, vitamin A, calcium, magnesium, potassium, folate, and zinc as well as a correlation to BMI (table 3.4). Dietary intake data compared to the NRVs also showed that vitamin D and calcium, potassium and iron intake were low (table 3.3). The results obtained in this study therefore support the view that overweight and obesity have a poor micronutrient status, thus adding to the body of knowledge surrounding this issue is important. It is

necessary to establish a baseline measure of nutritional status for overweight and obese populations in order to better manage obesity and its comorbidities.

Similarly, limited studies have examined the effects of high fibre supplementation on micronutrient levels in overweight and obesity. Fibre has demonstrated physiochemical properties such as fermentability, viscosity and gel formation, water-holding capacity, bulking and binding ability and varying solubility (37). These properties of fibre have been shown to have the ability to alter the bioavailability and absorption of micronutrients in the gastrointestinal tract. Furthermore, high intakes of dietary fibre sources have been linked to deficiencies of calcium, iron, zinc, magnesium, vitamin D and vitamin E (38). Thus it is important to assess high fibre supplementation on micronutrient absorption in this subset of the population who are already at increased risk of nutritional deficiency (23). In addition, PGX® is still a relatively new fibre product for weight loss, and has not been as extensively studied as psyllium, so contributing new information regarding the effects of this fibre type and its application is essential.

5.6 Limitations

One of the limitations of this study is that data from a 3-month period of fibre supplementation only were examined (chapter 4). Deficiency states may occur over a longer period of time depending on the micronutrient, so this time-frame may not show the impact of fibre supplementation over the longer-term. In addition, participants were only consuming an extra 15 g of dietary fibre in supplement form each day. A higher fibre dosage may also have impacted the serum micronutrient levels more significantly. Future studies would be advised to increase the study period and potentially increase the fibre dosage to get a wider scope of the impact dietary fibre may have on micronutrient status in overweight and obese Australian adults.

The form of the fibre (supplement versus dietary) is also another possible limitation, as psyllium and PGX® were given in sachet form prior to consuming a meal. Therapeutic doses of highly viscous fibre prior to food consumption reduces the physical volume able to be consumed and may further impact the bioavailability of micronutrients within the food matrix. However a high fibre diet from food alone does not appear to have that same potential negative effects on micronutrient status.

Typically high fibre foods such as fruit, vegetables and wholegrains are nutrient dense, which suggests that the inhibitory effects of high fibre on nutrient bioavailability are potentially offset by the higher micronutrient content, although this may not be true for all micronutrients (39).

Another limitation is the use of serum micronutrient values to determine micronutrient status, which may not be an accurate assessment measure for all of the micronutrients examined in the present study (chapter 3 and 4). For example, bone mineral density is thought to be a more accurate measure of calcium status than serum calcium levels, as serum calcium concentration is tightly regulated within the blood. Consequently if serum calcium levels fluctuate this is not necessarily an indication of status, but rather something is affecting bone calcium release or renal processing and reabsorption (40). In addition, iodine status was extrapolated using thyroglobulin values, whereas 24-hour urinary iodine values are a more accurate measure of iodine status. Higher thyroglobulin levels in the blood suggest that the thyroid is working harder to compensate for low iodine levels and may be an indication of iodine deficiency (41). However, for the purposes of this study, serum measurement of micronutrients were the most practical method available.

Finally, using 3-day food diaries was a limitation in this study as potential bias can be introduced due to participants misreporting dietary intake, for a variety of reasons. Studies have shown overweight and obese individuals with a BMI ≥ 30 to be likely to under report their dietary intake (42). Therefore, while food diaries may be a useful tool for use in weight management trials in theory, in practice the data gained from them may contain inaccuracies in micronutrient values due to misreporting. More accurate values can be obtained by providing meals to participants and monitoring the amount consumed, as in a lab setting, however this dietary assessment strategy also has limitations, often making it an impractical method to adopt for clinical trials with free-living individuals.

5.7 Significance

The results obtained from this study support the use of fibre supplementation as a relatively easy and noninvasive strategy to support weight management for overweight and obese individuals. There was no significant impact to serum micronutrient levels with fibre supplementation by adding 15 g of either PGX® or

psyllium fibre to the habitual diet every day for 3 months. When the current evidence is considered, increasing fibre intake can have far reaching health benefits, therefore excluding any possible adverse effects is important, particularly in groups at high risk of nutritional deficiencies, such as individuals with overweight or obesity.

5.8 Conclusions

The complexities of obesity as a disease are still unfolding. Previously over-nutrition in terms of excess calories was the main focus for health professionals and researchers, however high-calorie malnutrition in overweight and obese population is becoming recognised (1). Micronutrient deficiencies can develop as a result of poor diet; and overweight and obese individuals are particularly at risk. Generally this subpopulation tends to consume an energy dense, nutrient poor diet, based largely on highly processed foods. In recent years fibre supplementation has been promoted as an aid to weight loss as it can increase satiety, reduces cholesterol, postprandial glycaemia and improve MS risk factors (1, 43).

The results of the study in chapter 3 support the concern that overweight and obese individuals may have compromised micronutrient levels. Baseline serum micronutrients were compared to the AACB clinical reference intervals in chapter 3 and the majority of participants were outside of normal blood parameters (Table 3.2). A large proportion of the study sample fell below the clinical reference intervals for Calcium, vitamin D, vitamin A, potassium, magnesium, folate, zinc and sodium. Therefore, it is imperative that any weight loss strategy, such as high dietary fibre supplementation, does not further impair their nutritional status. The results from this study suggest that supplementing 15 g per day of either psyllium or PGX® fibre for 3 months does not have any deleterious effects for serum micronutrient levels, however dietary intake of micronutrients is affected by the addition of fibre supplementation (chapter 4). As, many nutritional deficiencies manifest over a long period of time, further studies of a duration greater than 3 months are needed to ascertain whether high-dose fibre supplementation would impair the serum micronutrient levels of individuals with overweight and obesity in the long term.

Further research is needed to gain a clearer understanding of the effects of nutrient-poor diets on the micronutrient status of overweight and obese individuals, particularly prior to undergoing any weight management programs, to minimise the

risk of further compromising their nutritional status with treatment. Research should focus on psyllium fibre, which is widely available and therefore a popular choice with consumers, as well as PGX® fibre, which is a relatively new fibre product and not yet as widely researched. The results of such research would assist with the development of evidence-based recommendations for fibre supplementation, to be used as an adjunct to weight management treatments for overweight and obese Australian adults.

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APPENDIX 1: Ethics approval statement

Curtin University

Memorandum

To	Associate Professor Sebely Pal, Public Health
From	Prof Stephan Millett, Chair, Human Research Ethics Committee
Subject	Protocol Approval **HR 41/2011**
Date	23 November 2011
Copy	Dr Suleen Ho, Public Health Dr Simone Radavelli Bagatini, Public Health

Office of Research and Development

Human Research Ethics Committee

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Thank you for your application submitted to the Human Research Ethics Committee (HREC) for the project titled "*Comparison of two different fibre supplements on body weight, body composition, metabolic and cardiovascular risk factors in overweight and obese individuals".* Your application has been reviewed by the HREC and is **approved**.

- You have ethics clearance to undertake the research as stated in your proposal.
- The approval number for your project is **HR 41/2011.** *Please quote this number in any future correspondence*.
- Approval of this project is for a period of twelve months **23-11-2011** to **23-11-2012**. To renew this approval a completed Form B (attached) must be submitted before the expiry date **23-11-2012**.
- If you are a Higher Degree by Research student, data collection must not begin before your Application for Candidacy is approved by your Faculty Graduate Studies Committee.
- The following standard statement **must be** included in the information sheet to participants:

  This study has been approved by the Curtin University Human Research Ethics Committee (Approval Number HR 41/2011). The Committee is comprised of members of the public, academics, lawyers, doctors and pastoral carers. Its main role is to protect participants. If needed, verification of approval can be obtained either by writing to the Curtin University Human Research Ethics Committee, c/- Office of Research and Development, Curtin University, GPO Box U1987, Perth, 6845 or by telephoning 9266 2784 or by emailing hrec@curtin.edu.au.

Applicants should note the following:

It is the policy of the HREC to conduct random audits on a percentage of approved projects. These audits may be conducted at any time after the project starts. In cases where the HREC considers that there may be a risk of adverse events, or where participants may be especially vulnerable, the HREC may request the chief investigator to provide an outcomes report, including information on follow-up of participants.

The attached **FORM B** should be completed and returned to the Secretary, HREC, C/- Office of Research & Development:
When the project has finished, or

- If at any time during the twelve months changes/amendments occur, or
- If a serious or unexpected adverse event occurs, or
- 14 days prior to the expiry date if renewal is required.
- An application for renewal may be made with a Form B three years running, after which a new application form (Form A), providing comprehensive details, must be submitted.

Regards,

[redacted]

Professor Stephan Millett
Chair Human Research Ethics Committee

APPENDIX 2:

SCREENING CHECKLIST - INCLUSION/EXCLUSION CRITERIA

OVERWEIGHT AND OBESE AUSTRALIAN ADULTS AND FIBRE SUPPLEMENTATION: ITS EFFECTS ON VITAMIN, MINERAL AND ANTIOXIDANT STATUS.

Date:……

Name:……..

Address:……..

…….

Phone No. (Circle best contact)

H:……W:…….Mobile:……..

EMAIL…….... DOB:…… Age:……

Male/Female Height:…… Weight:…… Waist:…….. BMI:……

Medical History	**Yes**	**No**	**Details**
Are you a Smoker			
Have you had your cholesterol, TG, glucose measured recently? Results?			
Medications: – warfarin, steroids, thyroid, lipid lowering etc., what for?			
Nutritional supplements			
Major operations			
Any foods you can't have?			
GI disorders eg. Irritable bowel syndrome, coeliac disease, diarrhoea			
Major illnesses/diseases: Do you have • Diabetic • Kidney disease (renal problems) • Liver disease • Heart disease			
Have you ever received a formal diagnosis by a professional for mental illness?			
Do you have High Blood Pressure			
Do you have asthma			
Allergies (esp. Bee stings)			
Do you play a sport or exercise regularly			
Supervised weight loss programs?			
Regularly have >2 alcoholic drinks/day			
Difficulty taking blood?			
Have you been involved in any other studies in the last 6 months?			

1. Are you able to get to Curtin University? Yes / No
2. Can we keep your contact details for future reference in clinical research? Yes / No

APPENDIX 3:

Supplement Intake Calendar

November 2013						
Sunday	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**
27 ( ) Breakfast ( ) Lunch ( ) Dinner	28 ( ) Breakfast ( ) Lunch ( ) Dinner	29 ( ) Breakfast ( ) Lunch ( ) Dinner	30 ( ) Breakfast ( ) Lunch ( ) Dinner	31 ( ) Breakfast ( ) Lunch ( ) Dinner	1 ( ) Breakfast ( ) Lunch ( ) Dinner	2 ( ) Breakfast ( ) Lunch ( ) Dinner
3 ( ) Breakfast ( ) Lunch ( ) Dinner	4 ( ) Breakfast ( ) Lunch ( ) Dinner	5 ( ) Breakfast ( ) Lunch ( ) Dinner	6 ( ) Breakfast ( ) Lunch ( ) Dinner	7 ( ) Breakfast ( ) Lunch ( ) Dinner	8 ( ) Breakfast ( ) Lunch ( ) Dinner	9 ( ) Breakfast ( ) Lunch ( ) Dinner
10 ( ) Breakfast ( ) Lunch ( ) Dinner	11 ( ) Breakfast ( ) Lunch ( ) Dinner	12 ( ) Breakfast ( ) Lunch ( ) Dinner	13 ( ) Breakfast ( ) Lunch ( ) Dinner	14 ( ) Breakfast ( ) Lunch ( ) Dinner	15 ( ) Breakfast ( ) Lunch ( ) Dinner	16 ( ) Breakfast ( ) Lunch ( ) Dinner
17 ( ) Breakfast ( ) Lunch ( ) Dinner	18 ( ) Breakfast ( ) Lunch ( ) Dinner	19 ( ) Breakfast ( ) Lunch ( ) Dinner	20 ( ) Breakfast ( ) Lunch ( ) Dinner	21 ( ) Breakfast ( ) Lunch ( ) Dinner	22 ( ) Breakfast ( ) Lunch ( ) Dinner	23 ( ) Breakfast ( ) Lunch ( ) Dinner
24 ( ) Breakfast ( ) Lunch ( ) Dinner	25 ( ) Breakfast ( ) Lunch ( ) Dinner	26 ( ) Breakfast ( ) Lunch ( ) Dinner	27 ( ) Breakfast ( ) Lunch ( ) Dinner	28 ( ) Breakfast ( ) Lunch ( ) Dinner	29 ( ) Breakfast ( ) Lunch ( ) Dinner	30 ( ) Breakfast ( ) Lunch ( ) Dinner

**Please tick off each box after each meal

**If you miss a fibre supplement, don't tick the box

Bring your completed calendar to your next long clinical visit

Long Clinical Visit Date: _____

APPENDIX 4:

3-Day Food and Drink Diary Instructions

Please **record everything** that you **eat** and **drink** for **three** consecutive days

Start a new page for each day.

TIME

Record the time each time you eat. Each day begins at 12 midnight.

LOCATION

Record the location of each meal, snack, drink. For example, home, work ,school, restaurant.

AMOUNT

Record the amount of food and liquid consumed.

Liquids: record cups, glasses (in mls). Liquids include water, juice, coffee, milk, tea, soft drinks, alcohol.

Please record sweetners if used;

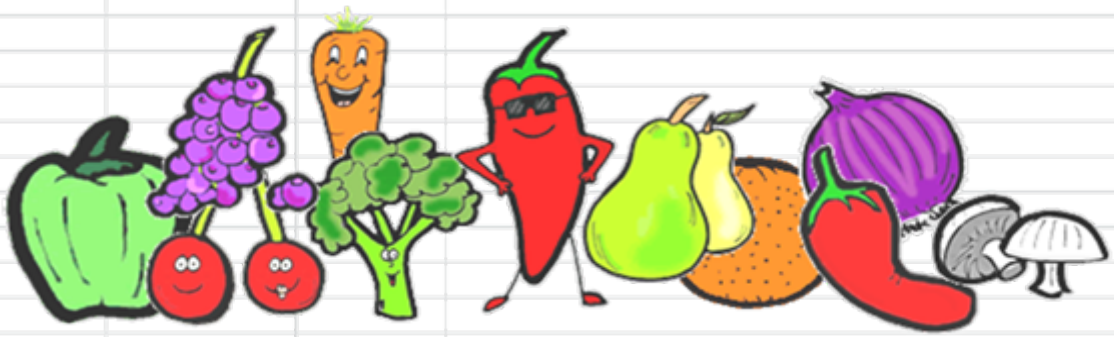
Foods: weigh foods in grams as much as possible. Tablespoons, teaspoons, standard servings (one slice of bread, small apple, etc.) and approximate dimensions are useful. Specify whether food is lean, fatty, diet, light, etc. Weigh food after cooking if possible.

DESCRIPTION AND METHOD PREPARATION

- identify if food is fresh, frozen, canned, unsweetened, sweetened, etc
- designate milk as whole, 2% lowfat, skim or nonfat, or chocolate
- identify cut of meat or poultry or type of fish. Indicate if you ate the skin or fat.
- for prepared food, identify brand and/or describe ingredients. For example, "Oatmeal cookies with raisins", "Kelloggs cornflakes" rather than "dry cereal", etc
- describe method of preparation (fried, baked, boiled, stewed, grilled, etc.)
- record any additional ingredients used in cooking.
- for homemade, prepared foods, describe ingredients and amounts.
- think of each meal in terms of individual foods and record each one separately. for example a ham and cheese sandwich is bread, ham, cheese.

ACCURACY

Write the % accuracy which you think describes your record.



3-day food & drink diary

3-day food diary			**Visit no: _____**
Participant name: _____			Participant ID: _____
Day: _____		Date: ____/____/____	
Begin your food diary at midnight			
Time	**Location**	**Amount**	**Food description and method preparation**
Notes, comments, recipes			
( ) Workday	( ) Non workday		Total fluid intake for the day (eg 2L) _____
Accuracy of food diary out of 100 _____			
If today`s food intake was innacurate, please describe why:			

APPENDIX 5

To Whom It May Concern

I, *Jenny-Lee McKay*, contributed *(80% to each component of the research reported in the publication)* to the paper/publication entitled

McKay J, Jane M, Ho S, Pal S. Comparing baseline (Blood) micronutrients with the clinical reference intervals to determine the nutritional status of overweight/obese Australian adults. 2019. Unpublished. Submitted to Obesity.

(Signature of Candidate)

Jenny-Lee McKay

I, as a Co-Author, endorse that this level of contribution by the candidate indicated above is appropriate.

Assoc. Prof Sebely Pal (Signature of Co-Author 1)

Dr Suleen Ho (Signature of Co-Author 2)

Dr Monica Jane (Signature of Co-Author 3)

APPENDIX 6

To Whom It May Concern

I, *Jenny-Lee McKay*, contributed *(80% to each component of the research reported in the publication)* to the paper/publication entitled

McKay J, Jane M, Ho S, Pal S. Between group comparisons in (blood) micronutrients to determine if psyllium or PGX supplementation altered micronutrient status of overweight and obese after 3 months of treatment. 2019. Unpublished. Submitted to BMC Public Health.

(Signature of Candidate)

Jenny-Lee McKay

I, as a Co-Author, endorse that this level of contribution by the candidate indicated above is appropriate.

Assoc. Prof Sebely Pal (Signature of Co-Author 1)

Dr Suleen Ho (Signature of Co-Author 2)

Dr Monica Jane (Signature of Co-Author 3)

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Review article

Effects of daily consumption of psyllium, oat bran and polyGlycopleX on obesity-related disease risk factors: A critical review

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ABSTRACT

The persistent obesity crisis, with its increased risk for the metabolic syndrome (MetS), type 2 diabetes, and cardiovascular disease (CVD), continues to damage the health of populations globally, including children. Diets rich in the fiber provided by fruit and vegetables support good metabolic health, although few adults and children achieve the recommended daily target. Daily fiber supplementation, particularly with soluble fiber products, such as psyllium, oat bran, or a newer product such as PolyGlycopleX, may provide a convenient solution. Literature searches were conducted to identify original research articles, systematic reviews, and meta-analyses with the search terms *psyllium, oat bran, PolyGlycopleX,* and *PGX*, AND *adults* and *children* AND *overweight, obesity,* and *metabolic syndrome*. Data source was Embase and PubMed from 1980 to 2017. The results show that the addition of a soluble fiber product, most notably psyllium, improves blood lipid profiles, particularly total and low-density lipoprotein cholesterol, as well as glycemic response, and increases satiety, and by thus improving MetS and CVD risk factors, may augment the processes initiated by weight reduction diets. Although less studied than psyllium, the available evidence has shown that β-glucan present in oat bran has a beneficial effect on MetS and CVD risk factors, particularly blood lipids and glycemia. Early research has found PolyGlycopleX to provide similar benefits to other soluble fiber products, and suggest it may also assist with weight loss. This critical review demonstrates that soluble fiber supplements used as an adjunct to dietary and lifestyle modifications may assist with the treatment of CVD and MetS risk factors. More research is needed to further clarify the benefits of PolyGlycopleX in particular, as well as to develop safe and efficacious recommendations for fiber supplementation of all types for children in general.

Introduction

Obesity is the cause of many debilitating physical, emotional, social, and economic consequences [1]. Worldwide rates of obesity have nearly doubled in the past 3 decades [2], a trend that shows no sign of abating [3,4]. More specifically, abdominal or central obesity is strongly associated with the development of metabolic syndrome (MetS) [1]. This is thought to be due to the effects of the increased release of free fatty acids, inflammatory cytokines, and other byproducts from the adipocytes, generated by excess adiposity [1,5]. These effects have also been observed in children who are overweight or obese [3,6,7].

MetS has been defined by the International Diabetes Federation as the presence of central obesity as well as any two of the following factors: elevated blood pressure (BP; hypertension), reduced high-density lipoprotein (HDL), raised triacylglycerols (TGs; hypertriglyceridemia), and elevated fasting plasma glucose (hyperglycemia) [8]. The same applies to children and adolescents, with the presence of central obesity identified as a waist circumference greater than the 90th percentile [9]. Having MetS leads to an increased risk for developing type 2 diabetes (T2D) and cardiovascular disease (CVD) [1,5,10,11]. The risk for CVD-related mortality is also higher in those identified as having MetS [11,12].

High dietary fiber intake is known to have a protective affect against CVD, T2D, hypertension, and obesity [13]—the latter being a risk factor for developing these conditions [5]—and to lower serum cholesterol [13–16]. A diet rich in fiber from fruit, vegetables, legumes, and whole grains is considered beneficial for health in adults [13,14] and children [17,18]. It is recommended that

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adults consume 25 to 30 g/d, whereas children should consume between 14 and 28 g/d of dietary fiber [19,20], although recommendations for children vary widely depending on the country and the evidence base used [21]. However, relatively few individuals achieve the targeted amount of fiber in their diets [22,23], as demonstrated in the U.S. Continuing Survey of Food Intakes by Individuals 1994–96, 98 [23], and more recently the 2011–12 Australian Health Survey [22]. Therefore, adding fiber may provide a convenient and cost-effective alternative for increasing the fiber content of a diet without the need for other major dietary modifications.

Psyllium has been extensively researched due to its inclusion in many popular over-the-counter laxatives [24]. It is known to promote bowel regularity and improve blood lipids [24,25]; however, less is known about the role of psyllium on weight and other MetS risk factors. Oat bran is another commonly consumed food product, but the potential health benefits have not been studied as widely. PolyGlycoplex (PGX) is a fiber product relatively new to the market, and early research has shown it has a potential for weight loss benefits; however, studies relating to the effects of PGX on other MetS risk factors are limited. Although the majority of research has been undertaken on adults and those at risk for MetS and CVD, the effects of dietary or soluble fiber products have been less explored in children [26]. The aim of this review is to critically examine the available evidence and assess the benefits of the aforementioned fiber products, and to evaluate their effects on weight loss and MetS risk factors in adults and children. This is required to clarify safe and effective fiber supplementation recommendations with particular reference to improvements in weight as well as MS and CVD risk factors in adults as well as children, particularly as fiber supplementation for the prevention (and treatment) of these conditions may be more preferable to medical interventions in the young. This critical review also identifies areas where further research is needed. Literature searches were conducted to identify original research articles, systematic reviews, and meta-analyses with the search terms "terms *psyllium, oat bran, PolyGlycopleX,* and *PGX*, AND *adults* and *children* AND *overweight, obesity,* and *metabolic syndrome*." Data sources were Embase and PubMed from 1980 to 2017. The bibliographies of all articles located were searched for further studies. The disproportionate amount of evidence available regarding psyllium compared with oat bran and PGX is reflected in this review.

Commercially available soluble fiber products

Dietary fiber is derived from indigestible polysaccharides in fruit, vegetables, and whole grains [24], and provides a prebiotic function in the large intestine via fermentation [15], a byproduct of which is short-chain fatty acids (SCFA), beneficial for gastrointestinal health [13]. Diets high in fiber tend to have lower energy density and thus promote decreased energy intake [27,28]. Fiber is classified as either soluble or insoluble depending on its water-holding capacity or degree of viscosity in solution [29] and contains a number of bioactive compounds [15]. A variety of soluble fiber preparations are readily available in most grocery stores, pharmacies, and health food stores. For the purposes of simplifying recommendations to patients, three readily identified and accessible soluble fiber products were selected: psyllium, oat bran, and PGX. Unprocessed psyllium and oat bran packaged in bags as a food ingredient appear to be the most cost-effective option. These can be added to water, juice, or yogurt; used in cooking; or added to breakfast cereals or muesli. For those who find consuming unprocessed fiber inconvenient or unpalatable, flavored powders are available. For example, psyllium is flavored with orange, berry, or lime under the brand name Metamucil, a commercially available fiber supplement. Psyllium can be obtained in capsule form as well.

A recent addition to the fiber product market is PGX, a proprietary complex of three natural polysaccharides, packaged as granules in dose-size sachets that can be added to food or drinks. Currently, PGX can only be obtained at retail outlets in the United States and Canada, but it is available online in other regions. Much of the research into PGX tested the granules. A PGX gel capsule preparation is available, but these contain a small amount of fatty acids derived from coconut oil [30], and at this stage the effect of this particular ingredient on satiety, weight loss, and MetS risk factors is unclear. Therefore, this product was not included in this review.

Mechanisms of action

Blood lipids

The viscosity of chyme appears to interfere with the absorption of bile acids in the large intestine, which increases fecal bile acid elimination [15], stimulating additional bile acid production and intracellular cholesterol [31]. The lipid-lowering effect may occur by reducing the rate of intestinal cholesterol uptake [32], possibly trapped in the viscous gel formed [15], and reducing the amount of circulating chylomicrons [33], thus reducing cholesterol in circulation [24]. Additionally, lower postprandial insulin secretion may result in reduced lipogenesis [31], and lower circulating TGs [24]. Undigested soluble fiber is subject to bacterial fermentation in the large intestine [24]. This results in the production of SCFAs, including propionate, which travels directly via the portal vein to the liver, where it is thought to hinder cholesterol synthesis [34].

Glycemic response

Satiety, as a result of having eaten, is a state in which continued eating is suppressed [27]. Consumption of soluble fiber increases the bulk and reduces the energy density of the chyme, thus delaying gastric emptying [35]. Soluble fiber also raises the viscosity of chyme, which has the effect of widening the unstirred water layer in the small intestine, thus delaying energy and nutrient (e.g., carbohydrate) absorption [24]. These delays reduce postprandial insulin secretion [35], moderate postprandial blood glucose concentration [24], and are thought to promote satiety [24,35], which may assist with weight management. Supplementation with soluble fiber may improve insulin sensitivity via this or other as yet unidentified mechanisms; however, further evidence is needed to elucidate this factor.

Blood pressure

So far, research evidence favors insoluble over soluble fiber consumption as a means of moderating blood pressure (BP) [36,37]. A variety of mechanisms haa been considered to explain the effect of fiber consumption on BP, such as moderation of the glycemic response due to enhanced insulin sensitivity [13] and increased production of the vasodilator nitric oxide, leading to improved endothelial function [38].

Soluble fiber and children

Before the 1990s, a concern among some pediatricians was that increasing dietary fiber intake in children may displace more energy-rich carbohydrates and thus reduce the amount of energy available for normal growth and development [39]; however, later

studies have refuted this claim [40]. Additionally, recent data show that many children do not get enough fiber from the diet [22]. Evaluation of epidemiologic evidence from Europe, Oceania, and North America by Edwards et al. found that most children did not consume the recommended amount of daily dietary fiber set within their country [21]. Although the number of studies available for review was modest, these investigators found some evidence to suggest that higher fiber intakes in children may have a protective effect against obesity, MetS, insulin resistance, and hypertension [21]. A cohort study of Japanese children 10 to 11 y of age (N = 5873) found dietary fiber consumption to be inversely associated with total cholesterol (TC), overweight, and obesity [41]. Nevertheless, there is insufficient evidence with which to make dietary fiber intake recommendations, specific for age and growth [42], and much of the existing recommendation appears to be extrapolated from research with adults [21]. Although supplementing fiber may be an acceptable alternative to pharmaceutical treatments in the treatment and prevention of MetS in children [43], efficacious dosages are yet to be determined.

Psyllium

Psyllium seed husk is a viscous, water-soluble, gel-forming mucilage from the *Plantago ovata* plant and has advantages over other soluble fiber sources because it is less readily fermented and therefore causes less abdominal bloating [44]. Psyllium has been shown to be an effective supplement for decreasing CVD risk [45] and elevated BP (in hypertensives) [46], improving blood lipid profiles, regulating the bowel [25,47].

Weight loss

A variety of studies have tested psyllium supplementation for weight loss. One such study showed no significant changes in body mass index (BMI) in 49 slightly overweight participants with T2D consuming 10.2 g of psyllium for 8 wk compared with a placebo [48], whereas other longer-term have studies produced contrasting results. A study that examined the supplementation of 21 g/d of psyllium for 3 mo in 57 overweight or obese participants reported significant changes in BMI ($P = 0.010$), weight ($P = 0.007$), and total body fat ($P = 0.002$) [49]. Additionally, improvements in these measures were greater when psyllium was combined with a healthy diet ($P = 0.001$, for the three outcomes) [49]. Another study of 141 overweight individuals with hypertension reported improvements in BMI (-1, $P = 0.01$) after a dose of 7 g/d of psyllium after 6 mo compared with a standard diet [50]. Additionally, a 12-mo study of overweight and obese adults conducted by Pal et al. reported statistically significant reductions in weight (2.6%, $P = 0.002$), body fat percentage ($P = 0.038$), and waist circumference ($P = 0.01$), after supplementation of 15 g/d of psyllium compared with placebo [51]. It would appear that duration of psyllium consumption may be a more influential factor than dosage where weight loss is concerned.

Satiety is an important factor for individuals undertaking weight management. Postprandial gastrointestinal appetite hormones ghrelin and peptide YY (PYY) have been shown to decrease 2 h after consumption of psyllium-enriched meals by healthy-weight participants, which may explain the weight loss effects of this fiber product [52]. Another study of non-restrained eaters found a significant reduction in 1 h postprandial fullness ratings, along with a decrease in total fat and energy intake during the day after two premeal doses of psyllium (separated by 3 h) [53]. The satiety-inducing effect of psyllium has been found to be similar to other fiber sources [54].

Blood lipids

Research has suggested that psyllium supplementation may provide cardiovascular benefits via its effect on blood lipids [13,55–57], perhaps due to its viscosity [29]. A study by Khossousi et al. evaluated the 6-h postprandial effects of the consumption of 3 g of psyllium taken with a low-fiber meal versus a high-fiber meal (15 g) in 10 overweight and obese men [58]. Serum TG concentration was significantly lower 4 h after consumption of the high-fiber meal compared with the low-fiber meal ($P < 0.05$); plasma concentration of apolipoprotein B48 (a marker for chylomicrons) was significantly lower 1 h after the high-fiber meal ($P < 0.05$) and remained lower for the entire 6-h postprandial period [58]. The authors suggest that a single acute dose of psyllium can decrease arterial exposure to TGs and modify chylomicron responses in the postprandial period [58].

Longer-term effects also have been observed. At the end of a 6-wk study of 20 individuals with T2D given 14 g/d of psyllium in a test breakfast, researchers observed a significant decrease in TC and low-density lipoprotein (LDL), 7.7% and 9.2%, respectively ($P < 0.05$) [59]. A daily dose of 10.2 g psyllium increased HDL significantly ($P < 0.05$), and decreased the LDL-to-HDL ratio (an indicator of CVD risk), in the 8-wk study of T2D patients mentioned earlier [48]. In 1988, Anderson et al. conducted a study of 26 men with mild to moderate hypercholesterolemia, whereby participants added 10.2 g/d of either psyllium or cellulose (placebo) to meals for 8 wk and found psyllium to reduce serum TC by 14.8%, LDL by 20.2%, and the ratio of LDL to HDL by 14.8%, relative to baseline values [60]. Similar findings resulted in later studies conducted by Anderson with other researchers [56,61].

More recently, the 12-wk study by Pal et al. demonstrated that supplementing a habitual diet with 21 g/d of psyllium was sufficient to result in improvements in TC (-15%, $P = 0.003$) and LDL (-23%, $P = 0.001$) in overweight and obese individuals [49]. A longer-term clinical trial conducted by Pal et al. found significant reductions in TC (4.8%, $P = 0.006$), and TG (12.7%, $P = 0.023$) at 6 mo compared with baseline in overweight and obese participants supplementing 15 g/d of psyllium [62].

Glycemic response

The viscosity of soluble fibers such as psyllium has been implicated as a moderator of the glycemic response [29,57,63]. An 8-wk study of 34 men with T2D given 10.2 g/d psyllium or cellulose (control) reported the lunch postprandial glucose concentration to be 19.2% lower and the all-day glucose concentrations to be 11% lower in the psyllium group than in the control group [64]. A previously discussed, an 8-wk study found 5.1 g/d of psyllium significantly improved fasting blood glucose and glycosylated hemoglobin compared with the control ($P < 0.05$), in T2D patients, and although insulin levels did not change, these results suggest improved gylcemic control [48]. Nevertheless, another study found that either a healthy diet alone, or together with psyllium supplementation, reduced insulin (5%, $P = 0.05$; 8%, $P = 0.02$, respectively) compared with the control group in overweight and obese participants after 12 wk; although psyllium added to the habitual diet did not [49]. Although a 12-mo trial with overweight and obese adults found no significant difference in blood glucose after 15 g/d of psyllium compared with the control, there was a significant difference in insulin (-9.4%, $P = 0.029$) [62].

Blood pressure

Studies measuring changes in BP after psyllium consumption have produced contradictory results. A systematic review and

meta-analysis found that psyllium consumption was not associated with lower BP [65]. No significant improvements in vascular function or BP were found by Pal et al. in their 12-wk study of the effect of consuming 21 g/d psyllium in obese and overweight individuals [66]. Another study examined the effect of dietary protein (raised to 25% of total energy intake) and/or 12 g psyllium added to a low-fiber diet in 41 treated hypertensive patients, and found that systolic and diastolic BP (SBP and DBP, respectively) decreased with exposure to either protein (SBP: −2.9 mm Hg; DBP: −2.5 mm Hg) or fiber (SBP: −2.4 mm Hg; DBP: −1.9 mm Hg); an additive effect was found when the two were combined (SBP: −10.5 mm Hg; DBP: −3.6 mm Hg) in the 24-h postprandial period, relative to a low-fiber, low-protein control group [67]. A concomitant reduction in hypertension may not necessarily be found with improvements to blood lipids due to the influence of other factors on BP [68].

Children

Studies examining the effect of psyllium supplementation on weight and other MetS risk factors in children are scarce. Early indications suggest that psyllium improves lipid profiles and glycemic response in children and adolescents who are overweight or obese [7]. More recently, a 6-wk study of 45 healthy adolescent participants (15–16 y of age; 44% overweight or obese), given 6 g/d of psyllium, reported improvements in LDL ($P = 0.042$), and a 4% reduction in the ratio of android to gynoid fat (or adiposity; $P = 0.019$), but no meaningful changes in BP [69]. Further studies are needed to determine the health benefits of psyllium supplementation in children of all age groups given that it has lipid-, glucose-, and insulin-lowering effects in adults.

Oat bran

Oat bran has attracted the attention of food and nutrition scientists in recent years; however, studies examining the health benefits of this fiber source are less abundant compared with psyllium. Oats, derived from the *Avena sativa* plant, is a cereal containing a complex array of compounds [70]. One constituent of interest is β-glucan, a viscous, soluble fiber found in the endosperm, consisting of glucose molecules with β-(1→3) and β-(1→4) linkages [70]. It is thought that these physiochemical properties may assist in the reduction of hypertension, thus reducing CVD and stroke risk [70,71] as well as MetS risk factors [72]. Oat bran may contribute to stool weight by delivering highly viscous β-glucan to the large intestine for fermentation, but does not appear not to contribute to improved laxation [24]. Consumption of oat bran β-glucan has been shown to result in dose-dependent improvements in the blood lipid profiles of middle-aged adults [73], as well as increase glycemic control in patients with T2D [74].

Weight loss

Studies regarding the effects of oat bran on weight are few, with most trials assessing changes to blood glucose and blood lipid profiles after oat bran consumption. A 3-mo trial with 56 overweight women randomized into groups adding either 0, 5–6, or 8–9 g of β-glucan to an energy-restricted diet, showed a significant reduction in weight (m = 4.1 kg, $P < 0.001$) and waist circumference ($P < 0.001$) in all groups [75]. The amount of weight loss expected in the 8- to 9-g group was 6 to 6.5 kg; therefore, the authors were unable to draw any firm conclusions about the effects of β-glucan consumption on weight [75].

To examine the effect of oat bran on weight management, Juvonen et al. assessed pre- and postprandial plasma levels of appetite-regulating hormones ghrelin and PYY, along with self-reported appetite ratings of three test meals of varying fiber composition (10 g oat bran, 5 g oat bran with 5 g wheat bran, or 10 g wheat bran, and a control) in 20 young, healthy-weight participants [76]. This study reported no significant between-group differences in plasma ghrelin, PYY measurements, or self-rated appetite; however, the researchers postulate that different results may be achieved with overweight and obese individuals [76].

A 12-wk trial examined the effect of oat bran on weight loss in 144 hypercholesterolemic overweight and obese individuals, whereby an oat-based cereal product (3 g/d of β-glucan) was added to a weight loss program and compared with a low-fiber control group [77]. Statistically significant reductions in waist circumference were seen and may have been due to improved gastrointestinal health as opposed to a reduction in abdominal adiposity. Weight loss, however, was not significant and therefore could not be attributed to the effect of β-glucan [77].

Blood lipids

Several studies have found that oat bran increases bile acid excretion and synthesis, and reduces serum TC [71]. More specifically, β-glucan has been shown to reduce fasting and postprandial lipoproteins [70]. One possible mechanism is that β-glucan consumption increases the viscosity of the outer layer of chyme, which hinders the absorption of cholesterol and the reabsorption of bile acids in the small intestine [71], thus increasing bile acid excretion, stimulating bile acid synthesis, and reducing serum LDL [70,71], similar to the actions of soluble fiber in general [15].

To examine this effect in the short term, a 3-d study of nine outpatients with conventional ileostomies tested two types of extruded oat bran cereal (75 g)—one with native β-glucan (11.6 g), the other with hydrolyzed β-glucan (4.5 g)—and measured bile acid and cholesterol excretion [78]. The results showed that native β-glucan cereal increased median bile acid excretion by 144% ($P = 0.008$), and lowered cholesterol absorption by 19% ($P = 0.013$) [78]. Native β-glucan may perform these functions better than an extruded analog, as the extraction process may alter the physiochemical properties of β-glucan [79].

In a 2-wk study of 24 healthy individuals who consumed a diet containing 10.2 g/d of oat bran (equivalent to 6 g/d of soluble fiber) versus a low-fiber diet reported that oat bran reduced circulating TC by 14% compared with the low-fiber control diet (4%, $P < 0.001$) and LDL by 33% compared with the control (9%, $P < 0.01$). The oat bran diet also resulted in reduced hemostatic factors and blood-clotting regulators implicated in CVD [80].

Although the duration and sample sizes in these studies are small, they support the view that β-glucan in food can positively alter blood lipids, and thus reduce CVD risk factors, in a relatively short time. Further evidence from a longer-term trial with 204 overweight and obese participants given 3 g/d of β-glucan compared with a low fiber control demonstrated a significant reduction in LDL (-8.7 ± 1 versus $-4.3 \pm 1.1\%$, $P = 0.005$) and TC (-5.4 ± 0.8 versus $-2.9 \pm 0.9\%$, $P = 0.038$) after 12 wk [77] and further support these results.

Glycemic response

Oat bran may lower a meal's glycemic index by reducing its energy digestibility, in a similar manner to fiber in general. Juvonen et al. reported a reduction in postprandial plasma glucose ($P = 0.001$) and insulin ($P < 0.001$) with 10 g of oat bran in a meal [76]. Another study demonstrated that β-glucan reduced the postprandial glycemic response ($P < 0.002$) in 12 patients with T2D, after adding 30 g of oat bran flour to a 25-g glucose load [81].

However, whether there is a long-term or additive effect on glycemic response or T2D with regular consumption of oat bran/β-glucan remains to be validated by further studies.

Blood pressure

Although one systematic review reported less convincing results with regard to the effect of oat bran β-glucan on BP [82], a slightly more recent systematic review and meta-analysis reported an inverse association between higher β-glucan intake and lower BP [65]. Specifically, diets higher in β-glucan (median difference of 4 g between high and low) were shown to reduce SBP and DBP by averages of 2.9 and 1.5 mm Hg, respectively [65]. However, different results can be found in individual trials. A 12-wk study of 110 participants with moderately high BP demonstrated non-significant decreases in SBP (1.8 mm Hg) and DBP (0.8 mm Hg) after 8 g/d of oat bran compared with a low-fiber diet [83]. Additionally, a 6-wk study with 100 g oat bran added to daily meals compared with a low-fiber meal equivalent found no significant effect on either SBP or DBP on normotensive participants [84].

Children

Minimal evidence exists of the effects of oat bran supplementation on MetS risk factors in children; however, some studies conducted in the early 1990s that examined its effects in children with elevated cholesterol provide limited information. One such study of 20 hypercholesterolemic children 5 to 12 y of age reported a significant reduction in LDL and a significant increase in HDL after daily consumption of oat bran compared with soy (dosages: 1 g/kg body weight) after 7 mo ($P < 0.05$) [85]. Another study of 49 children (mean age 10 y) with elevated cholesterol found that although the overall lipid profiles did not differ significantly, 38 g/d of oat bran for 4 wk significantly reduced apolipoprotein B ($P = 0.05$) and resulted in greater improvements in apolipoprotein A1 compared with the control [86]. These results may suggest that over a longer duration, oat bran consumption may improve lipid profiles in children with high cholesterol.

PGX

PolyGlycopleX is a non-starch polysaccharide product consisting of three natural fiber components (glucomannan, sodium alginate, and xanthan gum), manufactured by a proprietary process known as EnviroSimplex [87]. Together these constituents form a highly fermentable complex with a very high viscosity and high water-holding and gel-forming properties, making it approximately seven times more viscous than psyllium [87].

A study with healthy individuals to determine the tolerability of PGX found no major adverse events, with participants reporting gastrointestinal discomfort (e.g., flatulence, bloating, intestinal rumbling, or abdominal pain), which was no different to the effects of the placebo (skimmed milk powder) [88]. Such gastrointestinal responses to higher fiber consumption are well documented in the literature [24] and are considered normal consequences of increasing dietary intake of fruits and vegetables and fiber in general [89]. The small number of studies of PGX available for review reflect the relatively recent addition of this product on the fiber supplement market.

Weight loss

Recent trials have examined the effect of PGX on weight loss. Solah et al. found that when participants consumed 12.2 g PGX daily for 12 wk per protocol they experienced 1.4 kg weight loss compared with participants on a rice flour diet ($P < 0.01$); however, this change was not significant with intention-to-treat analysis [90]. A 14-wk study of 29 overweight and obese participants supplemented with 15 g/d of PGX, along with weight loss dietary and lifestyle modifications, experienced a 6.44% reduction of body weight, a 6.02% reduction of total body fat, and an 11.65% decrease in waist circumference compared with baseline ($P < 0.05$) [91]. However, without a control group to offset the effects of the diet and lifestyle modifications, it is not possible to determine the exact role of PGX on these outcomes. Nevertheless, similar results were reported in another 14-wk placebo-controlled trial of 64 overweight and obese adults in Japan, with significant reductions in waist circumference of 1.96 cm ($P < 0.008$) and visceral adiposity (in women only; $P = 0.045$) observed after consumption of 15 g/d of PGX [92]. A 15-wk study examining the effect of daily consumption of 15 g PGX on weight in 60 overweight and obese men and women compared with participants supplemented with 15 g/d inulin reported a significant weight loss of 1.6 kg in the PGX group women ($n = 14$; $P = 0.016$) [93].

A 12-mo trial by Pal et al. compared the effects of either 15 g of PGX or 15 g psyllium daily against a placebo with measurements collected at 3-mo intervals and found both PGX and psyllium improved weight and total body fat, although participants in PGX group had slightly better results (−2.8%, $P = 0.012$ and $P = 0.008$, respectively), and these changes were maintained more consistently throughout the trial period [51].

Blood lipids

More seems to be known regarding the influence of PGX on blood lipids, which has been shown to have lipid-lowering effects in both healthy and overweight or obese participants. A trial with 54 healthy participants given 5 g/d of PGX for the first week followed by 10 g/d of PGX for the following 2 wk reported a significant reduction of TC of 0.70 mmol/L and LDL of 0.48 mmol/L compared with controls (*P*-values not reported) [88]. The first of the 14-wk trials discussed previously showed significant reductions in TC and LDL levels of 19.26% and 25.51%, respectively, after 15 g/d of PGX compared with baseline measurements ($P < 0.05$) [91]. Similarly, the other 14-wk trial cited previously reported significant reductions in TC of 6.6% and LDL of 12.2% after 15 g of PGX daily over the trial period ($P < 0.05$) [92].

Further results from the long-term trial mentioned earlier found both PGX and psyllium to improve blood lipids; however, the PGX group showed better results for the 12-mo duration [62]. The cholesterol-lowering effects found in these studies may be due to SCFAs produced by microbiotic digestion of PGX in the large intestine, as demonstrated in a simulation experiment conducted by Reimer et al. [94].

Glycemic response

Some of the studies conducted so far also examined the effect of PGX on glycemic response. One study demonstrated that 2.5 to 5 g of PGX added to a meal was effective in lowering the glycemic index of food, thus reducing postprandial glycemia and modifying appetite-regulating hormones ghrelin and PYY ($P = 0.043$) in healthy adults [95]. A study of 10 participants compared meals containing either 2.5, 5, or 7.5 g PGX, or 5 g inulin (control), found increased self-rated satiety after the PGX meals, as well as a dose-dependent reduction in glycemic response over a 2-h postprandial period compared with the control meal [96]. However, matching inulin dosages in the control meals may yield different results.

Table 1
Range of the effects of the different fiber supplements on key disease factors

	Psyllium		Oat bran/β-glucan		PolyGlycopleX	
	Dose (g/d)	Effect	Dose (g/d)	Effect	Dose (g/d)	Effect
Total cholesterol (%)	10 to 21	−4.8 to −15	3 to 6	−5.4 to −14	10 to 15	−6.6 to −19.3
LDL-cholesterol (%)	10 to 21	−9.2 to −23	3 to 6	−8.7 to −33	10 to 15	−12 to −25.5
Fasting blood Glucose (%)	10	19.2*	IC	IC	IC	IC
Systolic BP (mm Hg)	12	−2.4*	8	−1.8*	NE	NE
Diastolic BP (mm Hg)	12	−1.9*	8	−0.8*	NE	NE

BP, blood pressure; LDL, low-density lipoprotein; IC, studies inconclusive; NE, no evidence.
*Data sourced from a limited number of studies.

Blood pressure

The available studies have not reported any effects regarding PGX supplementation and BP.

Children

To our knowledge, there have been no studies examining the effects of PGX supplementation in young or overweight children. Interestingly, however, a postprandial study of 31 healthy adolescents (16–17 y of age) compared the effects on appetite of one of three 5-g fiber preloads—PGX, glucomannan, or cellulose—administered 90 min before an ad libitum pizza meal. The study found PGX to produce a significantly greater reduction in subsequent pizza consumption (in grams) compared with the two comparison preloads ($P = 0.008$) [97]. No other outcome measures were collected in this study. Further studies are required to clarify the effects of PGX supplementation in children of different ages.

The quantitative results discussed here are presented in Table 1.

Recommendations for clinical practice

The following is a list of recommendations based on the evidence discussed.

Psyllium

Psyllium supplementation may be a suitable alternative to lipid-lowering medication with minimal side effects, particularly for overweight/obese adults and children at low to moderate risk. Adding psyllium to a healthy diet or weight management program would maximize the benefits of this strategy. A minimum of 10 g of psyllium husk or powder (~2 teaspoons) or the equivalent in capsules, taken with 250 mL of water twice daily would be suitable for adults, and a minimum of 5 g of psyllium taken with 250 mL of water once or twice daily may be suitable for children (≥12 y of age) at low risk. If palatability is a problem, psyllium powder or husks can be added to meals; however, extra water should be consumed throughout the day.

Oat bran

A 10-g dose of oat bran (~6 teaspoons) added to a meal appears to be sufficient to moderate the glycemic response after a meal and may assist adults at low risk in managing their blood glucose levels. No recommendations can yet be made for oat bran supplementation in children.

PGX

Although preliminary evidence suggests that 5 g supplementation of PGX taken with at least 250 mL of water twice or three times a day may augment a weight management program in overweight adults, further research is required to determine recommendations to assist with MetS risk factors. No recommendations can yet be made about supplementation of PGX in children. Additionally, this product is only available online in some places, and the price may be prohibitive.

Conclusions

Overall, soluble fiber preparations such as psyllium have demonstrated positive improvements in serum TC, LDL, and body composition, as well as postprandial glycemic response. Based on the evidence discussed here, 10 to 15 g/d of psyllium added to a weight management program for a minimum of 6 mo may augment the improvements in MetS risk factors desired for overweight and obese individuals. Although the effects of psyllium and, to a lesser degree, oat bran have had relatively more attention from researchers, a small number of studies with PGX have reported improvements to some of the aforementioned disease risk factors. Many unknowns remain regarding the effect of specific fiber products on BP, another risk factor for both MetS and CVD. Elucidating these effects appears problematic, possibly due to difficulty in separating the effects of other dietary and lifestyle factors on BP.

The results of this critical review demonstrate that the addition of a fiber product, most notably psyllium, to the daily diet can improve blood lipid profiles, particularly TC and LDL cholesterol, moderate glycemic response, and increase satiety. Although the weight loss results are inconsistent, by improving these cardiovascular and MetS risk factors, this fiber product may augment the processes initiated by dietary and lifestyle modifications. PGX is a relatively new fiber product; therefore, more research is needed to confirm the effects noted here and should include an examination of the different types available (e.g., granules versus gel capsules) as well as the required dosages.

Although it is still unclear whether fiber supplementation will reduce overall MetS and CVD risk on its own, it may be a useful strategy to support other risk reduction measures such as lifestyle changes, pharmaceutical treatments, or both [98]. The heterogeneity of studies into fiber supplementation for the treatment of obesity-related risk factors should be taken into consideration when evaluating the evidence presented in this review. Further randomized, controlled clinical trials are needed before any definitive safe and efficacious recommendations can be made regarding fiber supplementation of all types in children of all age groups.

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