THE ROLE OF ZINC IN CHRONIC DISEASE

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Declaration

The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968.

Khanrin P Vashum Date

Thesis by publication statements

I hereby certify that this thesis is submitted in the form of a series of publications of which I am first author. I have included as part of the thesis a written statement from each co-author; and endorsed by the Deputy Head of Faculty (Research), attesting to my contribution to the joint publications.

Khanrin P Vashum Date

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I have been very privileged to be surrounded by supportive and caring colleagues over the past 4 years. There are a number of people who I wish to thank.

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To my friends, I would like to say thank you once again for all your support and help during my PhD years and in finishing this thesis. Without any names being taken, you all know who you are; I appreciate every helping hand you all have extended towards me. Thank you for always being there for me.

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List of publications

1. **Khanrin Phungamla Vashum**, Mark McEvoy, Abul Hasnat Milton, Md. Rafiqul Islam, Stephen Hancock and John Attia. **Is Serum Zinc Associated with Pancreatic Beta Cell Function and Insulin Sensitivity in Pre-Diabetic and Normal Individuals? Findings from the Hunter Community Study**. *PLOS One* January 08, 2014 (DOI: 10.1371/journal.pone.0083944)

2. **Khanrin Phungamla Vashum**, Mark McEvoy, Zumin Shi, Abul Hasnat Milton, Md. Rafiqul Islam, David Sibbritt, Amanda Patterson, Julie Byles, Deborah Loxton and John Attia. **Is dietary zinc protective for Type 2 diabetes? Results from the Australian Longitudinal Study on Women's Health.** *BMC Endocrine Disorders* 2013, **13**:40 (DOI: 10.1186/1472-6823-13-40)

3. **Khanrin Phungamla Vashum**, Mark McEvoy, Abul Hasnat Milton, Patrick McElduff, Alexis Hure, Julie Byles and John Attia. **Dietary zinc is associated with a lower incidence of depression: findings from two Australian cohorts.** Journal of AffectiveDisorders166 (2014) 249–257 (DOI: 10.1016/j.jad.2014.05.016)

4. **Khanrin Phungamla Vashum**, Mark McEvoy, Abul Hasnat Milton, Patrick McElduff, Alexis Hure, Julie Byles and John Attia. Is **dietary zinc associated with a higher incidence of cardiovascular disease: findings from two Australian cohorts.** Submitted to the 'Nutrition, Metabolism & Cardiovascular Diseases' for publication.

List of additional publications with relevance to this thesis

1. Md. Rafiqul Islam, Iqbal Arslan, John Attia, Mark McEvoy, Patrick McElduff, Ariful Basher, Waliur Rahman, Roseanne Peel, Ayesha Akhter, Shahnaz Akter, **Khanrin P Vashum** and Abul Hasnat Milton. **Is serum zinc level associated with prediabetes and diabetes? A cross-sectional study from Bangladesh.** *PLOS One* April 17, 2013 (DOI: 10.1371/journal.pone.0061776)

2. Jun Shi Lai, Annette Moxey, Gabriel Nowak, **Khanrin Vashum**, Kylie Bailey, Mark McEvoy, **The Efficacy of Zinc Supplementation as Therapy for Depression: Systematic review of randomized controlled trials.** *Journal of Affective Disorders*. (DOI: 10.1016/j.jad.2011.06.022)

Statement of Contribution of Authors

We the undersigned co-authors attest that the research higher degree candidate **Khanrin Phungamla Vashum** contributed to conceptualization of the idea, study design, analyzed and interpreted the data, and developed all of manuscript included in this '**thesis by publication**'.

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Chapter 2

Synopsis

This thesis by publication is composed of a background, rationale & aims, brief literature review, four papers, and a final chapter providing conclusions. All but one of the papers relates to prospectively investigating the role of dietary zinc in chronic disease, which has been examined inadequately in the literature. At this stage of the thesis, three of the four papers have been accepted for publication in peer-reviewed journals. The final paper has been currently submitted to a peer-reviewed journal that is internationally recognised.

Chapter 1 outlines the background, structure of the thesis and describes the rationale and aims of this research.

Chapter 2 provides a summary of the general literature on the current understanding of the role of zinc in normal human physiology and chronic disease in particular. This chapter does not review literature specific to each health outcome investigated, which is presented and discussed in each of the chapters dealing with these health outcomes.

Chapter 3 (Paper 1), "Is Serum Zinc Associated with Pancreatic Beta Cell Function and Insulin Sensitivity in Pre-Diabetic and Normal Individuals? Findings from the Hunter Community Study" reports the association between serum zinc concentration and Homeostasis Model Assessment (HOMA) parameters cross-sectionally in a random sample of 452 older community-dwelling men and women in Newcastle, NSW, Australia. HOMA parameters were found to be significantly different between normoglycaemic and prediabetes groups ($p<0.001$). In adjusted linear regression, higher serum zinc concentration was associated with increased insulin sensitivity ($p = 0.01$) in the prediabetic group and a significant association between smoking and worse insulin sensitivity was also observed. This paper has been published in PLOS ONE.

Chapter 4 (Paper 2), "Is dietary zinc protective for type 2 diabetes? Results from the Australian longitudinal study on women's health," reports the longitudinal association of dietary zinc with incident type 2 diabetes in 8921 women, aged 50-55 years at baseline over 6-years of follow-up. 333 incident cases of type 2 diabetes were identified at the end of follow-up and after adjustment for dietary and non-dietary factors, the

highest quintile of dietary zinc intake had almost half the odds of developing type 2 diabetes (OR = 0.50, 95% C.I. 0.32–0.77) compared with the lowest quintile. Similar findings were observed for the zinc/iron ratio; the highest quintile had half the odds of developing type 2 diabetes (OR = 0.50, 95% C.I 0.30-0.83) after adjustment of covariates. This paper has been published in BMC Endocrine Disorders.

Chapter 5 (Paper 3), "Dietary zinc is associated with a lower incidence of depression: Findings from two Australian cohorts" reports the longitudinal association of dietary zinc with incident depression in two large Australian cohort aged 50 and above over 6-years of follow-up. Both studies showed that low dietary zinc intake is associated with a greater incidence of depression in both men and women, after adjusting for potential confounders. Compared to those with the lowest zinc intake, those with the highest zinc intake had significantly lower odds of developing depression with a reduction of about 30–50%. This paper has been published in Journal of Affective Disorders.

Chapter 6 (Paper 4), "Prospective Study of Dietary Zinc Intake and Risk of Cardiovascular Disease in Women," reports the longitudinal association of dietary zinc and cardiovascular disease (CVD) over 6-years of follow-up in a cohort of women aged 50-55 years at baseline. The study showed that risk of CVD increases with increased intake of dietary zinc. Compared to those in the lowest quintile of zinc intake those with in the highest quintile of zinc intake had significantly higher odds of developing CVD (OR= 1.67, 95% CI 1.08, 2.62) at the end of the follow-up. The same finding was also observed between energy-adjusted zinc to iron ratio and risk of developing CVD.

This has been submitted to the 'Nutrition, Metabolism & Cardiovascular Diseases' journal in July 2014.

Conclusions (Chapter 7). This program of research provided formative assessment of the potential role of dietary zinc in the following chronic diseases: Type 2 diabetes, depression and cardiovascular diseases. Given that this thesis studies were carried out in an Australian population, additional prospective cohort studies in other populations are needed to support the causal relationship between dietary zinc and these health outcomes. Hence, research that employs a longitudinal design, and rigorous

randomized controlled trials aimed at determining the efficacy of zinc in the prevention of chronic disease are needed. Furthermore, studies looking into the precise role and mechanisms for the effects of zinc compared to other essential nutrients from diet are needed to establish and reinforce the importance of dietary zinc in this chronic disease and other diseased state.

SECTION 1 OVERVIEW

Chapter 1 Background, aims and structure of thesis

1.1 Background and Rationale

Chronic disease is a global problem recognized by the World Health Organization as one of the leading causes of both morbidity and mortality. Type-2 diabetes, cardiovascular disease, and depression are increasing in both prevalence and incidence worldwide; contribute to the majority of the global burden of disease and are the chronic diseases with the highest and rising cause of morbidity, mortality and health care cost as portrayed in the brief literature review (chapter 2). Given that these conditions are largely preventable, world health agencies are focusing their efforts on identifying potentially modifiable risk factors with the aim of preventing these diseases. Diet and nutrition are important factors in the promotion and maintenance of good health throughout the entire life course. Poor diet plays an important role in the development and advancement of several chronic diseases. Adequate micronutrient intake is vital to normal physiological function and a deficiency of essential micronutrients is a global problem that has been linked to chronic disease and increased mortality. The micronutrient zinc is essential for the optimal function of a variety of biochemical and physiological processes. It is required for the catalytic activity of approximately 300 enzymes including members of all enzyme classes and plays a vital role in wound healing, immune function, protein synthesis, mitotic cell division as well as DNA and RNA repair. Although zinc is naturally available in food, the daily intake in many persons may be suboptimal.

Given that zinc is important for proper functioning of all body systems, a number of animal and human studies have examined the association of zinc status with type-2 diabetes, cardiovascular disease, and depression. Most of these studies have been small observational studies of poor quality. Considering that there is scarceness of longitudinal research to examine the association between dietary zinc intake and chronic disease outcomes such as type-2 diabetes, cardiovascular disease, and depression, this PhD thesis uses data from two large Australian cohorts to investigate

these associations. This thesis also provided greater insight into the role of zinc in type-2 diabetes by investigating serum concentration in normoglycemic, pre-diabetic and type-2 diabetic groups with the aim of determining if serum zinc concentration is a predictor of beta cell function and insulin sensitivity.

1.2 Aims and Hypotheses

The primary aim of this thesis was to examine the association of dietary zinc with chronic disease longitudinally. Detailed surveys including dietary habits using Food Frequency Questionnaire (FFQ) and clinical information were collected about a range of health outcomes in a prospective population-based cohort study in the Hunter Community Study (HCS) and Australian Longitudinal Study on Women's Health (ALSWH). These data and fasting blood samples analyzed for the concentration of serum zinc from the HCS were used to examine these associations.

Specific hypotheses tested in the thesis include:

- Lower serum zinc is associated with impaired pancreatic beta cell function and insulin sensitivity in pre-diabetic and normoglycaemic individuals (investigated in Chapter 3).

- Lower dietary zinc intake is associated with higher incidence of type-2 diabetes over 6 years in mid-age Australian women (investigated in Chapter 4).

- Lower dietary zinc intake is associated with higher incidence of depression over 5-6 years in a mid-aged and older population of Australian men and women (investigated in Chapter 5).

- Lower dietary zinc is associated with the higher incidence of cardiovascular disease over 6 years in mid-age population of Australian women (investigated in Chapter 6).

1.3 Structure of this thesis

This thesis is divided into 3 major sections.

1.3.1 Section 1 (Chapters 1 to 2):

- Outlines the structure of the thesis and describes the rationale and aims of this research (Chapter 1)
- Describes the candidate's contribution to this research (Chapter 1)
- Contains a brief literature review summarizing the current burden of chronic disease and understanding of the role of zinc in chronic disease (Chapter 2)

1.3.2 Section 2 (Chapters 3 to 6):

- Investigates the association between serum zinc concentration and pancreatic beta cell function and insulin sensitivity in pre-diabetic and normoglycaemic individuals (Chapter 3), dietary zinc intake and the risk of type-2 diabetes (Chapter 4), depression (Chapter 5), and cardiovascular disease (Chapter 6).

1.3.3 Section 3 (Chapter 7):

- Summarizes the observed associations, and describes future research needs related to zinc in diet, health and chronic disease.

1.4 Candidate's contribution for this thesis and to the peer-reviewed publications originating from PhD study

Presented below is a summary of the main sections, the chapters associated with each section, the papers they are based on, and my role undertaken in each section.

Chapter 1 describes the background, and rationale as well as the aims of this thesis.

Chapter 2 is a summary of the general literature relevant to chronic disease and zinc; the burden of chronic disease and the role of zinc in health and chronic disease in particular. This chapter does not review literature exclusive to each chronic disease examined – this is presented and examined in each of the chapters dealing with these health outcomes.

Chapter 3 assesses the association between serum concentrations of zinc and HOMA parameters in pre-diabetic and normoglycaemic participants of older adults. This is a secondary data analysis of existing cohort study data and not an independent research

study however; they are a priori hypothesis that was tested using this existing data. This chapter is based on:

Khanrin Phungamla Vashum, Mark McEvoy, Abul Hasnat Milton, Md. Rafiqul Islam, Stephen Hancock and John Attia. Is Serum Zinc Associated with Pancreatic Beta Cell Function and Insulin Sensitivity in Pre-Diabetic and Normal Individuals? Findings from the Hunter Community Study. *PLOS One* January 08, 2014

Chapter 4 assesses the association between dietary zinc, zinc to iron ratio and incident type 2 diabetes in mid age Australian women. This chapter is based on:

Khanrin Phungamla Vashum, Mark McEvoy, Zumin Shi, Abul Hasnat Milton, Md. Rafiqul Islam, David Sibbritt, Amanda Patterson, Julie Byles, Deborah Loxton and John Attia. Is dietary zinc protective for Type 2 diabetes? Results from the Australian Longitudinal Study on Women's Health**.** *BMC Endocrine Disorders* 2013, **13**:40

Chapter 5 assesses the association between dietary zinc and incident depression in mid age and older Australian population. This chapter is based on:

Khanrin Phungamla Vashum, Mark McEvoy, Abul Hasnat Milton, Patrick McElduff, Alexis Hure, Julie Byles and John Attia. Dietary zinc is associated with a lower incidence of depression: findings from two Australian cohorts**.** *Journal of Affective Disorders* 166 (2014) 249–257

Chapter 6 assesses the association between dietary zinc, zinc to iron ratio and incident cardiovascular disease in mid aged Australian women. This chapter is based on:

Khanrin Phungamla Vashum, Mark McEvoy, Abul Hasnat Milton, Patrick McElduff, Alexis Hure, Julie Byles and John Attia. Prospective Study of Dietary Zinc Intake and Risk of Cardiovascular Disease in Women. Submitted to the 'Nutrition, Metabolism & Cardiovascular Diseases' journal for publication.

Chapter 7 summarizes the observed associations and describes future research needs related to dietary zinc intake in health and chronic disease.

Table 1 Candidate's Contributions to the Study[1]

Activities	Primary Role	Others involved
Overall study	KPV, MM, AHM, JA	JB, AH, PM, SH, DS, ZS, AP, DL, MRI
Ethics application to the University of Newcastle	KPV, MM, AHM, JA	
Conceptualization of the idea	KPV, MM, AHM, JA	MRI
Study design	KPV, MM, AHM, JA	AH
Literature search	KPV	
Data analysis	KPV	PM
Data interpretation	KPV, MM,	JA
Scientific write-up and publication	KPV, MM, AHM, JA,	JB, AH, PM, SH, DS, ZS, AP, DL, MRI

[1] KPV: Khanrin Phungamla Vashum, MM: Mark McEvoy, AHM: Abul Hasnat Milton, JA: John Attia, JB: Julie Byles, AH: Alexis Hure, PM: Patrick McElduff, MRI: Md. Rafiqul Islam, SH: Stephen Hancock, DS: David Sibbritt, ZS: Zumin Shi, AP: Amanda Patterson, DL: Deborah Loxton

Chapter 2 Brief literature Review

The purpose of this review is to emphasize the burden of chronic disease and the importance of micronutrient, zinc to human nutrition and health and provide evidence supporting the role of zinc in the development of human disease states, particularly in chronic disease. It is not meant to be a systematic review of this literature as this has been done by many in the past (Prasad 1998; Brown, Wuehler et al. 2001; Temple and Masta 2004) and has recently been updated (Rink 2011; Chasapis, Loutsidou et al. 2012).

Rink 2011 & Chasapis, Loutsidou et al. 2012 concluded that zinc played a major role in the pathophysiology of many chornic diasease and some age-related disease. They also emphasized on the importance of zinc being vital for the proper functioning of the immune system, and it's multifunctional role in maintaining satisfactory growth and well-being both during childhood and ageing indicating that further research is required in the field of zinc. This reviews highlighted the importance of assessing zinc status and exploring the possibilty of zinc as a useful and inexpensive drug in medicine. Hence, the following review will provide an insight into:-

i. what is the current burden of chronic disease especially type 2 diabetes, depression and cardiovascular disease?
ii. What is the role of zinc in the development of human disease states, particularly in chronic disease?

2.1 Search Strategy

A literature review was conducted to locate studies that have examined the burden of chronic disease, associations between zinc and chronic disease, and health outcomes. The literature review was updated with relevant papers published up to June 2014. Published studies were identified through searches of Medline, PubMed, Cochrane Library, EMBASE, Australian government websites and Google scholar. The literature search was conducted using key words (eg: dietary zinc, zinc, micronutrients, chronic disease, diabetes, depression, cardiovascular disease, stroke, heart attack, coronary

artery disease, chronic disease (s), non-communicable disease etc.) or sentences (eg: role of zinc in health, zinc and health, zinc and chronic disease, dietary zinc and chronic disease zinc and depression etc.) and each term was included in one or more searches. All associated terms were combined using "OR" and then "AND" to obtain a total number of relevant study from each database. The search was first carried out in September 2010, without limitations on the time and language of publication. Reviewing the bibliographies of retrieved publications also identified articles in the grey literature. The search was last updated in June 2015 to locate new studies and data published since the initial search. The search strategy and terms used for Medline is provided in appendix 1. The same strategy was also applied to the other electronic databases where relevant.

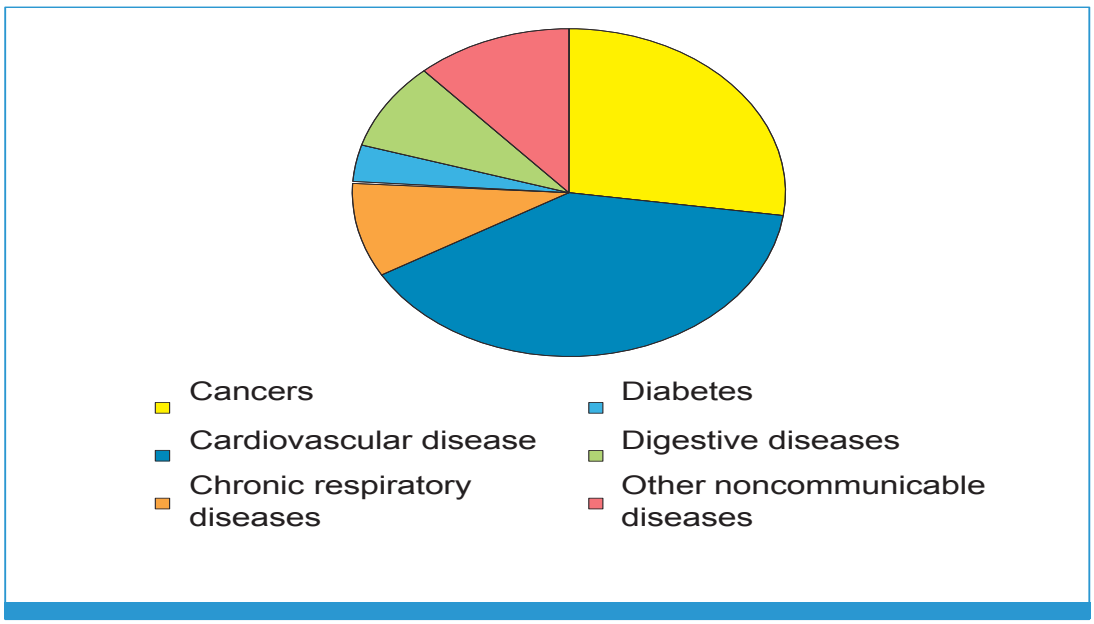
2.2 Current evidence on burden of chronic disease

Many illnesses and health conditions can be classified under the broad category of chronic disease or non-communicable disease. Chronic diseases are usually characterized by long duration and slow progression. Generally, chronic diseases are mostly characterized by complex causality, multiple risk factors, long latency periods, a prolonged course of illness, and functional impairment or disability (AIHW 2013). Chronic diseases develop over one's lifetime, with clinical sequel occurring many years after the underlying pathogenesis of the disease has occurred. Up until the nineteenth century, infectious diseases and injury dominated the health of all population even though descriptions of chronic diseases and conditions such as coronary heart disease, atherosclerosis and diabetes among affluent sections of the society were beginning to accumulate (Cohen 1989). However, by the second half of the twentieth century chronic disease became the main public health problems of industrialized countries. World Health Organization (WHO) data showed that by the end of the century, non-communicable conditions were responsible for between 83% and 89% of death in the very low mortality countries of European and Western Pacific regions and the Americas (Reinhardt and Cheng 2000).

Chronic diseases have now become a major public health problem worldwide and are by far, the leading cause of mortality in the world representing 63% of all deaths in

2008 (Alwan, MacLean et al. 2010) with half of these deaths in people less than 70 years of age (figure 1) and its contribution continues to rise (WHO 2011). According to a WHO projection, chronic disease death will increase by 15% globally between 2010 to 2020 and the regions that are to have the greatest total number of chronic disease deaths in 2020 are South-East Asia and the Western Pacific (WHO 2008). Population growth and increased life expectancy are leading to increasing numbers and proportions of older people. Hence, an ageing population is emerging as a significant trend in many parts of the world due to which annual non-communicable disease deaths are projected to rise substantially, to 52 million in 2030. In comparison, annual infectious disease deaths are projected to decline by around 7 million over the next 20 years. Chronic disease will be responsible for three times as many disability-adjusted life years (DALYs) and nearly five times as many deaths as communicable diseases, maternal, perinatal and nutritional conditions combined, by 2030 (Mathers, Fat et al. 2008).

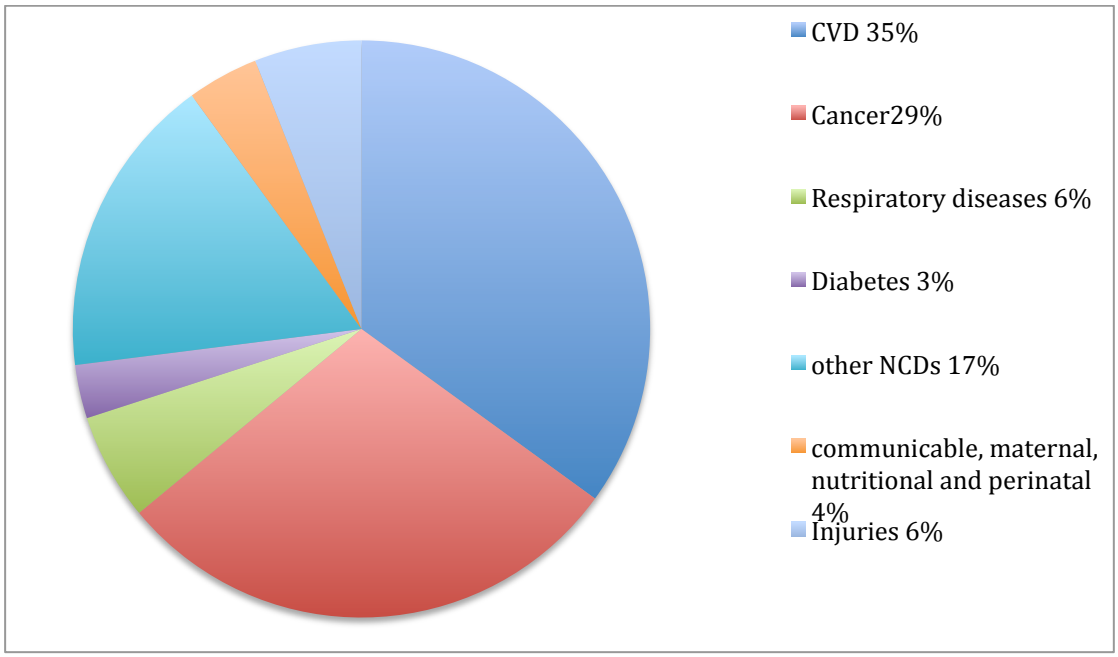
Figure 1. Proportion of global NCD deaths under the age of 70, by cause of death, 2008 adapted from WHO global report 2010.



Source: Global status report on noncommunicable diseases 2010. Chapter 1 – Burden: mortality, morbidity and risk factors (pg-11). Copyright World Health Organization (2011)

In Australia, chronic disease has been a major cause of death in the elderly and is a growing cause of concern; the latent effects of health risk factors and an ageing population contribute to increasing prevalence of chronic conditions (AIHW 2010). The burden of morbidity and mortality cause to individuals and their families, and generally to the Australian community is enormous and increasing. Premature death caused by chronic diseases can be measured by potential years of life lost (PYLL), which counts the number of years of life lost for each death before the age of 75 years. In 2007, 83% of all premature deaths (that is, deaths among people aged less than 75 years) were due to chronic disease of which more than 3 in 5 premature deaths (64%) were potentially avoidable (AIHW 2010). According to Noncommunicable Diseases (NCDs) Country Profiles by WHO (figure 2), NCDs are estimated to be responsible for about 90% of all deaths In Australia. It also showed that 22.6% of all NCDs deaths occurred in those less than 60 years of age (2008 estimates)(WHO 2011). In 2003, the burden associated with premature mortality, disability, illness and injury due to cancer, cardiovascular diseases, mental disorders, neurological diseases, chronic respiratory diseases and diabetes amounted to 74% of the total burden of disease and injury (Begg, Vos et al. 2007) showing a rising trend in NCDs. Also, apart from the enormous expenditure on chronic disease, loss to the economy through diminished participation in the labour force has also been related with the prevalence of, and fatality due to, chronic disease (AIHW 2009a). Chronic disease thus presents an enormous burden to society both globally and in different societies by increasing human suffering and health care costs.

Figure 2: Proportional mortality (% of all deaths, all ages) in Australia. Adapted from WHO country profile 2011. NCDs are estimated to account for 90% of all deaths.



Source: Noncommunicable Diseases Country Profiles 2011 (pg-23). Copyright World Health Organization (2011)

2.2.1 Burden of Type 2 Diabetes

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces, leading to chronic elevation of blood glucose concentration (hyperglycemia). In type 2 diabetes (T2D), hyperglycemia arises due to insulin deficiency and insulin resistance of various tissues including muscle and adipose tissues. If left untreated it results in substantial morbidity and mortality primarily from microvascular and macrovascular effects.

Type 2 diabetes comprises 90% of all people with diabetes around the world and results from the body's ineffective use of insulin. Currently it represents one of the most challenging public health problems of the 21st century. In 2004, an estimated 3.4 million people died from consequences of high fasting blood sugar (WHO 2009) and currently there are 347 million people worldwide with diabetes (Danaei, Finucane et al. 2011).

Diabetes increases the risk of heart disease and stroke; 50% of people with diabetes die of cardiovascular disease (Morrish, Wang et al. 2001) and is among the leading causes of kidney failure (WHO 2011). WHO projects that diabetes will be the 7th leading cause of death in 2030 (WHO 2011) and that the overall risk of dying among people with diabetes is at least double the risk of their peers without diabetes (Roglic, Unwin et al. 2005).

In Australia diabetes mellitus is a major problem that significantly affects health. In 2011-2012, 4.6 % of the population had some form of diabetes (excluding gestational diabetes) and 3.9% of these were with type 2 diabetes (ABS 2013). The prevalence of diabetes in Indigenous Australians is even higher (8.6% in 2012-2013) with a proportion of 3 to 1 compared to the non-indigenous population and in 2008-2009, 2.3% ($1, 507 million) of all health expenditure was spent on diabetes alone (AIHW 2013). In 2008 diabetes was the underlying cause for 4,191 (2.9%) deaths registered in Australia and it contributed to 14,461 (10%) deaths as either an underlying or associated cause of death (ABS 2010). According to the latest available data for 2012, diabetes was the 6th leading cause of all registered deaths while it was the 7th in 2007 (ABS 2014) further emphasizing the urgent need for prevention and control of diabetes.

2.2.2 Burden of Depression

Depression is a common mental disorder worldwide, characterized by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, feelings of tiredness, and poor concentration (WHO 2014). Depression is different from common mood fluctuations and short-lived emotional responses to everyday challenges in life. It can be long lasting or recurrent with moderate or severe intensity, extensively impairing the affected individual's ability to function at work and cope with daily life. Depression does not only affect the individual, but it also indirectly affects those around the individual such as family situations and in turn, can lead to more stress and dysfunction, worsening the affected person's life and depression itself.

Globally, more than 350 million people of all ages suffer from depression and is the leading cause of disability worldwide, contributing majorly to the global burden of disease (WHO 2012). The World Mental Health Survey conducted in 17 countries

found that on average about 1 in 20 people reported having an episode of depression in the previous year (Marcus, Yasamy et al. 2012). Depression is the leading cause of disability worldwide in terms of total years lost due to disability because it often starts at a very young age reducing their performance and is recurring. Depression can also lead to cardiovascular disease and vice versa. It has been shown that one out of every five patients with cardiovascular disease (CVD) suffers from major depressive disorder (Elderon and Whooley 2013). Depression at its worst can lead to suicide and results in an estimated 1 million deaths each year, which is equivalent to 3000 suicide deaths every day with 20 or more making an attempt for every persons who commits suicide (Marcus, Yasamy et al. 2012).

In 2011-2012 there were 3 million (13.6%) Australians reported to have a mental or behavioural condition (AIHW 2013). The most prevalent were mood (affective) problems, which includes depression with 2.1 million people or 9.7% of the population, followed by anxiety related problems (850,100 people or 3.8%). In 2007-2008 it was 11.2% and 9.6% in 2001, indicating a rising trend in mental and behavioral conditions over the years (AIHW 2013). In 2007, approximately 26% of young people aged 16-24 years had a mental disorder (ABS 2012) and 45% of Australians aged 16-85 years (or 7.3 million people) had experienced at least one mental disorder at some point in their lifetime (ABS 2009). Over half (52%) of all permanent residential aged care residents had symptoms of depression in 2012, further demonstrating that depression affects people of all ages (AIHW 2013). The Australian national expenditure on all mental health-related services in 2011–2012 was estimated to be over $7.2 billion with $322 per person, an increase from $282 per person in 2007-2008 (AIHW 2013).

The burden of depression and other mental health conditions is on the rise globally, affecting people of all ages within communities stressing the need for prevention and exploring other sources of antidepressant activity.

2.2.3 Burden of Cardiovascular Disease

Cardiovascular diseases (CVDs) are caused by a group of disorders of the heart and blood vessels that include coronary heart disease (heart attacks) and cerebrovascular disease (stroke). Heart attacks and strokes are usually acute events and are mainly

caused by a blockage that prevents blood from flowing to the heart or brain. Addressing risk factors of which unhealthy diet is one of the most important behavioral risk factor can prevent most cardiovascular diseases.

Annually more people die from CVDs than from any other cause and are the greatest cause of death globally (WHO 2011). In 2008, it was estimated that 17.3 million people died from CVDs, representing 30% of all global deaths (WHO 2011), of which an estimated 7.3 million were due to coronary heart disease and 6.2 million were due to stroke (WHO 2011). CVDs are projected to remain the single leading cause of death (Mathers and Loncar 2006) and that the number of people who die from CVDs, mainly from heart disease and stroke, will increase to reach 23.3 million by 2030 (Mathers and Loncar 2006; WHO 2011). Each year 9.4 million deaths or 16.5% of all deaths can be attributed to high blood pressure (Lim, Vos et al. 2013) which includes 51% of deaths due to strokes and 45% of deaths due to coronary heart disease (WHO 2008). Low and middle-income countries are disproportionally affected; over 80% of CVD deaths take place in low and middle-income countries and occur almost equally in men and women, often in their most productive years (WHO 2011).

In Australia, cardiovascular disease remains the largest cause of death despite dramatic reductions since the late 1960s (AIHW 2010). About 3.5 million Australians had a long-term cardiovascular disease in 2007–2008. In 2008, nearly 50,000 deaths were attributed to CVD; responsible for more deaths than any other disease group (34% of the total), was the main cause for 475,000 hospitalisations (2007–2008) and played a secondary role in a further 797,000 (AIHW 2011). Hence, CVD remains the most expensive disease group in Australia, costing about $5.9 billion in 2004–2005 with just over half of this money spent on patients admitted to hospital (AIHW 2011). Even in Australia discrepancies exist; lower socioeconomic groups, Aboriginal and Torres Strait Islander people and those living in the remote areas of Australia had the highest rates of hospitalization and death resulting from CVD (AIHW 2011).

CVD is also associated with diabetes (Bloomgarden 2003) and depression (Göthe, Enache et al. 2012) which further increases the burden of CVD. Thus, there is a need for development of more cost effective interventions that are feasible and easily

implemented even in low resource settings, for prevention and control of cardiovascular diseases.

2.3 Determinants of chronic disease

Many people with a chronic disease often die of another chronic disease and therefore, chronic disease mortality should be considered in terms of both underlying and associated causes. The underlying cause is the one that initiates the sequence of events leading to death, whereas an associated cause contributes to the set of events leading up to death.

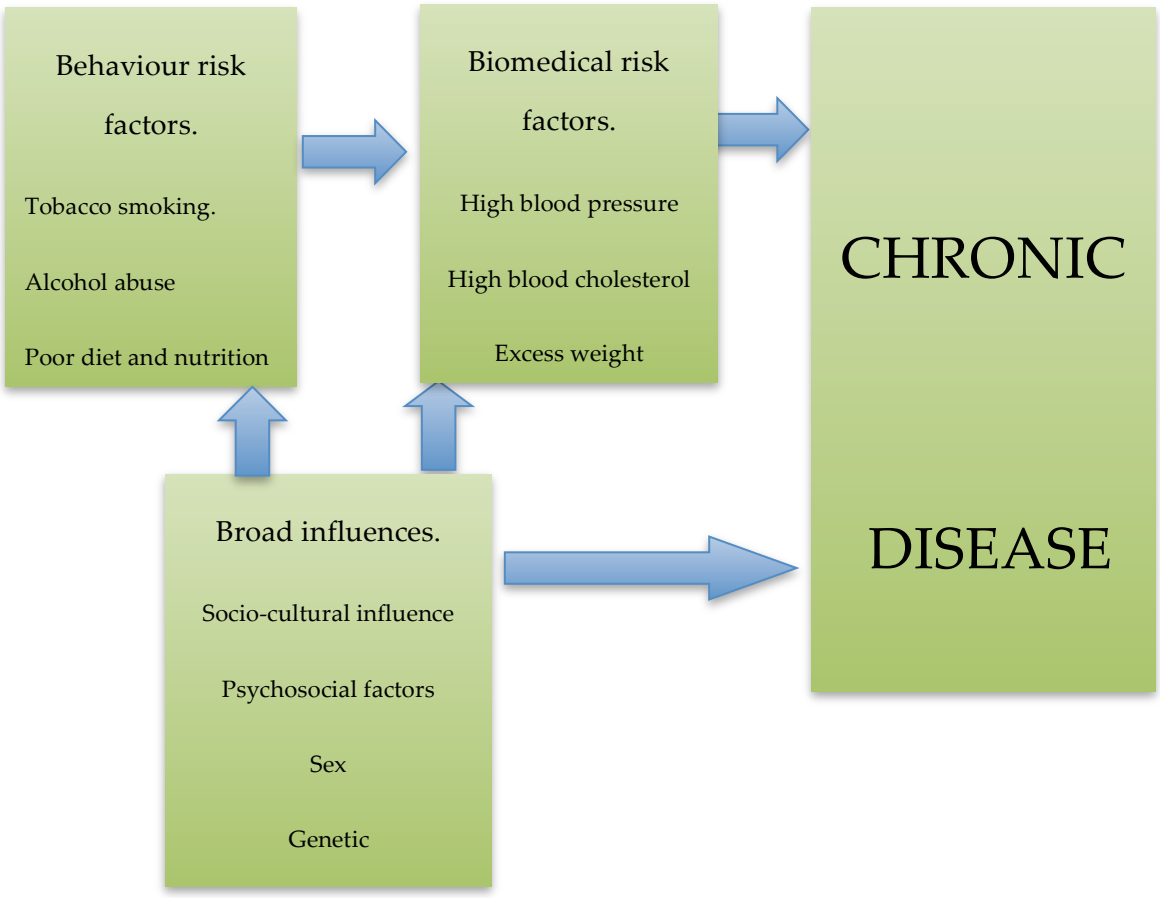


Figure 3: Relationship between risk factors and chronic disease.

Biomedical risk factors (hypertension, obesity, high blood cholesterol etc.) with complex social, economic, technological and cultural changes (globalization, urbanization, population ageing) along with early childhood have exerted, and will

continue to bring to bear a significant influence on the health of the population. However, these are the underlying determinant of chronic disease that is the "causes of causes" but common modifiable behavior risk factors trigger the major chronic diseases. These risk factors in combination with the non-modifiable risk factors such as age and heredity explain the vast majority of chronic disease deaths at all ages, in men and women, and in all parts of the world. The main behavioral risk factors include:

- **Unhealthy diet and excess energy intake.** An unhealthy diet is one of the major risk factors for a range of chronic diseases, including cardiovascular diseases, cancer, diabetes and other conditions linked to obesity. In 2014, more than 1.9 billion adults, 18 years and above were overweight. Of these over 600 million were obese which has doubled since 1980 (WHO 2014). Low fruit and vegetable intake is among the top 10 selected risk factors for global mortality and approximately 1.7 million (2.8%) of deaths worldwide are attributable to low fruit and vegetable consumption (WHO 2004). Increased prosperity has led to the perils of overconsumption and overindulgence, the creation of an 'obesogenic' environment (Swinburn, Egger et al. 1999). Once associated with high-income countries, obesity is also prevalent in low and middle-income countries now.

- **Physical inactivity.** Physical inactivity has been identified as the fourth leading risk factor for global mortality causing an estimated 3.2 million deaths (WHO 2014) and a key risk factor for chronic disease. In Australia about two-thirds of adults do not exercise enough to confer a health benefit. Over 2 million of these (15% of people aged 18-75 years) were sedentary, that is they did no physical activity in their leisure-time (AIHW 2013) and 3 in 5 Australian adults are overweight or obese (based on BMI), which is over 12 million people (AIHW 2013).

- **Tobacco use.** Tobacco kills nearly 6 million people each year with more than five million of those deaths as a direct result of tobacco use, while more than 600,000 are the result of non-smokers being exposed to second-hand smoke (WHO 2014). WHO has projected that the annual death toll could rise to more

than eight million by 2030 unless urgent action is taken (WHO 2014). In Australia, tobacco smoking is the largest single preventable cause of death and disease (The Cancer Council 2006). In 2004-2005, 23% of adults were current smokers, about 3.5 million persons (ABS 2006) and almost half (47%) of Indigenous persons aged 15 years and over were current smokers in 2008 (AIHW 2011).

Behavioral risk factors account for the loss of about 21% of DALYs in Australia. Increased activity and independently decreased sedentary periods have profound positive effects on obesity, diabetes, and CVD risk factors (Dunstan, Mori et al. 1999; Dunstan, Salmon et al. 2007). Tobacco smoking is the risk factor responsible for the greatest burden of chronic disease in Australia causing 7.8% of total DALYs (9.6% among males and 5.8% among females) followed by physical inactivity with 6.6% of total DALYs (Begg, Vos et al. 2007) and poor diet (as measured by lack of fruit and vegetables, 3% of total DALYs) (Mathers, Vos et al. 1999).

Many other behavioral risk factors have been identified for chronic diseases but they account for a lesser proportion of chronic disease. Excessive alcohol use is an important contributor to the global burden of disease although evidence from studies suggests that low to moderate consumption of alcohol has a protective effect against the development of cardiovascular diseases (Sacco, Elkind et al. 1999). Alcohol consumption causes death and disability relatively early in life. In the age group of 20-39 years approximately 25% of the total deaths are alcohol-attributable and an overall 5.1% of the global burden of disease and injury is attributable to alcohol as measured in disability-adjusted life years (WHO 2014). The relationship of alcohol use to chronic disease is complicated. Misuse of alcohol is associated with mental and behavioral disorders and has contributed significantly to major chronic diseases such as chronic liver disease, some cancers and cardiovascular diseases (Ridolfo and Stevenson 2001; WHO 2014). Despite a decline in alcohol consumption in the last three decades; Australia still ranks tenth amongst 20 developed countries in terms of per capita alcohol consumption (De Looper 1997) and the net harm from alcohol consumption attributes to 2% of total DALYs (Mathers, Vos et al. 1999).

A number of misconceptions have contributed to the neglect of chronic disease in the past. Conversely, the vast majority of current evidence dispels the notion that chronic diseases are a distant threat and are less important and serious than infectious diseases. Many perceive genes as the major cause of chronic disease however, it is now well recognized that genetic factors predispose the individual, but environmental factors determine the phenotypic expression of the disease. As previously pointed out by Booth et al. 2000, "100% of the increase in the prevalence of Type 2 diabetes and obesity in the United States during the latter half of the 20th century must be attributed to a changing environment interacting with genes, because 0% of the human genome has changed during this time period" (Booth, Gordon et al. 2000; Booth, Chakravarthy et al. 2002). Evidence for this phenomenon includes data from secular and migration studies (Kato, Tillotson et al. 1973) and studies on the Tarahumara Indians (McMurry, Cerqueira et al. 1991) and the Pima Indians (Bennett 1999).

Chronic diseases are often viewed as primarily affecting old people. We now know that almost half of chronic disease deaths occur prematurely, in people under 70 years of age. One quarter of all chronic disease deaths occur in people under 60 years of age (WHO 2005). In low and middle-income countries, middle-aged adults are especially vulnerable to chronic disease. Certain chronic diseases, especially heart disease, are often viewed as primarily affecting men. The truth is that chronic diseases, including heart disease affect women and men almost equally. Some people having adopted a negative outlook and believe that nothing can be done to prevent chronic disease. However, most of the major risk factors of chronic diseases are known, and if these were eliminated, at least 80% of all heart disease, stroke and type-2 diabetes would be prevented (WHO 2005).

2.4 Role of diet in chronic disease

Diet and nutrition are important factors in the promotion and maintenance of good health throughout the entire life course. Their role as determinants of chronic disease is well established and they therefore occupy a prominent position in prevention activities (WHO 2002; Waxman 2005).

Poor diet plays a vital role in the development and advancement of several chronic diseases like coronary heart disease, stroke and Type 2 diabetes (Roberts and Barnard 2005) and depression (Bonnet, Irving et al. 2005; Rienks, Dobson et al. 2012). It also contributes to a number of other health risk factors such as excess weight, high blood pressure and elevated blood cholesterol (International and Research 1997; Brundtland 2002). There is strong evidence that poor diet and nutrition mostly leads to the development of chronic disease through a range of intermediary risk factors. For example, high intake of saturated fat increases blood cholesterol levels, high overall fat intake contributes to overweight and obesity, and high salt use contributes to high blood pressure, all of which are intermediate factors that increase the risk of coronary heart disease (McMurry, Cerqueira et al. 1991).

The National Health and Medical Research Council (NHMRC) publication of dietary guidelines clearly states the significance of nutrition in prevention of disease and health maintenance (NHMRC 2013). The guidelines emphasize that a full set of nutrients is required; vitamins, minerals, carbohydrates, fiber, protein, and fats. Several organizations in the United States have also issued dietary recommendations aimed at chronic disease prevention (Kushi, Byers et al. 2006; Lichtenstein, Appel et al. 2006) and researchers have evaluated their effects and various other diet on disease risk and mortality in both men and women (Kant, Schatzkin et al. 2000; McCullough, Feskanich et al. 2002; Knoops, de Groot et al. 2004). In the United States, a study looking at actual cause of death showed that poor diet and physical inactivity caused 400,000 deaths in 2000 ranking second only to tobacco, and that it is likely that inactivity and diet will soon rank as the leading cause of death (Mokdad, Marks et al. 2004). This may be an underestimate as it mirrors deaths attributable only to those with obesity and physical inactivity, whereas inappropriate diet impacts on mortality at any BMI (Blair, Kohl et al. 1995). A more recent study showed that mortality from high dietary salt (102,000), low dietary omega-3 fatty acids (84,000) and high dietary trans fatty acids (82,000) were the dietary risks with the largest mortality effects in the United States (Danaei, Ding et al. 2009). A variety of chronic disease was identified by the Australian Burden of Disease and Injury Study and according to a study by Mathers et al., it was estimated that in 1996 in Australia 2,541 (3.7%) and 1,516 (2.2%) male and female deaths respectively due

to chronic disease were attributable to nutritional deficiency (Mathers, Vos et al. 1999). Physical activity and diet are effective interventions, for which Booth and co-workers have coined the term "the war on chronic disease" (Booth, Gordon et al. 2000). Undoubtedly, there is overwhelming evidence from over the past 20 years from a variety of sources, including epidemiological, prospective cohort, and intervention studies linking most chronic diseases seen in the world today to inappropriate dietary consumption, and that diet interventions can mitigate progression of chronic disease and in fact reverse existing disease.

2.5 Zinc

Vitamins and minerals are essential for human health and development. Inadequate intake of them is now recognized as an important contributor to the global burden of disease through increased rates of illness and death from diseases, and of disability such as mental impairment. It has been estimated that 2 billion people worldwide suffer from at least one form of micronutrient deficiency (UNICEF and M.Initiative 2004). According to WHO, 19% of the 10.8 million child deaths globally per year are attributable to iodine, iron, vitamin A, and zinc deficiencies and approximately 6% of global DALYs (Ezzati, Lopez et al. 2002; WHO 2002). It is estimated that zinc deficiency affects about one third of the world's population and the associated loss of disability-adjusted life years (DALYs) attributable to zinc deficiency amounts to more than 28 million (WHO 2002). Studies have shown that zinc deficiency in the developing countries is fairly prevalent; affecting about two billion people and that growth retardation commonly observed in these countries may indeed be due to zinc deficiency (Brown, Peerson et al. 2002).

Many chronic diseases share common modifiable risk factors like physical inactivity, smoking, unhealthy diet and obesity. The important underlying mechanisms for these factors to increase risk for disease include oxidative damage, inflammation and 1-carbon metabolism. Various in vitro and animal studies have suggested favorable effects of micronutrient on these process and on angiogenesis, immunity, cell differentiation, proliferation and apoptosis (Kelloff, Boone et al. 1994; Reddy 1995; Epstein, Diaz et al. 1997).

Given the high prevalence of micronutrient deficiencies globally and the growing epidemic of chronic disease, the role of micronutrients, especially that of zinc warrants a closer examination.

2.5.1 What is zinc and where do we get it?

Zinc is an essential trace element vital for the optimal function of a variety of biochemical and physiological processes. It is the second most abundant transition metal ion in living organism after iron however, if hemoglobin bound iron is not considered, then zinc becomes the most abundant transition metal (Vašák and Hasler 2000). The average amount of zinc in the adult body is about 1.4-2.3g (Prasad 2009; Bhowmik and Chiranjib 2010). Zinc is required for the catalytic activity of approximately 300 enzymes including members of all enzyme classes (Vallee and Falchuk 1993; Wellinghausen, Kirchner et al. 1997), plays a vital role in wound healing, immune function, protein synthesis, mitotic cell division as well as DNA and RNA repair (Prasad 2008).

Zinc is found in all body tissues and fluids and approximately 85% of the total body zinc content is found in bone and muscle, 11% in the skin and liver, and remaining in all other tissues with highest concentrations found in the choroid of the eye and in prostatic fluids (FAO 2002; Chasapis, Loutsidou et al. 2012). Zinc is absorbed in the small intestine, is concentration dependent and is excreted via the kidneys, skin and intestine (in faeces) (King and Turnlund 1989). The endogenous intestinal losses can vary depending on zinc intake whereas urinary and skin losses depends less on normal variations in zinc intake (King and Turnlund 1989). Starvation and muscle catabolism increase zinc losses in urine while strenuous exercise and elevated ambient temperatures could lead to losses by perspiration.

There are no body stores of zinc in the conventional sense and so daily intake of zinc is needed to maintain adequate body levels. However, human experimental studies with low-zinc diets have shown that circulating zinc levels and activities of zinc-containing enzymes can be maintained within normal range over several months (Lukaski, Bolonchuk et al. 1984; Milne, Canfield et al. 1987) highlighting the efficiency of the zinc homeostasis mechanism. Further studies showed that changes in the endogenous

excretion of zinc through the kidneys, intestine, and skin and changes in absorptive efficiency are how body zinc content is maintained (Johnson, Hunt et al. 1993; King, Shames et al. 2000).

Zinc is naturally available in foods. Meats, fish and poultry are the major contributors to the diet but cereals and dairy foods also contribute substantial amounts. It is also present in pulses, nuts, legumes and shellfish. Oyster is known to be the richest source of zinc containing more zinc per serving than any other food (National Institute of Health 2013). Zinc is also present in some fortified food product, is available as supplements alone or with other multivitamins/micronutrients in a number of different forms, including zinc sulfate, zinc gluconate and zinc acetate.

2.5.2 Recommended value of dietary zinc intake

Experimental studies have clearly shown that the body has a pronounced ability to adapt to different levels of zinc intakes by changing the endogenous zinc losses through the kidneys, intestine, and skin (Lukaski, Bolonchuk et al. 1984; Milne, Canfield et al. 1987). The dietary requirement of zinc differs according to age and gender due to the relationship between absorption and intestinal and non-intestinal losses. The table below (table 1) shows the recommended dietary intake and estimated average requirement of adult male and female for Australia and New Zealand (NHMRC 2006). Assessment of requirements was based on estimates of the minimal amount of absorbed zinc necessary to match total daily excretion of endogenous zinc. Estimates were made using a factorial approach that involves calculation of both intestinal and non-intestinal losses.

Table 1 Adults zinc intake recommended by National Health and Medical Council (NHMRC), Australia

Age	*EAR	*RDI	EAR	RDI
	MEN		**WOMEN**	
19-30 yr	12 mg/day	14 mg/day	6.5 mg/day	8 mg/day

31-50 yr	12 mg/day	14 mg/day	6.5 mg/day	8 mg/day
51-70 yr	12 mg/day	14 mg/day	6.5 mg/day	8 mg/day
>70 yr	12 mg/day	14 mg/day	6.5 mg/day	8 mg/day

*EAR: Estimated average requirement, *RDI: Recommended dietary intake.

The estimated average requirement (EAR) is defined as the daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group while recommended dietary intake (RDI) is the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a particular life stage and gender group (NHMRC 2006). RDI is derived from the set EAR. As half the population requires more zinc than EAR, Rink stated that a greater proportion of women and elderly are at risk of developing zinc deficiency compared to men (Rink 2011). The application of EAR and RDI are summarized in the table below that was adapted from https://www.nrv.gov.au/node/50

Table 2 Nutrient reference values uses by National Health and Medical Council (NHMRC), Australia

Nutrient Reference Value	For individuals:	For groups:
Estimated Average Requirement (EAR)	Use to examine the probability that usual intake is inadequate	Use to estimate the prevalence of inadequate intakes within a group
Recommended Dietary Intake (RDI)	Usual intake at or above this level has a low probability of inadequacy	Do not use to assess intakes of groups

2.5.3 Overview of zinc absorption and factors influencing it

Although zinc is naturally available in food, the daily intake in many persons may be suboptimal. The presence of zinc in foods as a complex rather than as free ions affects its bioavailability. The prime site of absorption of exogenous zinc in the human is understood to be in the proximal small bowel, either the distal duodenum or proximal jejunum (Lee, Prasad et al. 1989; Krebs 2000). Essential to maintenance of zinc homeostasis is the gastrointestinal system, especially the small intestine, liver and pancreas.

The mechanisms of uptake of exogenous zinc in the subcellular level remain unclear but both saturable, carrier-mediated processes, and non-saturable processes are still thought to be involved (Reyes 1996, Rink 2011). In the past 15 years rapid advancement has been made in the knowledge of zinc transporter family, which has led to an understanding of the basic contributions of zinc transporters (ZnT) to maintain zinc homeostasis in an integrative systems. ZnT1 was the first mammalian zinc transporter to be identified and characterized (Palmiter and Findley 1995). ZnT1 is a ubiquitously expressed protein that has been found to be present in tissues (Rink 2011) and in the villi of the proximal small bowel (McMahon and Cousins 1998a). In polarized cells, a basolateral membrane site has been identified, especially in rat enterocytes and cell lining the thick ascending and distal convoluted renal tubules. These findings and other observations suggest that ZnT1 has a major role in zinc enteric absorption and renal reabsorption (Krebs 2000, Rink 2011). Another protein is metallothionein (MT), an intracellular metal binding protein and plays an important role in metal homeostasis. Zinc is mostly bound to this protein whose role in the regulation of zinc absorption is not yet well defined, specifically in conjunction with the zinc transporters. Dietary zinc supplementation and inflammation besides others stimulates hepatic and intestinal MT synthesis while dietary restriction results in reduced MT synthesis. In experiments with knockout and transgenic mice, the rise in serum zinc after a single dose of zinc was much greater in the MT knockouts mice. On the other hand, the observed serum zinc of the MT transgenic animals was weak compared with that of the control. The

expression of ZnT1 was also measured and observed to be associated with serum zinc levels but unaltered by MT levels (Davis et al. 1998). Hence, MT may function in cellular responses to limit free zinc concentrations within quite narrow ranges (Cousins 1996) and function as a zinc pool (Davis et al. 1998). DCT1, a transmembrane polypeptide that is found in the duodenum in the crypts and lower villi is another transporter that might be potentially involved in zinc and uptake of several metal ions (McMahon and Cousins 1998).

In the whole body process, the environment within the gastrointestinal tract, which can be affected by other dietary constituents, markedly influences the solubility and absorptive efficiency of zinc (Cousins 1989; Lönnerdal 1989). There are a number of dietary factors that influences zinc absorption in a normal physiological state. The known dietary factors influencing zinc absorption are: -

- **Zinc intake**. The amount of zinc in the diet itself affects absorption, which was shown by Sandstom and Cederbald (1980) that with increasing amount of zinc in meal, fractional zinc absorption (%) decreases (Sandström and Cederblad 1980). It is likely that the reduced fractional absorption of zinc at higher doses is due to saturation of the transport mechanisms of zinc. The mechanism of this saturable transport mechanism has been investigated in experimental animals (Coppen and Davies 1987) and humans (Lee, Prasad et al. 1989) showing an inverse relationship between percentage absorption and dietary zinc intake. The absorption of zinc also differs based on whether it is absorbed from water solutions or meals. Data from zinc absorption studies showed that the amount of zinc absorbed from a single meal levels off at 18-20μmol whereas water solution showed zinc absorption reaching levels of 80-100μmol (Sandström and Sandberg 1992; Lonnerdal 1997). This level suggests that special attentions must be paid in administering zinc as a supplement, when zinc is added to food as a fortificant and when it is a part of a meal.
- **Protein.** The type, quality and quantity of protein also affect the bioavailability of zinc. A study showed that zinc absorption increases with increase in quantity of protein in meals (Sandström and Sandberg 1992). Zinc absorption was also

found to be higher in infant milk formula than with cow's milk indicating that casein in milk has a negative effect on zinc absorption (Sandström, Cederblad et al. 1983). Two milk formulas differing only in whey to casein ratio when compared showed that zinc absorption was considerably higher from the whey predominant formula than from the casein predominant formula (Lönnerdal, Cederblad et al. 1984). Studies on the effect of various sources of protein on zinc absorption is often confounded by the fact that proteins often contain other constituents that may affect zinc absorption (Lönnerdal 2000). Hence, the results or findings from studies should be interpreted with care.

- **Phytate and fibre**. Inhibitory effect of phytate on zinc absorption have been observed very early on in animals (Vohra and Kratzer 1964). This was later confirmed in human after observing zinc deficiency in the Middle East as staple food in most part of that world contains phytate (Halsted, Ronaghy et al. 1972). It was further supported by the low bioavailability of zinc from soy-based infant formula compared to milk formula and human milk due to its phytate content (Sandström, Cederblad et al. 1983). Thus reduction in dietary phytate content is likely to increase zinc absorption.

  Fibre is usually said to have a negative effect on zinc absorption however, this is more likely to be due to the phytate content which is usually present in fibre containing foods as shown by Knudsen et al., who observed that a fibre rich diet was also high in phytate (Knudsen, Sandström et al. 1996). Reducing the content of phytate in fibre rich food product demonstrated a significant increase in zinc absorption indicating that it was phytate and not fibre affecting zinc absorption (Barbro, Brittmarie et al. 1985).

- **Minerals.** Interaction of zinc with other minerals is of particular concern as the use of supplements is continually increasing. The potential interaction of zinc with iron is of concern as many iron fortification and supplementation programs exist to combat iron deficiency, which is the most common nutrient deficiency. A number of studies have demonstrated a negative impact of iron supplementation on plasma zinc levels (Solomons and Jacob 1981; Hambidge, Krebs et al. 1987). Solomon and Jacob (Solomons and Jacob 1981) showed a

small inhibition of zinc absorption at 1 to 1 ratio, considerable inhibition at 2 to 1 and 3 to 1 of iron to zinc proportion with zinc sulphate as the source of inorganic zinc and ferrous sulphate as the source of nonheme iron. In contrast, no effect on zinc absorption was observed when heme iron, as heme chloride was ingested in a 3 to 1 and ferrous iron at a 2 to 1 iron to zinc ratio. A succeeding study found that ferric iron was significantly less inhibitory at 2 to 1 ratio. Addition of ascorbic acid increased the inhibitory effect (Solomons et al. 1983). However, some studies have suggested that inhibitory effect of iron on zinc is exerted only at a very high iron to zinc ratio suggesting that iron fortification will not affect zinc absorption if zinc and iron are administered in a supplement and is given apart from meals (Lönnerdal 2000). The various mechanisms concerning interaction between zinc and iron are not well understood. A competitive binding to the transporter protein DMT1 that participates in divalent metal transport, could explain this interaction (Gunshin et al. 1997) however, other studies performed in Caco-2 cells have questioned the role of DMT1 on zinc uptake (Garrick et al. 2006, Kordas and Stoltzfus 2004). It has also been postulated that there is a common pathway of iron and zinc uptake, different from the DMT1, located in the apical membrane of the intestinal cell (Tandy S, 2000) and depends on the total amount of both minerals present in the intestinal lumen (Olivares M, 2007).

Calcium per se has no negative effect on zinc absorption however, calcium content of the diet my affect zinc absorption from phytate containing meals (Lönnerdal 2000). The proposed reason for this is that calcium has the tendency to form complexes with phytate and zinc that are insoluble and consequently have an inhibitory effect on zinc absorption. This interaction is however complex and may be of limited predictive value despite the support of the concept by studies (Lönnerdal 2000).

Interaction of zinc with copper has been observed in animals in a very high ratio however, no negative effect on zinc absorption was observed in humans with modest increase in copper intake when zinc intake is satisfactory (August,

Janghorbani et al. 1989). The effect of copper on zinc absorption in those with zinc intake is required to explore this interaction further.

It is also possible that pathologic conditions that affect the gastrointestinal tract, especially in the distal small bowel have adverse effects on zinc nutriture because of interference with the normal conservation of endogenously secreted zinc (Krebs 2000). Some of the health conditions that can decrease zinc absorption are gastrointestinal diseases including ulcerative colitis, Crohn's disease, short bowel syndrome and chronic diarrhoea. Chronic liver and kidney disease, conditions causing fat mal-absorption and alcoholism (decreases zinc absorption and increases urinary zinc excretion) also affect zinc absorption (National Institute of Health 2013). Certain antibiotics such as quinolone and tetracycline antibiotics may also interact with zinc supplements, leading to reduced absorption of the supplement and the antibiotic (National Institute of Health 2013).

2.5.4 Role of zinc in human health

The role of zinc in biology was first recognized by Raulin in 1869 by showing that zinc was essential for growth of Aspergillus niger, the common bread mould (Raulin 1869). Subsequently zinc was found to be essential for plants (Chandler 1937) and for normal growth of rats and mice (Todd, Elvehjem et al. 1934). Until 1961 it was considered improbable that zinc deficiency in humans could occur and lead to any significant clinical problems, but studies later showed that zinc was essential for humans. Symptoms of severe anemia, growth retardation, hypogonadism, skin abnormalities, geophagia and mental lethargy described in men from Iran, were attributed to nutritional zinc deficiency (Prasad, Halsted et al. 1961). Afterwards, there were many other reports (Prasad, Miale et al. 1963a; Prasad, Miale et al. 1963b) and the recognition that nutritional zinc deficiency is a potentially widespread problem, not only in developing countries, but also in highly industrialized ones (Prasad 1991; Sandstead 1994; Bhowmik and Chiranjib 2010).

Zinc is fundamental for life and is the only metal that is a cofactor to more than 300 enzymes (Rink 2000). The formation of DNA, the basis of all life on our planet, would

not be possible without zinc (Bhowmik and Chiranjib 2010). On the cellular level, zinc plays three major biological roles: Catalytical, Structural and Regulatory.

- **Catalytic role.** Zinc is directly involved in catalysis and co-catalysis by enzymes that control many cell processes. It includes DNA synthesis, normal growth, foetal and brain development, reproduction, behavioural response, membrane stability, bone formation and wound healing (Chasapis, Loutsidou et al. 2012). Zinc dependent enzymes can be found in all known classes of enzymes.
- **Structural role.** Zinc due to its physico-chemical properties plays an important role in the structure of proteins and cell membrane and is critical for the function of a number of metalloproteins (Tapiero and Tew 2003). Zinc ions exist in the expression of genetic formation, storing, synthesis and action of peptide hormones and maintenance of chromatin and biological membranes (Tapiero and Tew 2003). Loss of zinc from biomembranes increases their vulnerability to oxidative damage and weakens their functions.
- **Regulatory role.** Zinc regulates both enzymatic activity and the stability of the protein as an activator or inhibitor ion (Mocchegiani, Muzzioli et al. 2000). Zinc also plays a role in cell signalling and has been found to influence hormone release and nerves impulse transmission and zinc finger proteins have been found to regulate gene expression by acting as transcription factors (Bhowmik and Chiranjib 2010).

In human health, zinc is critical for wound healing, taste acuity, immune system function, prostaglandin production, bone mineralization, proper thyroid function, blood clotting, cognitive functions, fetal growth and sperm production (Chasapis, Loutsidou et al. 2012). Zinc also regulates pH, promotes formation of collagen for hair, skin, nails and helps to enhance the memory and improve mental development, it maintains the normal function of prostrate and has significant implication in testosterone secretion (Bhowmik and Chiranjib 2010).

Zinc deficiency triggers apoptic neuronal death. In the nervous system, cell multiplication and apoptosis occur actively during the perinatal period and are essential for normal neurodevelopment. Variations in these closely regulated events can

disturb the normal function of the nervous system later in life. Cellular zinc depletion induces death in many cell lines and is described as an inhibitor of apoptosis (Seve, Chimienti et al. 2002). Low cellular zinc concentration can also trigger apoptosis in numerous cell types such as fibroblast, hepatocytes, T cell precursors, glioma and testicular cells. Zinc is also vital for immune response. It influences and interact with components of the immune system, a highly proliferative system (Wellinghausen and Rink 1998). Zinc binds to enzymes, proteins and peptides with different binding affinity due to which it is important for immunocompetence (Mocchegiani, Muzzioli et al. 2000). Impairment of immune function has been attributed to zinc deficiency and may be the most common cause of secondary immunodeficiency state in humans (Tapiero and Tew 2003). Immune dysfunction in natural immunity is seen in zinc deficiency. More specifically zinc deficiency reduces the lytic activity of natural killer cells, impairs their cytotoxicity and immune signaling, impacts the neuroendocrine immune pathway and alters cytokine production in mast cells (Muzzioli, Stecconi et al. 2009). Another well know function of zinc is the protection of cells from oxidation damage by free radicals. Human cells like all living organism require adequate levels of antioxidant defenses in order to avoid the harmful effect of an excessive production of reactive oxygen species and to prevent damage to the immune cells. Studies in animals and humans have shown that sustained reduction or excessive uncompensated loss of zinc is associated with increased level of oxidative damage, including protein lipid and DNA oxidation (Prasad 2009; Jomova and Valko 2011). Deficiency of zinc increases the levels of lipid peroxidation in mitochondrial and microsomal membranes and the fragility of erythrocytes membrane while the presence of zinc prevents it; hence, zinc plays a crucial role in protecting the cell from oxidative damage (Vallee and Falchuk 1993; Tapiero and Tew 2003).

The role of zinc as a multipurpose trace element in chronic diseases, such as diabetes, depression, cardiovascular disease and other age-related diseases have been recently reviewed in details (Rink 2011, Chasapis, Loutsidou et al. 2012). Low serum zinc levels and zinc deficiency were associated with increased prevalence of cardiovascular disease and diabetes (Singh, Niaz et al. 1998; Little, Bhattacharya et al. 2010). Low dietary intake and decreased serum zinc level intake have also been associated with depression

(Nowak, Szewczyk et al. 2005; Amani, Saeidi et al. 2010) and benefits of zinc supplementation in antidepressant therapy in major depression have been observed in some clinical studies (Nowak, Siwek et al. 2003; Whittle, Lubec et al. 2009).

2.6 Summary

The burden of chronic disease is global and continues to rise as shown in this review emphasizing the need for improvement and progress in early prevention and treatment of chronic disease such as diabetes, cardiovascular disease and depression, which are the leading cause of death worldwide. The importance of micronutrients in health is undisputable and the role of zinc whose deficiency may play an important role in disease has been increasingly gaining attention. There is growing evidence that as one of the most important trace elements in organisms, zinc plays a key pathophysiological role in major chronic disease from it involvement in proper functioning of the immune system, cellular growth, cell proliferation and apoptosis and in numerous zinc binding proteins. It is well known that prolonged deficiency of zinc results in growth impairment and is associated with risk factors of diseases and the existence of a number of disease states in humans. Many studies have explored the role of zinc in health, used zinc supplementation as a therapeutic tool or as an adjuvant to change the outcome of disease states in both animals and humans. However, there is a serious lack of research into the role of zinc in chronic disease as the existing studies are mostly observational or of poor quality and there is a severe lack of longitudinal studies.

Type-2 diabetes, cardiovascular disease, and depression are of great public health interest and are associated with zinc deficiency. Given that there is scarcity of longitudinal research to examine their association, this PhD thesis has explored the association of serum concentration level in different diabetic stage and dietary zinc intake with these chronic diseases.

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SECTION 2 ASSOCIATIONS

Chapter 3 Serum zinc and HOMA parameters

3.1 Is Serum Zinc Associated with Pancreatic Beta Cell Function and Insulin Sensitivity in Pre-Diabetic and Normal Individuals? Findings from the Hunter Community Study

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3.1.1 Abstract

3.1.1.1 Aim*:* To determine if there is a difference in serum zinc concentration between normoglycaemic, pre-diabetic and type-2 diabetic groups and if this is associated with pancreatic beta cell function and insulin sensitivity in the former 2 groups.

3.1.1.2 Method*:* Cross sectional study of a random sample of older community-dwelling men and women in Newcastle, New South Wales, Australia. Beta cell function, insulin sensitivity and insulin resistance were calculated for normoglycaemic and prediabetes participants using the Homeostasis Model Assessment (HOMA-2) calculator.

3.1.1.4 Result*:* A total of 452 participants were recruited for this study. Approximately 33% (N=149) had diabetes, 33% (N=151) had prediabetes and 34% (N=152) were normoglycaemic. Homeostasis Model Assessment (HOMA) parameters were found to be significantly different between normoglycaemic and prediabetes groups ($p<0.001$). In adjusted linear regression, higher serum zinc concentration was associated with increased insulin sensitivity ($p=0.01$) in the prediabetic group. There was also a significant association between smoking and worse insulin sensitivity.

3.1.1.5 Conclusion*:* Higher serum zinc concentration is associated with increased insulin sensitivity. Longitudinal studies are required to determine if low serum zinc concentration plays a role in progression from pre-diabetes to diabetes

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3.1.2 Introduction

Diabetes, a disorder of metabolism with defects in either insulin secretion, insulin action or both, is increasing globally due to population growth, aging, urbanization, unhealthy eating habits, and increasing prevalence of obesity and physical inactivity [1]. Diabetes is a leading cause of morbidity and mortality with an estimated 346 million adults being affected worldwide in 2011 [1]. The prevalence is continuing to rise and is expected to double between 2005–2030. [1, 2].

Type 2 diabetes is often asymptomatic and may remain undiagnosed for several years [3]. It is characterized by insulin resistance, hyperinsulinaemia, beta cell dysfunction and subsequent beta cell failure [4]. Numerous influences on the onset of diabetes have been proposed, one of which may be the abnormal homeostasis of trace elements such as zinc [5]. [6]. Zinc is involved in the synthesis, storage, and secretion of monomeric insulin, as well as conversion to a dimeric form for storage and secretion as crystalline insulin [7, 8]. Zinc is essential in insulin action and carbohydrate metabolism [9]. Oxidative stress also plays an important role in the pathogenesis of diabetes and its complications, and zinc is a structural component of key antioxidant enzymes such as superoxide dismutase, which is vital for intra- and extracellular antioxidant defence [10].

Animal models have shown that peripheral insulin resistance contributes to impaired glucose tolerance in non-diabetic, zinc deficient rats [11]. Oral administration of zinc complex in KKA(y) mice daily also caused significant improvements in hyperglycaemia, glucose intolerance and insulin resistance [12]. Conversely, a zinc deficient diet led to increased fasting blood glucose levels and reduced circulating insulin in db/db mice [13]. Human studies on the other hand

have had conflicting results regarding zinc supplementation in type 2 diabetes. A lower incidence of type 2 diabetes has been reported in women who had a higher intake of dietary zinc [14]. A Cochrane review found that there was insufficient evidence to suggest the use of zinc supplementation in the prevention of type 2 diabetes [15]. By contrast, a systematic review and meta-analysis of 25 articles, which included 22 studies on type 2 diabetes, concluded that zinc supplementation has beneficial effects on glycaemic control [16]. The review however had several limitations including differences in zinc doses, sample size, study duration, limited availability of data on zinc intake and variation in baseline parameters.

Pre-diabetes is a condition that increases the risk of developing type 2 diabetes and often precedes diabetes. In Australia the prevalence of pre-diabetes is estimated at 16.4%, more than double the prevalence of type-2 diabetes [17]. Pre-diabetic individuals have a six fold increased risk of developing type 2 diabetes compared with those with normal glucose values [17]. People with Pre-diabetes have impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or both; blood glucose is higher than normal but not high enough to be classified as diabetes and this state is reversible [17]. Reversion to normal glucose tolerance occurs in about 25% over 3–5 years of observation, while the rest remain stable or progress to overt diabetes. However, with longer observation, the majority of individuals with IFG or IGT appear to develop diabetes [18]. People in the pre-diabetic stage are not only at an increased risk of developing diabetes but also have an increased risk of developing cardiovascular and other macro- vascular disease [17].

This study therefore set out to determine if there is a difference in serum zinc concentration between normal, pre-diabetic and type-2 diabetic groups of community-dwelling men and women aged 55–85 years. A further aim was to determine if serum zinc concentration is associated with pancreatic beta cell function and insulin sensitivity in pre-diabetic and normal individuals. The use of a pre-diabetic group who has not yet developed nephropathy is an important improvement in establishing that low zinc is not simply an epiphenomenon due

to loss of zinc secondary to diabetic nephropathy.

3.1.3 Methods

A random sample of participants aged between 55 and 85 years was selected from the Hunter Community Study (HCS), a cohort of community-dwelling men and women in Newcastle, New South Wales (NSW), Australia. Approval to conduct the research was granted by the University of Newcastle Human Research Ethics Committees and Hunter New England Research Ethics. This study has been described in detail elsewhere [19]. In brief, participants were randomly selected from the NSW State Electoral roll and contacted between mid-December 2004 and May 2007. A modified Dillman recruiting strategy [20] was used whereby two letters of introduction and an invitation to participate were posted to the selected persons. An HCS research assistant telephoned persons who did not respond to initial postal contacts. If contact was not established after five attempts, the individual was classified as a non-responder. Persons who could not speak English and those living in a residential aged care facility were deemed ineligible. Once written consent to participate was obtained from eligible study subjects, they were asked to complete a series of self- reported postal questionnaires.
In addition to completing the postal questionnaires, participants were invited to attend the HCS data collection centre (clinic) to enable a series of clinical measures to be ascertained. The clinic measures included blood pressure, height, weight, waist-circumference and blood collection for routine biochemical analysis (including fasting blood glucose). The clinic measures assessed also included consent to link study information with data from Medicare Australia (Medicare and Pharmaceutical Benefit Scheme) and local health databases.

3.1.3.1 Classification of diabetic status

As part of the clinical assessment in the HCS, fasting blood glucose was measured and participants were categorized into three groups according to American Diabetic Association (ADA) guidelines:

- Normal (normoglycemic): fasting blood glucose concentration <5.6 mmol/L,

- Prediabetic: fasting blood glucose concentration 5.6–6.9 mmol/L, and
- Diabetic: fasting blood glucose concentration ≥ 7 mmol/L or previously diagnosed as diabetic or currently taking diabetic medications (oral hypoglycaemic agents, insulin etc.)

3.1.3.2 Measurement of serum zinc and serum insulin

Blood was collected in EDTA tubes and centrifuged at 4°C and 3000 g for 10 min. to separate serum, which was stored for three years at -80°C before analysis. Serum zinc concentration was measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Perkin Elmer Sciex) as ICP-MS has greater precision and sensitivity compared to the older classic atomic absorption spectroscopy (AAS) technique often used to measure zinc. Analysis of serum insulin was performed using a BECKMAN-COULTER 'DXI 800'.

3.1.3.3 Calculation of HOMA parameters

Measured serum insulin and fasting blood glucose concentration was used to calculate steady state beta cell function (%B), insulin sensitivity (%S) and insulin resistance (IR) for normal and prediabetic participants by using the Homeostasis Model Assessment (HOMA2) calculator (University of Oxford, website; http:// www.dtu.ox.ac.uk/homacalculator/index.php). The HOMA2 calculator is an updated version (computer model) of the original HOMA model (HOMA1). HOMA2 also accounts for variations in hepatic and peripheral glucose resistance and the reduction of peripheral glucose stimulated glucose uptake. However, the HOMA2 calculator is not appropriate for use in frank diabetes as it needs further validation in this group [21].

3.1.3.4 Measurement of other exposure variables

Participant's age, gender, education, income, smoking, alcohol intake, self-reported medical history (clinician diagnosis), self-reported medications and use of zinc supplements or multivitamins with zinc, Body Mass Index (BMI) and Glomerular filtration rate (GFR) were collected. Education was categorized as primary school only, secondary school not completed, secondary school

completed, trade or technical college qualification, and University or other tertiary qualification. Smoking was categorized as current, never, or former smoker. Alcohol intake was quantified and classified according to Australian National Health & Medical Research (NHMRC) guidelines [22] as number of drinks per month. BMI was calculated from participants' height and weight, which was measured during their clinic visit and grouped according to the guidelines for BMI classification by the World Health Organization (WHO).

3.1.3.5 Statistical analysis

To detect a mean difference as low as 1.7 mmol/L (±5 mmol/L) in serum zinc concentration among three groups (normal, prediabetic and diabetic) with 95% confidence interval and 80% power, a sample size of 375 was calculated. Assuming a 15% refusal and dropout rate, recruiting a total of 450 eligible participants was estimated to be sufficient to observe the differences for this study, i.e. 150 in each group. The statistical analysis was performed using STATA software version 11.0 supplied by STATA Corporation, Texas, USA. Initially, baseline characteristics of the study participants compared by the participants' fasting blood glucose status and presented as mean, standard deviation and proportion were analysed using a chi- square test for categorical variables and analysis of variance (ANOVA) for continuous variables. For markers that did not follow a normal distribution, non-parametric tests were performed. Homeostasis Model Assessment (HOMA2) calculator was used to calculate the HOMA parameters i.e. beta cell function (%B), insulin sensitivity (%S) and insulin resistance (IR) for normal and prediabetic participants. Multiple linear regression analyses were then performed for all three HOMA parameters for normoglycaemic and prediabetic group separately to determine factors associated with each outcome. Number of medications used was considered in the multivariate analyses, but dropped out of final models (except for insulin resistance in the pre-diabetic group).

3.1.4 Results

A total of 452 participants with measurement of fasting blood glucose concentration were randomly recruited for this study. The baseline characteristics of the participants according to their fasting blood glucose concentration are shown in Table 1. Of the participants, roughly a third (N = 149) were diabetic, a third prediabetic (N = 151) and a third (N = 152) were normoglycaemic. Participants were roughly evenly split between males and females. Age was found to be significantly different with an overall average age of 66.6 years (±7.5), however the normoglycaemic group (65.6 ± 7.3 years) was slightly younger compared to the diabetic group (67.9 ± 7.7 years). Significant differences among the groups were also seen in household income, BMI, hypertension status, use of antihypertensive medication and the number of medications that the participants were taking. Overall, more than 80% of the participants across all the groups were on some form of medication. Only 6% (N = 27) of the total participants were taking zinc supplements or multivitamins with zinc but this was not significantly different between the groups. More than 50% in the normal group had never smoked but the rate of current smokers was similar across all the groups. Alcohol intake (number of drinks/month) was similar across the three groups with roughly 50% being classified as safe drinkers. The mean glomerular filtration rate (GFR) was also found to be similar across the three groups.

Table 2 shows the median of the blood/serum laboratory findings and HOMA parameters, as these biochemical and HOMA parameters were not normally distributed. Median fasting serum glucose concentration was 4.8 mmol/L, 5.8 mmol/L and 6.8 mmol/L in normoglycaemic, pre-diabetic and diabetic participants respectively. The median serum zinc concentration was found to be similar across all three groups (13 μmol/L) however; median serum insulin concentration was different between normoglycaemic and prediabetic groups with this being higher in the prediabetic group (76 Vs 54 mIU/L). Furthermore, to examine whether serum zinc concentration was associated with the HOMA parameters, steady state beta cell function (%B), insulin sensitivity

(%S) and insulin resistance (IR) for normoglycaemic and pre-diabetic groups were calculated. The pre-diabetic group had a lower median of beta cell function and median insulin sensitivity than the normoglycaemic group and higher median insulin resistance compared to the normoglycaemic group.

The non-parametric Kruskal-Wallis test was used to test differences by glycaemic status (Table 2). As median serum zinc concentration was similar across all groups no statistical significant difference was found. However, median insulin concentration was significantly different between normoglycaemic and prediabetic groups ($p<0.001$). Moreover, all the HOMA parameters i.e, beta cell function (%B), insulin sensitivity (%S) and insulin resistance (IR) were found to be statistically significantly different between groups [$p<0.001$ (table 2)].

Multiple linear regression analyses for HOMA parameters was carried out separately by the participants' glycaemic status i.e normoglycaemic and prediabetics groups (Table 3 & 4). After adjusting for multiple possible confounders, the prediabetic group showed significant association between serum zinc concentration and insulin sensitivity ($p=0.01$) indicating that insulin sensitivity increases with an increase in serum zinc concentration. Significant positive association of household income with beta cell function ($p=0.03$) and insulin resistance ($p=0.04$) was also observed in the prediabetic group. The same analysis also showed that insulin resistance increases with increasing number of medications ($p=0.02$). However, similar stratified analysis for the normoglycaemic group did not show any significant association of HOMA parameters with serum zinc or household income.

We also found that beta cell function was higher in those with higher BMI in both the nomoglycaemic ($p=0.001$) and prediabetic ($p=0.009$) group. In the normoglycaemic group, insulin resistance ($p<0.001$) was also higher in those with higher BMI. Moreover, an association was found between smoking status and insulin sensitivity in the normoglycaemic group indicating that current smokers had lower insulin sensitivity ($P=0.004$) and that insulin resistance increases with an increase in the number of medications ($p=0.02$).

Table 1. Baseline characteristics by participants fasting blood glucose status.

Characteristic	Overall (n = 452)	Normal (n = 152)	Pre-diabetic (n = 151)	Diabetic (n = 159)	p-value
Age (mean±SD)	66.6 (7.4)	65.6 (7.3)	66.3 (7.0)	67.9 (7.7)	0.03
Sex (n, %)					>0.05
Male	238 (53.72)	69 (46.62)	89(60.54)	80 (54.05)	
Female	205 (46.28)	79(53.38)	58(39.46)	68(45.95)	
Education (n, %)					>0.05
Primary schooling only	14 (3.17)	7 (4.73)	1 (0.68)	6 (4.08)	
Secondary schooling completed	108 (24.43)	41 (27.70)	36 (24.49)	31 (21.09)	
Secondary schooling not completed	98 (22.17)	29 (19.59)	28 (19.05)	41(27.89)	
Trade qualification or TAFE	103 (23.30), 89 (20.14)	39 (6.35), 24 (16.22)	34 (23.13), 38 (25.85)	30(20.41), 27(18.37)	
University or other tertiary study	24 (5.43)	5 (3.38)	9 (6.12)	10 (6.80)	
Others	6 (1.36)	3 (2.03)	1 (0.68)	2 (1.36)	
Household income/year (n,%)					0.003
Less than 5000	8 (1.81)	1 (0.68)	2(1.36)	5 (3.40)	
$5000–$9,999 per year	22 (4.98)	7 (4.73)	8 (5.44)	7 (4.76)	
$10,000–$19,999 per year	98 (22.17)	25 (16.89)	31 (21.09)	42 (28.57)	
$20,000–$29,999 per year	92 (20.81)	31 (20.95)	26 (17.69)	35 (23.810	
$30,000–$39,999 per year	47 (10.63)	27 (18.240	11 (7.48)	9 (6.12)	
$40,000–$49,999 per year	38 (8.60)	13 (8.78)	15 (10.20)	10 (6.80)	
$50,000–$69,999 per year	51 (11.54)	22 (14.86)	17 (11.56)	12 (8.16)	
$70 000 or more per year	61 (13.80)	18 (12.160	30 (20.41)	13 (8.84)	
Missing	25(5.66)	4 (2.70)	7 (4.76)	14 (9.52)	
Smoking status (n, %)					>0.05
Smoked never	209 (47.18)	77 (52.03)	63 (42.86)	69 (46.62)	
Previous smoker	192 (43.34)	59 (39.86)	70 (47.62)	63 (42.57	
Current smoker	35 (7.90)	11 (7.43)	13 (8.84)	11 (7.43)	
Missing	7 (1.58)	1 (0.68)	1 (0.68)	5 (3.38)	
Alcohol use (n, %)					>0.05
No	91 (20.54)	33 (22.30)	19 (12.93)	39 (26.35)	
Safe drinker	236 (53.27)	83 (56.08)	81 (55.78)	71 (49.97)	
Moderate drinker	28 (6.32)	9 (6.08)	11 (7.48)	8 (5.41)	
Hazardous drinker-binge	27 (6.09)	8 (5.41)	11 (7.48)	8 (5.41)	
Hazardous drinker-chronic	18 (4.06)	5 (3.38)	9 (6.12)	4 (2.70)	
Missing	43 (9.71)	10 (6.76)	15 (10.20)	18 (12.16)	
Hypertension (n, %)					<0.001
Yes	237 (47.57)	61 (40.13)	80 (52.98)	96 (64.43)	
No	215 (52.34)	91 (59.87)	71 (47.02)	53 (35.57)	
BMI (n, %)					<0.001
<25	49 (10.89)	28 (18.54)	10 (6.67)	11 (7.38)	
25.00–29.99	185 (41.11)	75 (49.67)	63 (42)	47 (31.54)	
≥30	216 (48)	48 (31.79)	77 (51.33)	91 (61.07)	
Taking any medicines (n, %)					<0.001
Yes	387 (85.62)	122 (80.26)	126 (83.44)	139 (93.29)	
No	64 (14.38)	30 (19.74)	25 (16.56)	10 (6.71)	
Taking antihypertensive (n, %)					<0.001
Yes	243 (53.76)	60 (39.47)	80 (52.98)	103 (69.13)	
No	209 (46.24)	92 (60.53)	71 (47.02)	46 (30.87)	
Taking anti diabetic (n, %)					
Yes	78 (17.26)			78 (52.35)	

Table 1. Cont.

Characteristic	Overall (n = 452)	Normal (n = 152)	Pre-diabetic (n = 151)	Diabetic (n = 159)	p-value
No	374 (82.74)	152 (100)	151 (100)	71 (47.65)	
Taking zinc supplements or multivitamins with zinc (n, %)					>0.05
Yes	27 (5.97)	9 (5.92)	6 (3.97)	12 (8.05)	
No	425 (94.03)	143 (94.08)	145 (96.03)	137 (91.95)	
GFR (ml/mt) (mean± SD)	75.3 (17)	77.4 (17.3)	73.9 (14.4)	74.5 (18.6)	>0.05

Table 2. Laboratory findings and Homeostasis Model Assessment (HOMA) using for beta cell efficiency in normal and pre-diabetic groups.

	Laboratory findings			Beta cell efficiency using HOMA-2 calculator		
Patient status	**Mean fasting blood glucose mmol/l ± SD (Median)**	**Mean serum zinc, umol/ L ±SD (Median)**	**Mean serum insulin, mIU/ L ±SD (Median)**	**Mean % of beta cell function ±SD (Median)**	**Mean % of insulin sensitivity ±SD (Median)**	**Mean insulin resistance ±SD (Median)**
Normal (n = 152)	4.8±0.4 (4.8)	13.11±1.72 (13)	63.86±39.33 (54)	115.67±46.46 (110.7)	146.33±217.80 (98.5)	1.17±0.71 (1.01)
Prediabetic (n = 151)	5.9±0.3 (5.8)	13.15±1.67 (13)	89.95±61.86 (76)	93.55±46.16 (88.8)	141.08±310.30 (68.5)	1.72±1.14 (1.46)
Diabetic (n = 149)	7.1±2.1 (6.8)	13.46±3.61 (13)	-	-	-	-
Non-parametric Kruskal-Wallis P value	†<0.001	†>0.05	<0.001	<0.001	<0.001	<0.001

†Across all groups.

Table 3. Adjusted linear regression analysis for HOMA parameters in participants with normal fasting glucose.

Exposure indicators	HOMA2 Parameters								
	Beta Cell function			Insulin Sensitivity			Insulin Resistance		
	Coefficient	P value	95% CI†	Coefficient	P value	95% CI†	Coefficient	P value	95% CI†
Serum Zinc	1.43	0.55	−3.30 to 6.17	9.05	0.35	−9.94 to 28.03	−0.007	0.93	−0.064 to 0.059
Age	0.27	0.66	−0.93 to 1.47	−1.82	0.46	−6.62 to 3.00	0.003	0.65	−0.012 to 0.019
Gender	4.77	0.59	−12.51 to 22.04	−31.16	0.37	−100.41 to 38.08	−0.056	0.62	−0.28 to 0.17
BMI	2.91	0.001	1.27 to 4.56	−2.64	0.43	−9.22 to 3.95	0.050	<0.001	0.028 to 0.071
Education	−0.01	0.80	−0.07 to 0.05	0.03	0.84	−0.23 to 0.28	−0.0003	0.40	−0.001 to 0.0005
House hold income	−0.83	0.74	−5.72 to 4.05	3.73	0.71	−15.84 to 23.31	−0.007	0.82	−0.070 to 0.056
Smoking class	8.55	0.22	−5.12 to 22.22	−80.79	0.004	−135.60 to −25.98	0.22	0.02	0.04 to 0.39
Alcohol use	−5.61	0.22	−14.62 to 3.41	29.02	0.11	−7.13 to 65.17	−0.079	0.18	−0.19 to 0.037
Constant	−6.98	0.91	−128.90 to 114.92	256.87	0.30	−231.74 to 745.48	−0.398	0.62	−1.975 to 1.178

†CI: Confidence Interval.

Table 4. Adjusted linear regression analysis for HOMA parameters in pre-diabetic participants.

Exposure indicators	HOMA2 Parameters								
	Beta Cell function			Insulin Sensitivity			Insulin Resistance		
	Coefficient	P value	95% CI†	Coefficient	P value	95% CI†	Coefficient	P value	95% CI†
Serum Zinc	0.45	0.85	−4.34 to 5.25	48.63	0.01	10.59 to 86.67	0.135	0.06	−0.007 to 0.28
Age	0.83	0.20	−0.445 to 2.10	−4.50	0.36	−14.59 to 5.58	0.011	0.51	−0.022 to 0.043
Gender	12.45	0.16	−5.15 to 30.05	−103.95	0.12	−243.50 to 35.60	0.092	0.71	−0.40 to 0.58
BMI	2.18	0.009	0.560 to 3.80	−3.39	0.66	−16.27 to 9.48	0.030	0.17	−0.013 to 0.074
Education	−3.25	0.34	−9.92 to 3.41	12.23	0.53	−40.63 to 65.09	−0.128	0.18	−0.31 to 0.06
House hold income	4.81	0.03	0.50 to 9.12	−12.28	0.46	−46.45 to 21.88	0.126	0.04	0.003 to 0.248
Smoking class	3.64	0.57	−9.11 to 16.40	−96.90	0.06	−198.04 to 4.24	−0.017	0.92	−0.357 to 0.322
Alcohol use	0.42	0.92	−7.49 to 8.32	−17.34	0.59	−80.04 to 45.36	0.022	0.84	−0.201 to 0.246
No. of medicatons	_	_	_	_	_	_	0.114	0.02	0.018 to 0.210
Constant	−71.47	0.34	−218.43 to 75.50	168.84	0.77	−996.58 to 1334.25	−2.44	0.23	−6.474 to1.583

†CI: Confidence Interval.

3.1.5. Discussion

This study has demonstrated that higher serum zinc concentration is associated with increased insulin sensitivity even after adjusting for a number of potential confounders. Furthermore, we also found that the higher the BMI, the higher the beta cell functions in both groups and the higher the insulin resistance in the normoglycaemic group. Current smoking status in the normoglycaemic group was associated with lower insulin sensitivity and higher insulin resistance compared to those who had never smoked or were previous smokers. In the prediabetic group, increase in household income led to increase in beta cell function and insulin resistance.

Previous studies have reported reduced concentrations of serum zinc in prediabetics and diabetes mellitus [23,24] however, like other studies in both developed and developing countries [25,26], our study did not find any difference in serum zinc concentration amongst the diabetic, pre-diabetic and normoglycaemic groups. Zargar et al found that serum zinc concentration was not altered in diabetes mellitus whereas Ekmekcioglu et al found a higher zinc concentration in whole blood and plasma, although the levels between healthy and diabetic individuals did not differ statistically in these blood fractions. Serum zinc concentration varies under the influence of lifestyle factors such as smoking and alcohol consumption [27], as well as metabolic and hormonal influences [25].

Another method that is broadly used to assess zinc status is the determination of zinc concentration in urine. Hyperzincuria has been described to be a regular finding in type 2 diabetes and has been associated with hyperglycemia [5, 9]. Increased urinary loss can imply a decrease in total body zinc. The serum zinc concentrations in our participants were within normal limits (10–18 μmol/L); however as we did not measure zinc excretion, altered levels of zinc in the tissues cannot be ruled out. However, mean GFR was above 70 mls/min in all three groups and the absence of a significant difference in GFR between the groups suggests that there is no impairment in renal function and hence there is unlikely to be a difference in urinary zinc excretion secondary to diabetic

nephropathy.

Another explanation for these findings could be the dietary habits of the Australian study population; those involved in this study may all consume food with relatively high levels of zinc. Although zinc deficiency is widespread and 33% of the world's population is affected [28], a recent study on the global prevalence of zinc deficiency showed that in Australia, the estimated prevalence of inadequate zinc intake was <15% [29] whereas in developing countries it was >25%. In developing countries, pulses and cereals represent the major source of zinc. However, in the United States and other developed countries, meat provides 40–60%, pulses about 20–40% and dairy products about 10–30% of the dietary zinc [30]. Although the sample of people in this study was randomly selected, they may have been relatively healthy and hence zinc replete.

We also examined total serum iron concentration amongst the groups, as zinc is known to interact with iron and decrease zinc absorption [31]. The mean total serum iron was 17.47 (±5.38) μmol/L and there was minimal difference in the mean total serum iron concentration between the groups and no statistical difference was seen ($p=0.13$, results not presented).

The HOMA parameters findings however indicate that zinc may play a role in progression of diabetes with results showing that serum zinc concentration is associated with insulin sensitivity in the pre-diabetic group. There are many probable mechanisms for this; zinc is important with regard to metabolic diseases such as insulin resistance, metabolic syndrome (MS) and diabetes mainly because it is required for insulin storage in the pancreas and stabilization of insulin hexamers. The anti-oxidative properties of zinc may also delay progression of insulin resistance and diabetes [32]. Furthermore, some studies have suggested that zinc supplementation may improve insulin sensitivity [33, 34] and that zinc status affects some risk factors related to insulin resistance [35].

This study found that increasing BMI was associated with increasing beta cell function in both groups and also an increase in insulin resistance in the normoglycaemic group. Previous studies showed that higher BMI was associated with insulin resistance with or without other associated conditions

[36–38]. Release of chemokines and inflammatory cytokines from adipose tissue in obesity may result in chronic systemic low grade inflammation that leads to the development of insulin resistance [36]. On the other hand there are still areas of significant uncertainty and gaps of knowledge that limit a full understanding of beta cell function in obesity. It has been found that obesity is associated with a modest expansion of beta cell mass, however any effect of various factors (e.g. duration of obesity or recent changes in body weight) on beta cell mass in humans is unknown [39].

The result of this study also showed that there was an association between current smoking status and insulin sensitivity and insulin resistance. This finding is similar to a recent study, which showed that smokers were less insulin sensitive compared with nonsmokers; the mechanisms responsible for this are unclear [40]. Bergman et al. also reported that smoking cessation is associated with an improvement in insulin sensitivity in the absence of changes in adiposity or body weight. Since the association between the number of medications consumed and increased insulin resistance was only observed in the prediabetic group, this is likely an indication of metabolic disorders and associated risks, e.g. obesity, hypertension, lipid abnormalities, and atherosclerotic cardiovascular disease [41].

A strength of this study is the large sample size with a similar number of participants in all groups compared to previous studies. Zinc measurement was done using ICP-MS rather than atomic absorption spectroscopy (AAS) as studies have showed that ICP- MS provides much lower detection limits, high precision, high accuracy and reliable isotopic analysis compared to other methods for measuring trace elements [42,43]. Also, the coefficients of variation (CV%) for ICP-MS for serum zinc analysis was 13% which indicates low laboratory error. A potential limitation of our study is the absence of information on family history of diabetes and we did not consider other important trace elements such as copper, which may influence serum zinc concentration.

In conclusion, though no significant difference was found in the serum zinc concentration between normoglycaemic, prediabetes, and diabetes groups, this

study suggests that zinc may still plays a vital role in diabetes as higher serum zinc concentration is associated with increased insulin sensitivity. The associations of BMI with beta cell function, and insulin sensitivity with smoking status should be explored further as these are important risk factors that may be modified as a means of preventing the progression to full diabetes. Further studies are needed to confirm these findings and the results need to be replicated in longitudinal studies.

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Author Contributions

Conceived and designed the experiments: KPV MM MRI AHM SH JA. Performed the experiments: KPV MM JA AHM. Analyzed the data: KPV

3.1.6 References

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Chapter 4 Dietary zinc and type 2 diabetes

4.1 Is dietary zinc protective for type 2 diabetes? Results from the Australian longitudinal study on women's health

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4.1.1 Abstract

4.1.1.1 Background: Animal studies have shown that zinc intake has protective effects against type 2 diabetes, but few studies have been conducted to examine this relationship in humans. The aim of this study is to investigate if dietary zinc is associated with risk of type 2 diabetes in a longitudinal study of mid-age Australian women.

4.1.1.2 Methods: Data were collected from a cohort of women aged 45-50 years at baseline, participating in the Australian Longitudinal Study on Women's Health. A validated food frequency questionnaire was used to assess dietary intake and other nutrients. Predictors of 6-year incidence of type 2 diabetes were examined using multivariable logistic regression.

4.1.1.3 Results: From 8921 participants, 333 incident cases of type 2 diabetes were identified over 6 years of follow-up. After adjustment for dietary and non-dietary factors, the highest quintile dietary zinc intake had almost half the odds of developing type 2 diabetes (OR=0.50, 95% C.I. 0.32–0.77) compared with the lowest quintile. Similar findings were observed for the zinc/iron ratio; the highest quintile had half the odds of developing type 2 diabetes (OR=0.50, 95% C.I 0.30-0.83) after multivariable adjustment of covariates.

4.1.1.4 Conclusions: Higher total dietary zinc intake and high zinc/iron ratio are associated with lower risk of type 2 diabetes in women. This finding is a positive step towards further

research to determine if zinc supplementation may reduce the risk of developing type 2 diabetes.

Keywords: Diabetes, Australia, Women & Zinc

4.1.2 Background

Diabetes, a disorder of metabolism results in substantial morbidity and mortality, primarily from macrovascular (myocardial infarction, stroke, and peripheral vascular disease), and microvascular effects (retinopathy, nephropathy, and neuropathy). The World Health Organization (WHO) estimated that 3.4 million people worldwide died from consequences of high fasting blood sugar in 2010 [1]. Currently 347 million adults worldwide have diabetes; the prevalence is continuing to rise and is expected to be the 7th leading cause of death by 2030 [1]. This will have the greatest impact on the productive population of countries, as people under the age of 70 years constitute almost half of all diabetic deaths.

Molecular and cellular studies have demonstrated that the mineral zinc plays a key role in the synthesis and action of insulin under normal physiological conditions and in type 2 diabetes (T2D). Observational studies have reported an association between reduced serum zinc and established T2D [2]. Protective effects of zinc supplementation have also been demonstrated in rodent models of T2D [3], but this has not been properly tested in humans.

Epidemiological studies have observed an association between reduced zinc status and T2D [4, 5]. This association may simply be due to loss of zinc through the kidneys due to diabetic nephropathy; indeed, a number of studies have shown that urinary excretion of zinc is increased and serum levels decreased in T2D patients compared with controls [5]. A study by Marreiro et al found that 35.5% of study subjects with T2D had higher urinary zinc excretion than normal [6]. However, other lines of evidence support a more direct causative role of zinc in the pathogenesis of T2D. A few studies have examined the effect of zinc supplementation in T2D patients with many showing an improvement in

glycaemic control [7-9]. Only one study has examined the use of zinc supplementation for the primary prevention of T2D [6]. This study was identified and the only study included in a recent Cochrane review that examined the role of zinc supplementation for the primary prevention of T2D and found that there was insufficient evidence to suggest the use of zinc supplementation in the prevention of T2D [10]. Recent support for the use of zinc supplementation for reducing the risk of T2D comes from the Nurses' Health Study (n ~ 82,000) where higher dietary zinc intake was associated with a lower risk of developing T2D in women [11]. However, another study in a Chinese population did not find an association between dietary zinc intake and hyperglycaemia but did find an association with the dietary zinc to haeme iron ratio [12]. In light of these findings we have used a large population-based cohort of women to examine these associations in an Australian context. In addition, this study also examines dietary zinc to iron ratio as iron is known to interact with the absorption of zinc and a recent study showed that zinc to haeme iron ratio was inversely associated with risk of diabetes in US women [11].

4.1.3 Methods

The Australian longitudinal study on women's health

The Australian Longitudinal Study on Women's Health (ALSWH) is a prospective study examining the health and wellbeing of three cohorts of women aged 18–23 years (young), 45–50 years (mid-aged), and 70–75 years (older) at the time of the initial surveys in 1996. Women were selected randomly within each age group from the National Medicare Health Insurance Database (which includes all permanent residents of Australia regardless of age, including immigrants and refugees) with intentional overrepresentation of women living in rural and remote areas, which was achieved by sampling women in these areas at twice the rate of women in urban areas. Further details of the recruitment methods and response rates have been described elsewhere [13]. The study collects self-reported data using mailed surveys at 2 to 3 year intervals from 40,000 women living in all states and territories of Australia. The surveys include questions about health

conditions, symptoms, and diagnoses; use of health services; health related quality of life; social circumstances, including work and time use; demographic factors; and health behaviours. Complete details of each survey (S) are on the study website (available at http://www.alswh.org.au). Informed written consent was obtained from all participants in 1996, with ethical clearance for the study obtained from the University of Newcastle. Ethics committees at the Universities of Newcastle and Queensland approved the ALSWH. This article only includes data from the mid-aged cohort as this age group (45-50 years) were at higher risk of developing diabetes than the other two cohorts (18–23 and 70–75 years) of women and were the only age group with food frequency questionnaire (FFQ) data collected. There were five waves of data collection from 1996 to 2007 (S1, 1996; S2, 1998; S3, 2001; S4, 2004; and S5, 2007). Surveys 1, 2 and 3 consisted of 13716, 12338, and 11228 women respectively. The response rate for S3 of the mid age cohort was 83% of women who had completed S1 and had not died (n=115) or become too ill to complete further surveys (n = 21). Other non-respondents included those who were unable to be contacted (n = 930), were contacted but did not complete the survey (n = 998), and those who withdrew from the project (n = 242) [13]. Of the women who completed S3 (then aged 50–55 years), 11196 completed the food frequency questionnaire (FFQ) and 8921 participants remained in the study and were available for analysis by S5. Those who were lost to follow-up were not significantly different but were more likely to be born outside Australia, less educated, or a current smoker.

4.1.3.1 Dietary assessment

At S3, dietary intake was assessed using an FFQ known as the Dietary Questionnaire for Epidemiological Studies (DQES) Version 2. Both the development of the questionnaire [14] and its validation in mid-aged Australian women has been previously reported [15]. This questionnaire asks respondents to report their usual consumption of 74 foods and six alcoholic beverages over the preceding 12 months using a 10-point frequency scale. Additional questions are asked about the number of serves or type of fruit, vegetables, bread, dairy

products, eggs, fat spreads and sugar and further details are provided in Hodge et al. [15]. Nutrient intakes were computed from NUTTAB 1995, a national government food composition database of Australian foods NUTTAB95 [16], using software developed by the Cancer Council of Victoria. The validation of the FFQ against a 7-day weighted food record showed Pearson correlation coefficient = 0.4 for dietary zinc intake. The FFQ validation study deemed the correlation coefficient acceptable as it is of similar magnitude to those previously reported [15].

4.1.3.2 Ascertainment of type 2 diabetes

At each survey women were asked if a doctor had told them that they had T2D. At S1 they were asked if they had ever had a diagnosis of T2D. At S2, S3, S4, and S5 they were asked whether they had been diagnosed with T2D in the time period that had elapsed since the previous survey. Prevalence of T2D at each survey was defined as the proportion of these women who reported at that survey or a previous survey that they had been told they had T2D.

4.1.3.3 Measurement of non-dietary factors

Social and behavioural factors were based on information collected at S3 (AIHW 2001). Participants were asked to report frequency of engaging in vigorous (e.g., aerobics, jogging) and less vigorous (e.g., walking and swimming) exercise lasting for <20 min in a normal week. Responses were scored using approximate weekly frequencies of exercise (never = 0, once a week = 1, 2 or 3 times per week = 2.5, 4–6 times per week =5, every day = 7, and more than once a day = 10) and then weighted to reflect the intensity of the activity (vigorous = 5 and less vigorous = 3). The resulting physical activity scores ranged from 0 to 80 and were categorized as "nil/sedentary (<5)," "low (5 to 15)," "moderate (16 to 25)," or "high (>25)." A score of 15 is commensurate with the current recommendation of moderate intensity activity on most days of the week. This measure is described in more detail elsewhere [17] and has previously been shown to have acceptable test-retest reliability [18]. Standard questions were used to categorize respondents

as never-smoker, ex-smoker, or current smoker; the latter was grouped as smoking less than 10 cigarettes (c) per day (<10c/d), 10-19 c/d and > =20 c/d.

Body mass index (BMI) was calculated as self-reported weight (kg) divided by the square of estimated height (m^2). Medical history of arthritis, congestive heart disease (CHD), stroke, hypertension (HT), asthma and depression along with use of hormone replacement therapy (HRT) (coded as either yes or no) were all self-reported. The participants were also asked to report the number of supplements being used and categorised as taking multivitamin & mineral supplements (yes or no).

4.1.3.4 Statistical analysis

Chi square test was used to compare differences in categorical variables, and ANOVA was used to compare mean values of continuous variables between groups. Predictors of 6-year incidence of T2D were examined using forward stepwise multivariable logistic regression in stepped approach, with the main predictor being energy adjusted zinc and zinc/iron ratio measured at S3 used to predict incidence of T2D by S5. Women who first reported a diagnosis of T2D at S1, S2, or S3 (prevalent cases) were excluded from this analysis (N = 567). The macronutrient variables are adjusted for total energy intake by calculating their component of total energy (as a %). The micronutrients were adjusted for total energy by regressing (using linear regression) the natural log of the micronutrient on the natural log of total energy and extracting the standardized residuals.

The multivariable analysis controlled for dietary factors (energy adjusted fibre, fat and iron) and non-dietary factors (BMI, smoking status, HRT, exercise group, history (yes/no) of arthritis, CHD, HT, asthma and depression). P values for trends were conducted by treating quintile of energy-adjusted zinc as a continuous variable. Statistical significance was considered when 2 sided $p < 0.05$. STATA software version 11 was used for all statistical analyses.

4.1.4 Results

At the end of the 6 years follow-up 333 incident cases of T2D were identified out of 8,921 participants. Table 1 describes characteristics of participants at survey 3 by quintile of energy-adjusted zinc. Dietary zinc intake was divided into quintiles of energy-adjusted zinc with quintile 1 as the lowest intake and quintile 5 the highest intake. The dietary zinc intake of the lowest and highest quintile was 5.94 mg (95% CI 5.90 -5.99) and 17.35 mg (95% CI 17.12-17.59) respectively and the mean intake was 10.66 mg/day. At baseline (Table 1) non-dietary factors found to be significantly different across quintiles included smoking status, exercise, BMI, HRT, HT, asthma and depression. Energy-adjusted dietary factors found to be significantly different across quintiles includes carbohydrates, total protein, total fat (including cholesterol and saturated, polyunsaturated, monounsaturated fat), dietary fibre, minerals (iron, calcium, magnesium, sodium and potassium) and vitamins, which includes retinol, vitamin C and E. Those in the highest quintile of intake were more likely to smoke greater than 20 cigarettes per day and have a history of hypertension. Women in the lower quintiles were found to have a more sedentary life with nil or little exercise but interestingly those with higher intake of zinc also had higher BMI. Of particular interest in this investigation, those in the highest quintile of zinc intake also had the highest intake of dietary iron. The most commonly recorded dietary source of zinc was meat, fish and poultry as the major contributors, though cereals and dairy products were also a substantial source.

Table 2 shows the median value, along with the minimum and maximum values of energy-adjusted zinc for each quintile of dietary zinc. These values represent the standardized residuals after adjusting for total energy intake. For example, the median values of energy-adjusted zinc in Q1 and Q5 are 1.25 and 1.24 respectively indicating that the middle value for energy-adjusted zinc in Q5 is much higher than that of Q1. In an age adjusted analysis there was no significant association across quintiles between energy-adjusted zinc and risk of T2D (Table 2). After adjustment for non-dietary factors including age, BMI,

smoking, HRT, exercise group, history of arthritis, CHD, HT, asthma and depression, an overall decrease in the odds of developing T2D across the quintiles was observed, which was statistically significant ($p = 0.010$). Further adjustment for dietary factors (energy adjusted fibre, iron and fat) strengthened the association ($p = 0.004$). Additional adjustment for alcohol intake and use of supplements (multivitamins or minerals) also showed a statistically significant decrease in the odds of developing T2D across quintiles ($P_{trend} = 0.006$). Compared with the lowest quintile of energy-adjusted zinc those in the highest quintile had almost half the odds of developing T2D (OR = 0.50, 95% C.I. 0.32 – 0.77).

The association between the energy-adjusted zinc/iron ratio and odds of developing T2D (Table 3) followed a similar trend to the association observed between total energy-adjusted zinc intake and T2D. After adjusting for age and non-dietary factors a borderline statistically significant association was observed between energy-adjusted zinc to iron ratio and the odds of developing T2D ($P_{trend} = 0.073$), however there was a consistent decrease in the odds of developing T2D across the quintiles. A statistically significant decrease in the odds of developing T2D across quintiles of energy-adjusted zinc to iron ratio was observed with further adjustment for energy-adjusted dietary factors ($p = 0.003$) and alcohol intake and use of supplements ($p = 0.004$). Compared with the lowest quintile of energy-adjusted zinc to iron ratio those in the highest quintile had half the odds of developing T2D (OR = 0.50, 95% C.I 0.30-0.83).

4.1.5 Discussion

In this longitudinal study we observed an inverse association between dietary zinc intake and risk of T2D in a mid-aged female population after adjusting for potential dietary and non-dietary confounders. In addition, we also observed a consistent inverse association between dietary zinc/iron ratio and risk of T2D. This suggests that the proportion of zinc intake in relation to iron is an important determinant of T2D risk. This makes sense given that iron competes with zinc for binding ligands within the intestinal lumen or for transport proteins on the brush border of enterocytes [19]. These findings are consistent

with a recent study of 82,297 women (mostly white nurses) aged 30 to 60 years reported by Sun et al. [11], but this is in contrast to the findings of a study conducted by Shi et al [12] in China of 1056 healthy men and women aged 20 years and above. As our study is a representative sample of mid-age, community-dwelling women from across Australia rather than a clinical sample it has high external validity.

Table 1 Characteristics of subjects at survey 3 by quintile of energy-adjusted zinc

Characteristic	Sub group/or mean(Std)	Quintile of energy-adjusted zinc			p-value
		Q1 Lowest (n = 1785)	Q3 Middle (n = 1784)	Q5 Highest (n = 1784)	
Smoking status	Never smoker	948 (53%)	1033 (58%)	922 (52%)	<0.001
	Former smoker	568 (32%)	531 (30%)	569 (32%)	
	smoker < 10 c/d	84 (4.7%)	75 (4.2%)	76 (4.3%)	
	smoker 10-19 c/d	77 (4.3%)	64 (3.6%)	81 (4.6%)	
	smoker > = 20 c/d	100 (5.6%)	76 (4.3%)	130 (7.3%)	
Exercise group	Nil/sedentary	321 (19%)	271 (16%)	323 (19%)	0.003
	Low	621 (37%)	683 (40%)	622 (36%)	
	Moderate	318 (19%)	355 (21%)	338 (20%)	
	High	437 (26%)	408 (24%)	433 (25%)	
Hormone replacement therapy	No	1250 (70%)	1193 (67%)	1160 (65%)	0.020
	Yes	535 (30%)	591 (33%)	624 (35%)	
Heart disease	No	1743 (99%)	1741 (98%)	1735 (98%)	0.077
	Yes	26 (1.5%)	28 (1.6%)	30 (1.7%)	
Hypertension	No	1498 (85%)	1495 (85%)	1449 (82%)	0.038
	Yes	271 (15%)	274 (15%)	316 (18%)	
Arthritis	No	1409 (80%)	1358 (77%)	1375 (78%)	0.342
	Yes	360 (20%)	411 (23%)	390 (22%)	
Asthma	No	1568 (89%)	1602 (91%)	1584 (90%)	0.038
	Yes	201 (11%)	167 (9.4%)	181 (10%)	
Depression	No	1538 (87%)	1605 (91%)	1597 (90%)	0.001
	Yes	231 (13%)	164 (9.3%)	168 (9.5%)	
Age	mean (SD)	52.6 (1.4)	52.6 (1.5)	52.4 (1.5)	0.004
Body mass index	mean (SD)	26.0 (5.4)	26.5 (5.1)	27.2 (5.4)	<0.001
Total energy intake	mean (SD)	6676 (2415)	6604 (2275)	6687 (2790)	0.508
Alcohol intake	mean (SD)	6.2 (11.0)	8.3 (12.1)	9.7 (14.9)	<0.001
Number of supplements	mean (SD)	1.1 (1.1)	1.1 (1.1)	1.0 (1.1)	0.163
Carbohydrates (% of energy)	mean (SD)	48.2 (5.8)	45.6 (5.8)	41.0 (7.5)	<0.001
Dietary fibre (% of energy)	mean (SD)	2.4 (0.7)	2.5 (0.6)	2.5 (0.8)	<0.001
Total protein (% of energy)	mean (SD)	17.1 (2.1)	20.8 (1.7)	25.1 (2.8)	<0.001
Total fat (% of energy)	mean (SD)	35.5 (5.5)	34.3 (5.9)	34.5 (6.6)	<0.001
Saturated fat (energy adjusted)	mean (SD)	14.0 (3.8)	13.5 (3.4)	13.9 (3.3)	<0.001
Polyunsaturated fat (% of energy)	mean (SD)	6.3 (2.3)	5.5 (1.9)	4.7 (1.5)	<0.001
Monounsaturated fat (% of energy)	mean (SD)	12.1 (2.2)	12.1 (2.4)	12.6 (2.8)	<0.001
Iron (energy adjusted)	mean (SD)	−0.605 (0.984)	0.022 (0.905)	0.543 (0.897)	<0.001
Cholesterol (energy adjusted)	mean (SD)	−0.584 (1.170)	−0.043 (0.827)	0.607 (0.855)	<0.001
Retinol (energy adjusted)	mean (SD)	0.451 (0.947)	0.030 (0.932)	−0.525 (0.993)	<0.001
Vitamin C (energy adjusted)	mean (SD)	−0.092 (1.198)	0.024 (0.957)	0.028 (0.895)	0.001
Vitamin E (energy adjusted)	mean (SD)	0.346 (1.094)	0.035 (0.923)	−0.407 (0.939)	<0.001
Calcium (energy adjusted)	mean (SD)	−0.374 (0.901)	0.126 (0.940)	0.089 (1.166)	<0.001
Magnesium (energy adjusted)	mean (SD)	−0.453 (1.001)	0.064 (0.901)	0.268 (1.069)	<0.001
Sodium (energy adjusted)	mean (SD)	−0.432 (1.030)	0.026 (0.930)	0.345 (0.999)	<0.001
Potassium (energy adjusted)	mean (SD)	−0.594 (1.072)	0.067 (0.891)	0.454 (0.915)	<0.001

Table 2: Stepwise approach to examine energy-adjusted zinc as an independent predictor of a new diagnosis of diabetes

	Quintile of energy-adjusted zinc					
	Q1	Q2	Q3	Q4	Q5	P
Number of women	1785	1784	1784	1784	1784	
Energy-adjusted zinc [median (min, max)]	−1.25 (-4.8, -0.79)	−0.48 (-0.79, -0.23)	0.01 (-0.23, 0.26)	0.50 (0.26, 0.79)	1.24 (0.79, 4.45)	
Number of diabetics	80	60	59	74	60	
Odds ratio						
• Age adjusted	1.00	0.74 (0.53 to 1.05)	0.73 (0.52 to 1.03)	0.99 (0.67 to 1.28)	0.75 (0.53 to 1.05)	0.319
• Age & non-dietary† factors adjusted	1.00 1.00	0.82 (0.56 to 1.19)	0.65 (0.44 to 0.96)	0.83 (0.58 to 1.19)	0.56 (0.38 to 0.83)	0.010
• Age, non-dietary† and dietary‡ factors adjusted	1.00	0.78 (0.53 to 1.15)	0.60 (0.40 to 0.91)	0.77 (0.52 to 1.13)	0.48 (0.31 to 0.75)	0.004
• Age, non-dietary† and dietary‡ factors adjusted plus alcohol intake and use of supplements		0.80 (0.54 to 1.17)	0.60 (0.40 to 0.90)	0.78 (0.53 to 1.15)	0.50 (0.32 to 0.77)	0.006

† Non-dietary factors were BMI; smoking status; HRT; exercise group; and history of arthritis, CHD, hypertension, asthma and depression.
‡ Dietary factors were energy-adjusted fiber, iron and fat.
Adjustment for family income in the models resulted in a loss of 1300 observations but the p-values for the test for trend were very similar (p = 0.010 for all 3 adjusted models).

Table 3 Stepwise approach to examine zinc/iron ratio as an independent predictor of a new diagnosis of diabetes

	Quintile of zinc to iron ratio					
	Q1	Q2	Q3	Q4	Q5	P
Number of women	1785	1784	1784	1784	1784	
Zinc/Iron ratio [median(min, max)]	0.69 (0.28, 0.77)	0.84 (0.77, 0.90)	0.95 (0.90, 1.00)	1.06 (1.00, 1.12)	1.21 (1.12, 1.75)	
Number of diabetics	60	72	71	65	65	
Odds ratio						
• Age adjusted	1.00	1.21 (0.85 to 1.71)	1.19 (0.84 to 1.70)	1.09 (0.76 to 1.56)	1.09 (0.76 to 1.56)	0.885
• Age & non-dietary† factors adjusted	1.00	0.91 (0.61 to 1.33)	0.91 (0.62 to 1.34)	0.73 (0.49 to 1.09)	0.74 (0.50 to 1.10)	0.073
• Age, non-dietary† and dietary‡ factors adjusted	1.00	0.76 (0.50 to 1.16)	0.71 (0.46 to 1.11)	0.54 (0.33 to 0.86)	0.50 (0.30 to 0.83)	0.003
• Age, non-dietary† and dietary‡ factors adjusted plus alcohol intake and use of supplements	1.00	0.75 (0.50 to 1.14)	0.72 (0.46 to 1.12)	0.54 (0.34 to 0.87)	0.50 (0.30 to 0.83)	0.004

† Non-dietary factors were BMI; smoking status; HRT; exercise group; and history of arthritis, CHD, hypertension, asthma and depression.
‡ Dietary factors were energy-adjusted fiber, iron and fat.

Zinc is required for various aspects of cellular homeostasis. It is involved in the catalytic activity of approximately 300 enzymes and plays a role in immune function, cell division, protein and DNA synthesis and apoptosis [2, 20]. The human body has no specialised zinc storage system [20] and so humans rely on a daily intake of dietary zinc to maintain health and prevent disease. It is well established that zinc has an insulin-like effect on all insulin sensitive tissues. Insulin exerts its effect by binding the insulin receptor and activating an intracellular signalling cascade mediated by the phosphoinositide 3'-kinase (PI3K)/Akt complex. Zinc (II) ions have been shown to activate this same complex in numerous human cell types [21]. Zinc (II) ions have also been shown to suppress protein tyrosine phosphatases associated with the insulin signalling cascade thus activating the insulin signalling cascade resulting in glucose uptake, increased glycogen synthesis, and decreased gluconeogenesis [21].

The importance of zinc in the euglycaemic state is highlighted by a number of animal studies that have demonstrated that zinc improves hyperglycaemia, glucose intolerance, and insulin resistance [22-24]. A number of molecular and cellular studies have shown that zinc inhibits lipolysis in adipocytes and stimulates glucose uptake in rat and murine adipocytes [24, 25]. Further studies have shown that gluconeogenesis was attenuated in rat hepatocytes exposed to zinc and in rat renal cortex slices cultured in the presence of zinc, while glycogen synthesis was stimulated in various cell types exposed to zinc. Moreover, zinc attenuates the high insulin secretory response to glucose in isolated pancreatic islet cell [26]. Considering that zinc is required for the synthesis and release of insulin from pancreatic β cells [27] it follows that low levels of bioavailable zinc may have a significant impact on glucose metabolism and ultimately the risk of T2D.

Studies have documented zinc deficiency in most patients with T2D leading to speculation that zinc may have beneficial effects when administered to diabetic patients [2,7], although the zinc loss may simply reflect increased urinary loss due to diabetic nephropathy. Epidemiological studies have observed that

hyperzincuria and hypozincemia are commonly seen in T2D although the mechanism for the urinary loss has not been fully identified [4, 5]. Costarelli et al [28] found that subjects with a lower dietary zinc intake display general impairment of their zinc status, an altered lipid profile and increased insulin production in comparison to obese subjects with normal zinc dietary intake. As mentioned above few studies have examined the effect of zinc supplementation in T2D patients with some showing an improvement in glycaemic control. A recent study [29] showed that supplementation either with zinc plus multi vitamin and mineral (MVM) or MVM alone in 96 patients with T2D resulted in a significant favourable effect on blood glucose parameters. This beneficial effect of the zinc and MVM supplements was dependent on the initial fasting blood sugar, indicating that zinc was more effective earlier in the diabetic spectrum and less effective once diabetes was established. A more recent systematic review and meta-analysis of 25 articles, which included 22 studies on T2D, concluded that zinc supplementation has beneficial effects on glycaemic control [30]. The review however had several limitations including differences in zinc doses, sample size, study duration, limited availability of data on zinc intake and variation in baseline parameters and so the conclusions remain in doubt. Despite all this study supporting the effect of zinc in glycaemic control in T2D, there are equally many studies that have found that zinc supplementation have no effect on glycaemic parameters such as Hb1Ac and glucose levels [31-33]. Another study also did not find differences in glycaemic control among patients with T2D treated with oral zinc compare to placebo despite improving zinc status [34]. Hence, despite the positive effects of supplemental zinc observed in animals and cellular studies the connection between dietary zinc supplementation and its role in cellular signalling in humans remains unclear and requires further investigation.

The main strengths of this study are the prospective design, where dietary assessment preceded the development of T2D, and the generalizability, this being a population-based cohort rather than a clinic sample. The main advantage of the prospective design is that it reduces selection bias and potential recall

bias. The large sample size also means that it is possible to obtain reasonably stable estimates of incidence rates between successive surveys.

Despite the good generalizability of this study there are some limitations. Though the Pearson correlation coefficient for dietary zinc intake was deemed acceptable because other studies have provided a similar estimate it is still below that observed using other dietary intake methods such as a weighted food record. This is one of the known limitations of using a FFQ to collect dietary information (especially micronutrients) and is due to the lack of homogeneity in food composition tables [35] and over or under reporting of certain foods/food groups. However, the use of FFQs to collect dietary information in large population-based samples is the most cost-effective and feasible method available and the interpretation of findings using an FFQ study needs to consider this limitation. In this study the use of an FFQ to estimate dietary zinc intake will have underestimated the amount of zinc consumed by study participants and biased the effect size towards the null.

Another concern is that the existence of additional environment sources of zinc that may have contributed to the overall intake of zinc. However given the random sample of subjects selected for this investigation this additional exposure is unlikely to have affected some quintiles of zinc intake more than others. Hence, the amount of zinc consumed by all study participants is likely to be an underestimate and this will bias the observed association between dietary zinc and T2D towards the null. Given that an FFQ was used to estimate dietary zinc intake and that there may by some environmental sources of zinc consumption the true estimate of the association will actually be larger than that observed in this study.

There is also the possibility that other confounding variables may not have been controlled for in the analysis and this suggests that our estimate of the effect of zinc on T2D risk may be subject to some residual confounding; however given that the most important dietary (including multivitamin use) and non-dietary confounders have been controlled for this residual confounding is likely to be small.

A further limitation is that dietary assessment was carried out at one time only in survey 3. However, the dietary habits of the participants are unlikely to have changed significantly over the 6-year period from survey 3 to survey 5, as they are mid-aged and dietary patterns are likely to remain stable. Instead of excluding participants who develop comorbidities during the follow-up period we controlled for these in the analysis. This was done to account for any change in dietary habit that may have occurred following the development of comorbid conditions.

Although adjustments were made for all known potential confounders, residual confounding cannot be entirely excluded.

This study also considered the use of supplementary zinc as and additional source and though the quantity of zinc in the supplements was not known, analysis showed that the proportion of people taking supplements were not significantly different between quintiles of zinc intake. Although multiple dietary assessments are useful to reduce random measurement error, given that single dietary assessment is likely to bias results toward the null the fact that we still observed a significant association is an indication that the association between dietary zinc and dietary zinc to iron ratio is likely to be robust and even stronger than that observed in this study.

Although T2D was self-reported we do not consider this a significant limitation as the kappa statistic for agreement between self-report of diabetes and New South Wales Admitted Patient Data Collection (APDC) hospital records in the mid-aged cohort was 0.75 while the prevalence adjusted figure is 0.93 (unpublished data).

4.1.6 Conclusions

In conclusion, the current study confirms the recent findings by Sun et al [11] that higher total dietary zinc intake and high zinc/iron ratio is associated with lower risk of T2D in women. Future research should examine this association in both men and women across different age groups. A positive finding in future studies

should prompt further research to determine if zinc supplementation may reduce the risk of developing T2D.

Abbreviations

ALSWH: Australian longitudinal study on women's health

DQES: Dietary questionnaire for epidemiological studies

FFQ: Food frequency Questionnaire

HRT: Hormone replacement therapy

HT: Hypertension

MVM: Multivitamin and minerals

S1: Survey 1

S2: Survey 2

S3: Survey 3

S4: Survey 4

S5: Survey 5

T2D: Type 2 diabetes.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KV: Conceptualization of the idea, study design, literature search, data interpretation and development of manuscript. MMcE: Conceptualization of the idea, study design, literature search, data interpretation and development of manuscript. AM: Conceptualization of the idea, study design and manuscript preparation. MRI: Conceptualization of the idea, study design and manuscript preparation. DS: Data analysis, data interpretation and manuscript preparation. AP: Original concept, design of the analysis and manuscript preparation. JB: Data collection and development of manuscript. DL: Data collection and development of manuscript. JA: Conceptualization of the idea and development of manuscript. ZS: Development of manuscript. All authors read and approved the final manuscript.

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Chapter 5 Zinc and depression

5.1 Dietary zinc is associated with a lower incidence of depression: Findings from two Australian cohorts

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5.1.1 Abstract

5.1.1.1 Background*:* Several animal and human studies have shown that zinc plays a role in reducing depression, but there have been no longitudinal studies in both men and women on this topic. The aim of this study was to investigate dietary zinc, and the zinc to iron ratio, as predictors of incident depression in two large longitudinal studies of mid-age and older Australians.

5.1.1.2 Methods*:* Data were self-reported, as part of the Australian Longitudinal Study on Women's Health (women aged 50–61 years) and Hunter Community Study (men and women aged 55–85 years). Validated food frequency questionnaires were used to assess dietary intake. Energy-adjusted zinc was ranked using quintiles and predictors of incident depression were examined using multivariate logistic regression.

5.1.1.3 Results*:* Both studies showed an inverse association between dietary zinc intake and risk of depression, even after adjusting for potential confounders. Compared to those with the lowest zinc intake those with the highest zinc intake had significantly lower odds of developing depression with a reduction of about 30–50%. There was no association between the zinc to iron ratio and developing depression in either study.

5.1.1.4 Limitations*:* Dietary assessment was carried out only at baseline and although adjustments were made for all known potential confounders, residual confounding cannot be entirely excluded.

5.1.1.5 Conclusions*:* Low dietary zinc intake is associated with a greater incidence of depression in both men and women, as shown in two prospective cohorts. Further studies into the precise role of zinc compared to other important nutrients from the diet are needed.

Keywords: Depression, Diet, Cohort, Australia, Zinc

5.1.2 Introduction

Depression is a mental disorder with high morbidity and mortality; more than 350 million people of all ages worldwide suffer from depression (WHO, 2012). Depression is a major risk factor for self-inflicted injury and, at its worst, can lead to suicide. It has been estimated that suicide is responsible for 1 million deaths every year (WHO, 2012). Depression is also associated with decreased productivity, poor psychosocial outcomes, and decreased quality of life and wellbeing. In Australia, mental disorders were identified as the leading cause of healthy years of life lost due to disability in 2003 (AIHW, 2008). Depression-associated disability costs the Australian economy $14.9 billion annually and depression is forecast to come second only to heart disease as the leading medical cause of death and disability within 20 years (The Department of Health, 2009).

Even though there are treatments for depression, pharmacotherapy is usually costly. Medications have the potential for adverse side effects (Gartlehner et al., 2007) and a significant proportion of people fail to achieve a reduction in their depressive symptoms (Mauskopf et al., 2009). Hence, there is a need to investigate alternative treatments and prevention strategies. In recent years, there has been an increasing interest in the role of nutrition in depression (Lai et al., 2014). Micronutrients of particular interest in depression are zinc and magnesium (Jacka et al., 2012a; Szewczyk et al., 2008). Zinc is an immunomodulatory trace mineral found in abundance in the human brain where it is required to regulate numerous aspects of cellular metabolism. It has been suggested that zinc supplementation enhances antidepressant therapy by lowering depressive scores in patients (Lai et al., 2012). Zinc may produce antidepressant-like effects through modulating the functions of serotonergic and N-methyl-D-aspartate (NMDA) recep- tors and increasing levels of brain derived neurotrophic factor (BDNF) (Szewczyk et al., 2009, 2011; Takeda and Tamano, 2009). In animal models of depression, zinc induces an antidepressant-like effect that appears to be mediated through its interaction with NMDA receptors (Kroczka et al., 2001; Rosa et al., 2003; Sowa-Kućma et al., 2008) and enhances the effect of antidepressant medication (Kroczka et al., 2001). In humans, recent population-based studies have shown an association between low dietary zinc intake and depression (Jacka et al., 2012a; Maserejian et al., 2012; Roy et al., 2010) in women and men (Lehto et al., 2013).

Although animal and human studies have demonstrated a role for zinc in reducing depression, there have been no longitudinal studies looking at the link between dietary intake of zinc and depression in both men and women. To date there has been only one prospective study carried out to look at dietary zinc intake and risk of incident depression (Lehto et al., 2013); however, this was only carried out in men. Therefore, the aim of this study was to determine if dietary zinc is associated with depression using Centre for Epidemiological Studies Depression Scale (CESD) scores in both men and women from two large Australian cohorts. In addition, this study examined

the association of dietary zinc to iron ratio with depression, because minerals with similar physical or chemical properties such as iron and zinc may compete with each other biologically (Hill and Matrone, 1970) and previous studies in humans have shown that iron interferes with the absorption of zinc (Solomons, 1986).

5.1.3 Methods

5.1.3.1 The Hunter Community Study (HCS)

Data for this study was obtained from the Hunter Community Study (HCS), a cohort of community-dwelling men and women aged 55–85 years of age in Newcastle, NSW, Australia. Approval to conduct the research was granted by the University of Newcastle Human Research Ethics Committees. This study has been described in detail elsewhere (McEvoy et al., 2010). In brief, participants were randomly selected from the NSW State Electoral Roll and contacted between December 2004 and May 2007. Data collection was carried out simultaneously during this period. Participants were asked to complete a series of self-reported postal questionnaires that included socio-demographics, self-reported medical and surgical history, complementary and alternative medicines use, medication use, and Centre for Epidemiological Studies Depression Scale (CESD) after written consent was obtained. In addition to completing the postal questionnaires, participants were invited to attend the HCS data collection centre (clinic) to collect measures of blood pressure, height, weight, waist-circumference and a blood sample.

The cohort was followed-up with repeat questionnaires and clinical assessment from 2010 to 2011 to update exposure and outcome information. Out of the 3281 participants, 2918 completed the food frequency questionnaire (FFQ) at baseline and 2092 participants completed the follow-up after 5 years.

5.1.3.2 Dietary assessment for HCS

At baseline, dietary data were collected using a twice-validated 145-item self-administered semi-quantitative FFQ. The Older Australian's FFQ was originally developed for the Blue Mountains Eye Study to measure the dietary intakes of older community dwelling older adults in Australia (Smith et al., 1998) and was modelled on an early FFQ by Willett et al. (1988). The Older Australians' FFQ was originally validated against three separate 4-day food records (Smith et al., 1998), and has recently been further validated using objective folate and carotenoid biomarkers (Lai et al., manuscript to be published). The Older Australians' FFQ shows high reproducibility in the short term, with correlations for most nutrients including zinc and iron at about 0.70–0.80. It is acceptably reproducible in the longer term, with correlations of mostly 0.60–0.70, and less than 1% of the subjects grossly misclassified, indicating good validity (Smith et al., 1998). Dietary data from the Older Australians' FFQs were coded into a custom-made nutrient analysis program based on NUTTAB 2006, an Australian nutrient composition database (FSANZ, 2007). Nutrient supplement information was obtained from manufacturers and added to the database.

5.1.3.3 The Australian Longitudinal Study on Women's Health (ALSWH)

The Australian Longitudinal Study on Women's Health (ALSWH) is national cohort examining the health and wellbeing of women of different ages. This paper uses data from 1946 to 1951 cohort, who were 45–50 years at the Survey 1 (baseline) in 1996. Ethical clearance for ALSWH was obtained from the University of Newcastle and University of Queensland. Details of ALSWH's methods and recruitment have been previously published (Brown et al., 1999; Lee et al., 2005). Briefly, women were randomly selected from Medicare, the National Health Insurance Database, which includes all permanent residents of Australia. ALSWH collects self-reported data using mailed and online surveys at roughly 3-year intervals and has linked the study data with administrative records after the women gave their consent to participate in the study. The

surveys include questions about: health conditions, symptoms, and diagnoses; use of health services; health-related quality of life; social circumstances, including work and time use; demographic factors; and health behaviours.

The response rate for the 1946–1951 cohort at Survey 3 in 2001 (then aged 50–55 years) was 83% of those who had completed Survey 1 (1996) and had not died (n=115) or become too ill to complete further surveys (n =21). Complete FFQ data were available for 11,196 women aged 50–55 years (Survey 3, 2001) and 9738 of these had data available for analysis when they were 56–61 years (Survey 5 in 2007).

5.1.3.4 Dietary assessment for ALSWH

ALSWH uses the Dietary Questionnaire for Epidemiological Studies (DQES) Version 2 FFQ. Both the development of the questionnaire (Ireland et al., 1994) and its validation in young Australian women has been previously reported (Hodge et al., 2000). This questionnaire asks respondents to report their usual consumption of 74 foods and six alcoholic beverages over the preceding 12 months using a 10-point frequency scale. Additional questions are asked about the number of serves or type of fruit, vegetables, bread, dairy products, eggs, fat spreads and sugar and further details are provided in Hodge et al. (2000). Nutrient intakes were computed from NUTTAB 1995, a national government food composition database of Australian foods NUTTAB95 (Lewis et al., 1995), using software developed by the Cancer Council of Victoria. The validation of the FFQ against a 7-day weighted food record showed Pearson correlation coefficient=0.40 for dietary zinc and 0.44 for dietary iron. The FFQ validation study deemed the correlation coefficient acceptable as it is of similar magnitude to those previously reported (Hodge et al., 2000).

5.1.3.5 Ascertainment of depression in HCS and ALSWH

Centre for Epidemiological Studies Depression Scale (CESD) (Radloff, 1977) is a screening test for depression and depressive disorder. It includes 20 items comprising six scales reflecting major dimensions of depression: depressed

mood, feelings of guilt and worthlessness, feelings of helplessness and hopelessness, psychomotor retardation, loss of appetite and sleep disturbance. Response categories indicate the frequency of occurrence of each item, and are scored on a 4-point scale ranging from 0 (rarely or none of the time) to 3 (most or all of the time). Higher scores indicate more depressive symptoms and a score of 16 or higher has been used extensively as the cut-off point for high depressive symptoms (Beekman et al., 1997; Weissman et al., 1977).

CESD scores were collected at both baseline and follow-up in HCS and ALSWH. In this paper, incident cases of depression at follow-up were classified as those with CESD ≥16 or those who started on antidepressant treatment, after removing those who self-reported a diagnosis of depression at baseline and/or were on antidepressant treatment.

5.1.3.6 Measurement of non-dietary factors

Social and behavioural characteristics were based on information collected at Survey 3 in 2001 for ALSWH and at baseline for HCS. Standard questions were used to categorise respondents as never-smoker, ex-smoker, or current smoker; Body mass index (BMI) was calculated as self-reported weight in kg, divided by height in m^2. Medical history of arthritis, heart disease, stroke, hypertension, diabetes, asthma and depression, along with use of antidepressant medication prescribed by a physician (coded as either yes or no) were all self-reported in both studies. In ALSWH use of supplements (multivitamins/minerals) and the number of supplements being used were reported, while in HCS use of multivitamin and mineral supplements was categorised into whether they contained zinc (yes or no) or included zinc supplement (yes or no). Alcohol intake was quantified and classified according to Australian National Health and Medical Research (NHMRC) guidelines (NHMRC, 2009). Marital status was classified as: married or *de facto* (living with a partner); separated, divorced or widowed; and never married or single. Household income was classified as annual household income with a slight

variation in sub classification of the yearly income for the two cohorts. Education was categorised as: no formal education: school or intermediate certificate; secondary school completed, trade or technical college qualification; and University or other tertiary qualification.

5.1.3.7 Statistical analysis

Zinc and iron were adjusted for total energy intake, using the residual method (Willett, 1998). The natural logs of zinc and iron were each regressed on the natural log of energy using linear regression and the standardised residuals from the model with a constant added to it; these were used as the values of zinc and iron. The zinc to iron ratio was calculated after energy-adjustment. Alcohol was not adjusted for total energy intake.

Associations between baseline characteristics and quintiles of energy-adjusted zinc were tested using chi-square for categorical variables and analysis of variance (ANOVA) for continuous variables. Quintiles of energy-adjusted zinc were obtained using the *xtile* command in Stata.

Logistic regression was used to examine the association between dietary zinc at Survey 3 (2001) and depression at Survey 5 (2007) for ALSWH and at baseline (2004–2007) and follow-up 2010–2011) for HCS after adjusting for potential confounders. Multivariate analysis included only those variables with p value < 0.20 in the univariate analysis. Statistical significance was considered when 2-sided $p < 0.05$. STATA software version 11.0 was used for all statistical analyses.

5.1.4 Results

Table 1 shows the baseline characteristics of the HCS and ALSWH participants by quintile of energy-adjusted dietary zinc (quintile 1= lowest intake; quintile 5= highest intake). Tables 2 and 3 show the predictors of incident depression (CESD ≥ 16 at follow-up) for HCS and ALSWH, respectively.

5.1.4.1 The Hunter Community Study

After five years 270 (12.9%) new cases of depression were identified from the total of 2092 participants. The dietary zinc intakes from the lowest and highest quintiles were 7.59 mg/day (95% CI 7.47, 7.71) and 21.08 mg/day (95% CI 20.60, 21.57) respectively, and the mean intake was 13.13 mg/day. At baseline (Table 1) the participant characteristics that were different across quintiles included age, energy-adjusted iron, gender, smoking status, alcohol, education, and heart attack or stroke, and self-reported depression and anxiety. Participants in the highest quintile of dietary zinc intake were more likely to be non-smokers, consume less alcohol and have better education. Those in the highest quintile were also less likely to have a history of heart attack or stroke. Use of multivitamins and/or minerals did not differ between quintiles even when multivitamins and/or minerals containing zinc were separated from zinc supplements alone. Participants in the lowest zinc quintiles were older than those in the higher quintiles and women had higher zinc intakes than men. Energy-adjusted iron increased as zinc intake increased, and interestingly those in the highest quintile of zinc also had higher rates of self-reported depression and anxiety, though CESD scores were not different between quintiles.

In the univariate logistic regressions (Table 2) there were no associations ($p > 0.20$) between the potential predictors: age, arthritis, heart attack or stroke, use of zinc supplements or multi- vitamins and/or minerals, and zinc to iron ratio; and the outcome of depression. After adjusting for all variables that were found to be associated with risk of depression in the univariate analysis, we observed that education, household income, hypertension, BMI, and quintiles of energy-adjusted zinc were significantly associated ($p < 0.05$) with the risk of depression however, gender was not associated with depression. Interestingly, even after multivariate adjustment for these significant predictors, being in quintiles 2 (OR= 0.43, 95% C.I. 0.26–0.71), 3 (OR= 0.52, 95% C.I. 0.32–0.84) or 4 (OR= 0.46, 95% C.I. 0.28–0.75) for energy-adjusted zinc was

associated with about a 50% reduction in the odds of developing depression, whereas the highest quintile of energy-adjusted zinc remained insignificant (OR= 0.75, 95% CI 0.47, 1.19).

A sensitivity analysis was carried out, removing all those participants with less than the recommended dietary intake of zinc, to check if the observed effect could be explained by being zinc deficient. We observed that the finding was similar to that as seen above after adjustment and remained significant across quintile 2 (OR= 0.65, 95% C.I. 0.41–0.98), 3 (OR= 0.62, 95% C.I.0.39–0.96) and 4 (OR= 0.52, 95% C.I. 0.33–0.82) with around 60% reduction in the odds of developing depression while Quintile 5 was insignificant (result not shown).

Further adjustment for iron to zinc ratio in addition to all the other significant variables did not affect the findings.

Table 1
Univariate association between baseline characteristics and quintile of energy-adjusted zinc in both cohorts.

Characteristic	Sub group/or mean(Std)	Quintile of energy adjusted zinc in HCS				Quintile of energy adjusted zinc in ALSWH			
		Q1 Lowest (n=581)	Q3 Middle (n=580)	Q5 Highest (n=580)	p-Value	Q1 Lowest (n=2125)	Q3 Middle (n=2125)	Q5 Highest (n=2124)	p-Value
Age	mean (SD)	67.74 (8.11)	66.72 (7.84)	65.39 (7.21)	<0.001	52.6 (1.4)	52.6 (1.5)	52.4 (1.5)	0.028
Body mass index (Kg/m^2)	mean (SD)	28.23 (4.57)	28.78 (4.82)	29.02 (5.14)	0.092	26.3 (5.6)	26.8 (5.3)	27.5 (5.6)	<0.0001
Energy adjusted iron	mean (SD)	13.25 (0.14)	13.42 (0.17)	13.67 (0.29)	<0.001	16.37 (0.21)	16.50 (0.22)	16.63 (0.25)	<0.0001
CESD at baseline[a]	mean (SD)	8.18 (8.36)	7.41 (7.94)	8.04 (9.04)	0.066	6.7 (5.7)	5.8 (5.2)	6.0 (5.2)	<0.0001
CESD at follow-up[a]	mean (SD)	8.95 (9.59)	8.36 (8.37)	8.82 (9.17)	0.056	6.1 (5.5)	5.4 (5.2)	5.8 (5.3)	<0.0001
Change in CESD[a]	mean (SD)	−1.06 (9.14)	−1.42 (8.72)	−1.16 (8.61)	0.924	−0.47 (5.12)	−0.27 (4.98)	−0.10 (4.75)	0.251
Gender	Female	219 (37.69%)	312 (53.79%)	375 (64.66%)	<0.001	N/A	N/A	N/A	
	Male	362 (62.31%	268 (46.21%)	205 (35.34%)		N/A	N/A	N/A	
Smoking status	Never	275 (49.11%)	326 (58.42%)	323 (56.87%)	<0.026	1111 (52.55%)	1206 (56.91%)	1069 (50.59%)	<0.001
	Ex smoker	229 (40.89%)	196 (35.13%)	209 (36.80%)		685 (32. 40%)	636 (30.02%)	689 (32.61%)	
	Current smoker	56 (10%)	36 (6.45%)	36 (6.33%)		318 (15.05%)	277 (13.07%)	355 (16.80%)	
Alcohol[b]	Abstainer/rarely drinks	377 (73.63%)	425 (83.83%)	433 (84.90%)	<0.001	960 (49.84%)	762 (39.08%)	748 (37.72%)	<0.001
	Low risk/moderate drinker	46 (8.98%)	35(6.90%)	32(6.27%)		902 (46.83%)	1088 (55.79%)	1049 (52.90%)	
	Binge/risky drinker	38 (7.42%)	31 (6.11%)	26 (5.10%)		53 (2.75%)	93 (4.77%)	156 (7.87%)	
	Chronic/ High risk drinker	51 (9.96%)	16 (3.16%)	19 (3.73%)		11 (0.57%)	7 (0.36%)	30 (1.51%)	
Education	No formal qualification	20 (3.66%)	16 (3.00%)	9 (1.70%)	0.032	417 (19.80%)	300 (14.23%)	365 (17.29%)	<0.001
	School or intermediate certificate	126 (23.08%)	129 (24.20%)	103 (19.43%)		597 (28.35%)	658 (31.21%)	764 (36.19%)	
	Secondary schooling completed	153 (28.02%)	141 (26.45%)	156 (29.43%)		343 (16.29%)	372 (17.65%)	342 (16.20%)	
	Trade qualification or TAFE	146 (26.74%)	140 (26.27%)	128 (24.15%)		415 (19.71)	435 (20.64%)	397 (18.81%)	
	University or other tertiary study	101 (18.50%)	107 (20.08%)	134 (25.28%)		334 (15.86%)	343 (16.27%)	243 (11.51%)	
Annual household income	$5000-$19,999 (HCS) $1-15,999 (ALSWH)	177 (30.46%)	163 (28.11%)	159 (27.41%)	0.378	127 (9.25%)	82 (5.27%)	86 (5.66%)	<0.001
	$20,000-$39,999 (HCS) $16,000-$36,999 (ALSWH)	161 (27.71%)	173 (29.82%)	159 (27.42%)		415 (30.23%)	398 (25.56%)	428 (28.18%)	
	$40,000-$69,999 (HCS) $37,000-$77,999 (ALSWH)	106 (18.24%)	110 (18.96%)	114 (19.65%)		472 (34.38%)	589 (37.83%)	545 (35.88%)	
	$70,000 or more (HCS) $78,000 or more (ALSWH)	89 (15.32%)	84 (14.48%)	99 (17.07%)		189 (13.77%)	276 (17.73%)	253 (16.66%)	
	Don't know or missing	48 (8.26%)	50 (8.62%)	49 (8.45%)		170 (12.38%)	212 (13.62%)	2017 (13.63%)	
Marital status	Married/Living with a partner	407 (71.40%)	433 (77.18%)	424 (75.23%)	0.408	1543 (72.99%)	1746 (82.48%)	1839 (86.99%)	<0.001
	Widowed/ divorced /separated	146 (25.61%)	113 (20.14%)	134 (23.43%)		466 (22.04%)	308 (14.55%)	235 (11.12%)	
	Never married	17 (2.98%)	15 (2.67%)	14 (2.45%)		105 (4.97%)	63(2.98%)	40 (1.89%)	
Hypertension	No	299 (53.20%)	282 (50.72%)	295 (52.49%)	0.777	1750 (83%)	1740 (83%)	1695 (81%)	0.031
	Yes	263 (46.80%)	274 (49.28%)	267 (47.51)		353 (17%)	364 (17%)	404 (19%)	
Heart attack/stroke	No	510 (90.75%)	513 (92.27%)	535 (95.20%)	0.004	2085 (98%)	2082 (98%)	2076 (98%)	0.534
	Yes	52 (9.25%)	43 (7.73%)	27 (4.80%)		40 (1.9%)	43 (2.0%)	48 (2.3%)	
Diabetes	No	510 (90.75%)	495 (89.03%)	493 (87.72%)	0.054	1724 (99.60%)	1790 (99.50%)	1719 (99.42%)	0.659
	Yes	52 (9.25%)	61 (10.97%)	69 (12.28%)		7 (0.40%)	9 (0.50%)	10 (0.58%)	
Arthritis	No	422 (75.09%)	411 (73.92%)	391 (69.57%)	0.300	1666 (79%)	1614 (77%)	1621 (77%)	0.284
	Yes	140 (24.91%)	145 (26.08%)	171 (30.43%)		437 (21%)	490 (23%)	478 (23%)	
Asthma	No	495 (88.08)	478 (85.97%)	464 (82.56%)	0.085	1869 (89%)	1896 (90%)	1878 (89%)	0.212
	Yes	67 (11.92%)	78 (14.03%)	98 (17.44%)		234 (11%)	208 (9.9%)	221 (11%)	
Self-reported Depression & anxiety	No	454 (80.78%)	425 (76.16%)	413 (73.49%)	0.042	1450 (81.14%)	1567 (84.84%)	1523 (85.75%)	0.001
	Yes	108 (19.22%)	133 (23.84%)	149 (26.51%)		337 (18.86%)	280 (15.16%)	253 (14.25%)	
Depression Medication	No	462 (90.06%)	459 (89.30%)	458 (88.93%)	0.895	1578 (91.11%)	1683(93.45%)	1633(93.74%)	0.002
	Yes	51 (9.94%)	55 (10.70%)	57 (11.07%)		154 (8.89%)	118 (6.55%)	109 (6.26%)	
Use of multivitamins/minerals	No	252 (97.67%)	283 (97.92%)	382 (94.79%)	0.064	957 (45.38%)	918 (43.47%)	989 (46.78%)	0.260
	Yes	6 (2.33)	6 (2.08)	21(5.21%)		1152 (54.62%)	1194 (56.53%)	1125 (53.22%)	

[a] Centre for Epidemiological Studies Depression Scale (CESD) at baseline: – 2005–2006 for HCS and 2001 for ALSWH. CESD at follow-up: – 2010–2011 for HCS and 2007 for ALSWH. Change in CESD: – Over 5 years for HCS and over 6 years for ALSWH.

[b] Alcohol intake classification: low risk/moderate drinker ≤ 5 standard drinks/week, Binge/risky > 5 standard drink on one occasion or drinks 3–4 days/ week and high risk/chronic are those who consume > 9 standard drink/ week or drinks > 2 standard drink for > 6 days f the week.

Table 2
A range of results from logistic regression models with incident depression at follow up as the outcome for HCS.

Characteristic	Sub group or mean(SD)	Univariate		[#]Variables with *p*-value < 0.20		Non-significant variable remove		Significant variables with zinc to iron ratio	
		OR (C.I.)	*p*-value	OR (C.I.)	*p*-value	OR (C.I.)	*p*-value	OR (C.I.)	*p*-value
Smoking status	Never	Ref		Ref					
	Ex smoker	1.03 (0.77, 1.37)	0.843	0.81 (0.57, 1.16)	0.255				
	Current smoker	1.73 (1.03, 2.89)	0.036	1.24 (0.64, 2.38)	0.528				
Alcohol use	Abstainer/rarely drinks	Ref							
	Low risk drinker	0.96 (0.57, 1.63)	0.894						
	Binge/risky drinker	1.36 (0.78, 2.37)	0.276						
	Chronic/ High risk drinker	.76 (0.36, 1.62)	0.480						
Education	No formal qualification	Ref		Ref		Ref		Ref	
	School or intermediate certificate	0.35 (0.15, 0.84)	0.018	0.27 (0.09, 0.78)	0.015	0.28 (0.09, 0.77)	0.014	0.28 (0.100. 0.77)	0.014
	Secondary schooling completed	0.30 (0.13, 0.69)	0.005	0.26 (0.09, 0.74)	0.011	0.26 (0.10, 0.72)	0.009	0.26 (0.10, 0.72)	0.009
	Trade qualification or TAFE	0.21 (0.09, 0.49)	< 0.001	0.21 (0.07, 0.59)	0.003	0.20 (0.07, 0.55)	0.002	0.19 (0.07,0.55)	0.002
	University or other tertiary study	0.18 (0.77, 0.44)	< 0.001	0.22 (0.07, 0.63)	0.005	0.21 (0.07, 0.60)	0.003	0.21 (0.07, 0.60)	0.003
Household income	$5000–$19,999 per year	Ref		Ref		Ref		Ref	
	$20,000-$39,999 per year	0.97 (0.68, 1.37)	0.848	1.04 (0.68, 1.59)	0.851	1.01 (0.68, 1.51)	0.961	1.01 (0.68, 1.51)	0.948
	$40,000–$69,999 per year	0.68 (0.46, 1.01)	0.056	0.78 (0.48, 1.28)	0.330	0.77 (0.49, 1.21)	0.257	0.76 (0.49, 1.21)	0.248
	$70,000 or more per year	0.36 (0.22, 0.59)	< 0.001	0.41 (0.22, 0.75)	0.004	0.40 (0.21, 0.66)	0.001	0.37 (0.21, 0.66)	0.001
Marital status	Married/Living with a partner	Ref		Ref					
	Widowed/divorced /separated	1.39 (0.99, 1.93)	0.053	1.09 (0.71, 1.71)	0.672				
	Never married	0.86 (0.36, 2.06)	0.736	0.87 (0.34, 2.24)	0.772				
Use of Multivitamin/mineral	No	Ref		Ref					
	Yes	1.20 (0.46, 2.96)	0.695						
Heart attack or stroke	No								
	Yes	1.09 (0.67, 1.78)	0.723						
Hypertension	No								
	Yes	1.32 (1.00, 1.74)	0.045	1.17 (0.84, 1.65)	0.349				
Diabetes	No								
	Yes	1.35 (10.88, 2.08)	0.174	1.11 (0.65, 1.89)	0.709				
Arthritis	No								
	Yes	1.17 (0.85, 1.60)	0.331						
Asthma	No								
	Yes	1.38 (0.94, 2.02)	0.095	1.31 (0.82, 2.08)	0.260				
Age	Mean (stderr)	1.00 (0.99, 1.03)	0.296						
Gender	Female	Ref							
	Male	0.82 (0.63, 1.06)	0.132	0.93 (0.65, 1.34)	0.707				
Body mass index	Mean(stderr)	1.04 (1.01, 1.07)	0.003	1.04 (1.01, 1.08)	0.017	1.05 (1.02, 1.08)	0.005	1.05 (1.01, 1.08)	0.005
Zinc/iron ratio	Mean(stderr)	1.27 (0.70, 2.30)	0.424					1.23 (0.57, 2.64)	0.600
Quintile of energy adjusted Zinc	Q1 (lowest)	Ref		Ref		Ref		Ref	
	Q2	0.63 (0.41, 0.96)	0.031	0.46 (0.27, 0.76)	0.003	0.43 (0.26, 0.71)	0.001	0.43 (0.26, 0.71)	0.001
	Q3	0.66 (0.43, 1.01)	0.057	0.54 (0.33, 0.87)	0.012	0.53 (0.33, 0.85)	0.008	0.52 (0.32, 0.84)	0.007
	Q4	0.63 (0.41, 0.96)	0.033	0.47 (0.28, 0.79)	0.004	0.47 (0.29, 0.76)	0.002	0.46 (0.28, 0.75)	0.002
	Q5 (highest)	0.97 (0.65, 1.46)	0.894	0.73 (0.44, 1.19)	0.210	0.75 (0.47, 1.19)	0.222	0.70 (0.42, 1.19)	0.186

OR: odds ratio, C.I.: Confidence Interval.
#Separate analysis was performed to examine quintile of zinc to iron ratio as an independent predictor of incident depression however, the findings remained insignificant (*p*-value > 0.05) across all quintile.

Table 3
A range of results from logistic regression models with incident depression at survey 5 as the outcome for ALSWH.

Characteristic	Sub group or mean(SD)	Univariate		#Variables with p-value < 0.20		Non-significant variable remove		Significant variables with zinc to iron ratio	
		OR (CI)	p-value	OR (C.I.)	p-value	OR (C.I.)	p-value	OR (C.I.)	p-value
Smoking status	Never	Ref		Ref		Ref		Ref	
	Ex smoker	1.06 (0.95, 1.19)	0.307	1.04 (0.87, 1.23)	0.696	1.05 (0.88, 1.25)	0.581	1.05 (0.88, 1.25)	0.581
	Current Smoker	1.62 (1.40, 1.87)	< 0.001	1.48 (1.18, 1.86)	0.001	1.49 (1.19, 1.86)	0.001	1.49 (1.19, 1.86)	0.001
Alcohol use	Abstainer/rarely drinks	Ref		Ref		Ref		Ref	
	Low risk/moderate drinker	0.62 (0.56, 0.70)	< 0.001	0.75 (0.64, 0.89)	0.001	0.76 (0.64, 0.89)	0.001	0.76 (0.64, 0.89)	0.001
	Binge/risky drinker	0.98 (0.77, 1.26)	0.891	1.11 (0.77, 1.59)	0.577	1.09 (0.76, 1.56)	0.643	1.09 (0.76, 1.56)	0.643
	Chronic/High risk drinker	1.01 (0.55, 1.82)	0.984	1.24 (0.56, 2.76)	0.602	1.19 (0.54, 2.65)	0.667	1.19 (0.54, 2.65)	0.667
Education	No formal qualification	Ref		Ref		Ref		Ref	
	School or intermediate certificate	0.58 (0.51. 0.67)	< 0.001	0.78 (0.62, 0.97)	0.029	0.76 (0.60, 0.95)	0.015	0.76 (0.60, 0.95)	0.015
	Secondary schooling completed	0.59 (0.50, 0.70)	< 0.001	0.78(0.60, 1.02)	0.068	0.76 (0.59, 0.98)	0.037	0.76 (0.59, 0.98)	0.037
	Trade qualification or TAFE	0.46 (0.36, 0.54)	< 0.001	0.70 (0.54, 0.91)	0.008	0.70 (0.54, 0.90)	0.006	0.70 (0.54, 0.90)	0.006
	University or other tertiary study	0.39 (0.32, 0.47)	< 0.001	0.60 (0.44, 0.82)	0.001	0.60 (0.45, 0.81)	0.001	0.60 (0.45, 0.81)	0.001
Household income	$1–15,999 per year	Ref		Ref		Ref		Ref	
	$16,000–$36,999 per year	0.71 (0.55, 0.91)	0.007	0.81 (0.60,1.10)	0.174	0.81 (0.60,1.10)	0.180	0.81 (0.60,1.10)	0.180
	$37,000–$77,999 per year	0.53 (0.42, 0.69)	< 0.001	0.69 (0.51, 0.94)	0.019	0.70 (0.52, 0.95)	0.022	0.70 (0.52, 0.95)	0.022
	$78,000 or more per year	0.46 (0.34, 0.60)	< 0.001	0.75 (0.52, 1.05)	0.097	0.73 (0.52, 1.03)	0.076	0.73 (0.52, 1.03)	0.076
Marital status	Married/Living with a partner	Ref		Ref					
	Widowed/divorced /separated	1.31 (1.14, 1.51)	< 0.001	1.17 (0.84, 1.64)	0.345				
	Never married	1.04 (0.77, 1.41)	0.785	1.01 (0.47, 2.19)	0.972				
Heart attack or stroke	No								
	Yes	1.41 (1.01, 1.97)	0.041	1.14 (0.68, 1.90)	0.618				
Hypertension	No								
	Yes	1.39 (1.22, 1.58)	< 0.001	1.30 (1.06, 1.58)	0.010	1.32 (1.08, 1.60)	0.006	1.32 (1.08, 1.60)	0.006
Diabetes	No								
	Yes	0.65 (0.17, 2.88)	0.613						
Arthritis	No								
	Yes	1.39 (1.23, 1.57)	< 0.001	1.34 (1.12, 1.61)	0.001	1.33 (1.11, 1.58)	0.002	1.33 (1.11, 1.58)	0.002
Asthma	No								
	Yes	1.38 (1.17, 1.63)	< 0.001	1.20 (0.94, 1.53)	0.148				
Use of multivitamin/mineral	No								
	Yes	0.81 (0.73, 0.90)	< 0.001	0.89 (0.76, 1.04)	0.129				
Age	Mean(stderr)	1.03 (0.99, 1.07)	0.114	0.99 (0.94, 1.04)	0.587				
Body mass index	Mean(stderr)	1.03 (1.02, 1.04)	< 0.001	1.02 (1.01, 1.03)	0.024	1.02 (1.01, 1.03)	0.011	1.02 (1.01, 1.03)	0.011
Zinc/iron ratio	Mean(stderr)	0.99 (0.99, 1.00)	0.952					0.99 (0.99, 1.00)	0.943
Quintile of energy adjusted Zinc	Q1 (lowest)	Ref		Ref		Ref		Ref	
	Q2	0.70 (0.60, 0.83)	< 0.001	0.73 (0.57, 0.93)	0.011	0.74 (0.59, 0.95)	0.016	0.74 (0.59, 0.95)	0.016
	Q3	0.69 (0.59, 0.82)	< 0.001	0.72 (0.56, 0.92)	0.008	0.74 (0.58, 0.94)	0.013	0.74 (0.58, 0.94)	0.013
	Q4	0.60 (0.51, 0.71)	< 0.001	0.64 (0.50, 0.82)	< 0.001	0.66 (0.51, 0.84)	0.001	0.66 (0.51, 0.84)	0.001
	Q5 (highest)	0.79 (0.68, 0.93)	0.005	0.70 (0.55, 0.90)	0.004	0.70 (0.55,0.89)	0.004	0.70 (0.55,0.89)	0.004

OR: odds ratio, C.I.: Confidence Interval.
#Separate analysis was performed to examine quintile of zinc to iron ratio as an independent predictor of incident depression however, the findings remained insignificant (p-value > 0.05) across all quintile.

5.1.4.2 The Australian longitudinal study on women's health

At the end of the 6 years follow-up 1830 (18.8%) new cases of depression were identified out of 9738 participants. The dietary zinc intakes of the lowest and highest quintiles were 5.94 mg/day (95% CI 5.90, 5.99) and 17.35 mg/day (95% CI 17.12, 17.59) respectively, and the mean intake was 10.66 mg/day. At baseline (Table 1) the participant characteristics found to be significantly different across quintiles included age, BMI, smoking status, alcohol intake, education, household income, marital status, self-reported hyper- tension, self-reported depression and anxiety (including CESD), and depression medication. Energy-adjusted dietary iron was also found to be significantly different across quintiles and followed a similar trend to that seen in HCS. Those in the highest quintile of zinc intake were more likely to be smokers, low-risk drinkers, and have a history of hypertension. Women in the higher quintiles were found to have a higher income, education and were more likely to be married. Those in the highest quintile were also less likely to be on depression medication or have self-reported depression or anxiety. Age was similar across all the quintiles and those in the lowest quintile had the highest CESD at both surveys.

Logistic regression results for ALSWH are presented in Table 3. After adjusting for all the variables that were significant in the univariate analysis, characteristics that were statistically significantly associated with the risk of depression were smoking status, alcohol use, education, household income, hypertension, arthritis, BMI and quintiles of energy-adjusted zinc.

In the adjusted analysis a significant and consistent reduction of about a 30% in the odds of developing depression was seen across quintiles 2–5 of zinc, compared to quintile 1. Sensitivity analysis with the zinc deficient participants removed showed a similar significant result with quintile 2 (OR= 0.77, 95% C.I. 0.60–0.97), 3 (OR= 0.76, 95%C.I. 0.60–0.97) and 4 (OR= 0.71, 95% C.I. 0.55–0.89) with

around 80% reduction in the odds of developing depression in Quintile 5 being insignificant (result not shown).

Further adjustment for zinc to iron ratio did not change the outcome.

5.1.5 Discussion

Findings from both cohorts showed dietary zinc intake was associated with a lower incidence of depression in men and women 50 years and older, even after adjusting for potential confounders. However, it was not a dose response relationship. For example, the men and women in quintile 2 who still had relatively low zinc intakes had roughly the same reduction in risk of depression as those in the higher intake quintiles (quintile 4 for HCS and 5 for ALSWH). We also observed no association between zinc to iron ratio and risk of depression in either of the studies. To the best of our knowledge this is the only longitudinal study that has looked at the relationship between zinc, and zinc to iron ratio, and depression in an adult population of both men and women and also in women alone.

The association between dietary zinc and depression raises the possibility that an increase in dietary zinc may reduce the risk of depression. This finding is consistent with findings from previous population based study in both genders and in females alone (Amani et al., 2010; Marcellini et al., 2006; Yary and Aazami, 2012). However these studies are limited by a cross-sectional design and do not show the direction of the relationship. The only other prospective study that looked at dietary intake and depression was carried out only in middle-aged men. The finding from this study is in contrast to our findings as the study found no association between energy-adjusted zinc intake and depression (Lehto et al., 2013). This study may have lacked power as the endpoint was defined as hospital discharge diagnosis of depression, and may have missed cases of depression not requiring hospitalisation.

There was no clear dose-response for zinc intake by quintile and the reduced odds of depression. It appears that only those in the lowest quintile of zinc are

at increased risk of developing depression; the remaining quintiles have largely equivalent reduced risks. This could be due to the fact that both cohorts are resident in Australia, a largely affluent Western society, with reasonably good nutrition and very little zinc deficiency. Lehto et al. (2013) in their study also stated that dietary zinc intake might not have relevance for depression prevention in middle-aged men with sufficient dietary zinc intake and that the connection may be more relevant in populations already suffering from depression (Lehto et al., 2013). We speculate that perhaps only those in the lowest quintile of zinc are below the threshold where zinc effects on depression can be seen; those in other quintiles are zinc replete and a ceiling effect may account for the similar risk across quintiles 2–4 for HCS and 2–5 for the ALSWH. The finding is not consistent at the highest quintiles (5) of zinc intake and this could be due a number of factors, such as gender and age group differences. ALSWH had a much larger sample size and hence greater power. The HCS also comprises people from the Hunter region alone whereas ALSWH samples from women across Australia. However, sensitivity analyses after removing those with zinc deficiency from both cohorts found very similar results so we cannot conclude that this association is solely driven by an increased risk of depression in those who are zinc deficient.

Analysis was carried out separately using both continuous and quintile of zinc to iron ratio as an independent predictor of incident depression but the findings remained non-significant in both cases. Dietary zinc to iron ratio was considered as part of the analysis in this study as iron is known to interact with the absorption of zinc (Whittaker, 1998). Whittaker also noted that when iron and zinc are given in a meal this interaction is not observed and Lonnerdal (1989) suggested that when the amount of iron to zinc ratio is equal 1:1, it may exclude the possibility of an interaction between iron and zinc (Lonnerdal, 1989; Whittaker, 1998); interestingly it was observed that those in the highest quintile of iron also had the highest quintile of zinc in both HCS and ALSWH. This could be due to the fact that there are many shared sources for iron and zinc

and the most commonly recorded dietary source of zinc was meat, fish and poultry as the major contributors, though cereals and dairy products were also a substantial source. A recent study in Australia on red meat intake and common mental disorders also showed a clear non-linear association between red meat intake and depressive or anxiety disorders (Jacka et al., 2012b). The study showed that participants consuming less than the recommended intake of red meat were more likely to have a diagnosed depressive or anxiety disorder and were also more inclined to have psychological symptoms. Jacka et al. (2012a,b) also observed that those consuming more than the recommended amount of red meat were more likely to have a depressive disorder once overall diet quality was taken into account.

We cannot be sure whether the zinc intake from these two cohorts is directly lowering the incidence of depression, as zinc intake tends to increase as other micronutrient intakes increase (for example, iron in this study), or diet quality improves more generally. In a recent meta-analysis, Lai et al. (2014) showed a healthy dietary pattern is associated with a 16% reduced odds of depression, which might account for some of the association we have seen. However, our effect size was much larger: a ~30% reduction in the ALSWH and ~50% reduction in depression in HCS. Furthermore, the experimental animal data and other observational human studies lend support to the biological plausibility that zinc plays a crucial role in the incidence of depression.

The main strengths of this study are the prospective design, where dietary assessment preceded the development of depression, and the generalizability, this being a population-based cohort of men and women rather than a clinic sample. The main advantage of the prospective design is that it reduces selection bias and potential recall bias. It is also the only prospective study so far that looks at both genders and also in women alone. The large sample size also means that it is possible to obtain reasonably stable parameter estimates and similar findings in

both studies confirm the consistency of the association between dietary zinc intake and risk of depression.

Despite the good generalizability of this study there are some limitations. Use of FFQ to collect dietary information (especially micronutrients) has limitations due to the lack of homogeneity in food composition tables (Liu et al., 2013) and over or under reporting of certain foods/food groups. However, the use of FFQs to collect dietary information in large population-based samples is the most cost-effective and feasible method available. In this study the use of an FFQ to estimate dietary zinc intake will have underestimated the amount of zinc consumed by study participants and biased the effect size towards the null.

A further limitation is that dietary assessment was carried out at one time only. However, the dietary habits of the participants are unlikely to have changed significantly over the 5–6 year period, as they are mid-aged and dietary patterns are likely to remain stable. Instead of excluding participants who developed comorbidities during the follow-up period we controlled for these in the analysis. This was done to account for any change in dietary habit that may have occurred following the development of comorbid conditions. Although adjustments were made for all known potential confounders, residual confounding cannot be entirely excluded. It may be that the lowest quintile of zinc is simply a marker of poor diet and other health behaviours.

This study also considered the use of supplementary zinc multivitamins/supplements containing zinc as an additional source and though the quantity of zinc in the supplements was not known, analysis showed that the proportion of people taking supplements were not significantly different between quintiles of zinc intake. Additionally in HCS we also looked at the use of multivitamins/supplements containing zinc and saw that those in the highest quintile of zinc also were more likely to take multi- vitamins/supplements containing zinc however, in the regression

analysis it was found to be insignificant. Although multiple dietary assessments are useful to reduce random measurement error, given that single dietary assessment is likely to bias results toward the null, the fact that we still observed a significant association is an indication that the association between dietary zinc is likely to be robust and even stronger than that observed in this study.

5.1.6 Conclusion

In conclusion, the results from the two independent cohort studies show that lower total dietary zinc intake is associated with a higher incidence of depression in both men and women. This finding is further reinforced by the sensitivity analysis, which showed that the association persists despite excluding those with zinc deficiency. The relationship between zinc to iron ratio with regards to depression needs to be explored further and future research should examine this association between dietary zinc and depression in both men and women across different age groups.

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The funding source had no role in the concept formation, study design and writing of the study manuscript.

The corresponding author declares that I had full access to all the data in the study and have the final responsibility to make the decision to submit for publication

Conflict of interest

All authors declare that they have no conflict of interest.

The corresponding author declares that I had full access to all the data in the study and have the final responsibility to make the decision to submit for publication.

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Chapter 6 Zinc and cardiovascular diseases

6.1 Prospective Study of Dietary Zinc Intake and Risk of Cardiovascular Disease in Women

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6.1.1 Abstract

6.1.1.1 Background and Aims: Several animal and human studies have shown that zinc is associated with cellular damage and cardiac dysfunction. The aim of this study was to investigate dietary zinc, and the zinc to iron ratio, as predictors of incident cardiovascular disease (CVD) in a large longitudinal study of mid-age Australian women.

6.1.1.2 Methods and Results: Data were self-reported, as part of the Australian Longitudinal Study on Women's Health (women aged 50-61 years). Validated food frequency questionnaires were used to assess dietary intake. Energy-adjusted zinc was ranked using quintiles and predictors of incident CVD were examined using stepwise logistic regression. After 6 years of follow-up 320 incident CVD cases were established**.** A positive association between dietary zinc intake, zinc to iron ratio and risk of cardiovascular disease was observed even after adjusting for potential dietary and non-dietary confounders. Compared to those with the lowest quintile those in the highest quintile of zinc (OR=1.67, 95% C.I=1.08-2.62) and zinc to iron ratio (OR=1.72, 95% C.I =1.05 to 2.81) had almost twice the odds of developing CVD (*P* trend=0.007).

6.1.1.3 Conclusions: High dietary zinc intake and zinc to iron ratio is associated with a greater incidence of CVD in women, as shown in this large prospective cohorts. Further studies into the precise role of zinc and iron and its sources from the diet compared to other important nutrients are needed.

Keywords: Diet, Cohort, Australia, Zinc, Women, Cardiovascular disease (CVD).

6.1.2 Introduction

The prevalence of chronic disease has been increasing in the past decades. Cardiovascular diseases (CVDs) are the number one cause of death globally; more people die annually from CVDs than from any other cause [1]. CVD has a strong genetic basis and it is modified by environmental factors. To date, the most accessible and easy way to improve CVD will be modification of lifestyle factors, mostly diet and exercise. A review in 1996 by Houtman about trace elements and CVD obtained from epidemiological, biochemical and cell biological studies concluded that zinc has the potential to counteract the development of cardiovascular disease [2], but the strength of this effect on public health is difficult to measure. Recently, there has been increasing interest in the relation of dietary intake, especially micronutrients present in the diet and risk of CVDs[3-5]. Zinc is one of those micronutients present in our diet whose deficiency may play an important role in the appearance of diseases with three major biological roles, as catalyst, structural, and regulatory ion [4]. Zinc has been reported to have antioxidant and antiatherosclerotic effects[4-6]. Zinc deficiency leads to cellular damage and atherosclerosis [5] and is known to cause sensitivity to oxidative damage, leading to an increased release of interleukin 1, tumor necrosis factor-α and to cause an increase in endothelial cell apoptosis[6].

Experimental studies have shown that zinc administration during reperfusion improves myocardial recovery to almost 100%, protects against cardiac mechanical and/or electrical dysfunction, decrease the incidence of arrhythmias and improved post-ischemic myocardial recovery[7]. Another recent experimental study also showed that decreased level of zinc in the diet could induce hypertension and cardiac dysfunction [8]. In Humans, a high prevalence of inadequate intake of zinc was observed in patients with heart disease[9] and trace element analysis of hair showed that CVD patients had

lower concentrations of zinc [10]. Similarly, decreased serum zinc level was also observed in ischemic stroke patients compared to normal patients[11].

Trials have explored the contribution of zinc supplementation in different conditions. In stroke patients, zinc supplementation has been shown to decrease the risk of mortality (Lixian trial) [12] and enhance neurological recovery [13]. However, according to a systematic review on the effect of zinc supplements in humans, besides the observed adverse effect on plasma high-density lipoprotein cholesterol (HDL-C) concentrations, the effect of zinc supplementation on heart disease risk remains unclear[14]. Apart from the inconsistency in trial findings, no prospective study has been carried out to look specifically at dietary zinc intake and risk of incident CVD in women to the best of our knowledge. Therefore, the aim of this study was to investigate if dietary zinc is associated with incident cardiovascular disease in a large cohort of Australian women. Additionally, this study also examined the association of dietary zinc to iron ratio with CVD, because minerals with similar physical or chemical properties such as iron and zinc may compete with each other biologically[15] and previous studies in humans have shown that iron interfere with the absorption of zinc[16].

6.1.3 Methods

6.1.3.1 The Australian Longitudinal Study on Women's Health (ALSWH)

The Australian Longitudinal Study on Women's Health (ALSWH) is a national cohort examining the health and wellbeing of women of different ages. This paper uses data from the 1946-1951 cohort, who were 45-50 years at the Survey 1 in 1996. Ethical clearance for ALSWH was obtained from the University of Newcastle and University of Queensland. Details of ALSWH's methods and recruitment have been previously published [***17***, ***18***]. Briefly, women were randomly selected from Medicare, the National Health Insurance Database, which includes all permanent residents of Australia. ALSWH collects self-reported data using mailed and online surveys at roughly 3-year intervals and has linked the study data with administrative records after the women gave their consent to participate in the study. The surveys include questions about: health conditions, symptoms, and diagnoses; use of health services;

health-related quality of life; social circumstances, including work and time use; demographic factors; and health behaviors.

The response rate for the 1946-1951 cohort at Survey 3 in 2001 (then aged 50–55 years) was 83% of those who had completed Survey 1 (1996) and had not died (n=115) or become too ill to complete further surveys (n=21). Food frequency questionnaire (FFQ) was distributed at survey 3 and complete FFQ data were available for 11,196 women aged 50–55 years (Survey 3, 2001) and 9264 of these women had data available for analysis when they were 56-61 years (Survey 5 in 2007).

6.1.3.2 Dietary Assessment

ALSWH uses the Dietary Questionnaire for Epidemiological Studies (DQES) Version 2 FFQ. Both the development of the questionnaire [19] and its validation in young Australian women has been previously reported [20]. This questionnaire asks respondents to report their usual consumption of 74 foods and six alcoholic beverages over the preceding 12 months using a 10-point frequency scale. Additional questions are asked about the number of serves or type of fruit, vegetables, bread, dairy products, eggs, fat spreads and sugar and further details are provided in Hodge et al. [20]. Nutrient intakes were computed from NUTTAB 1995, a national government food composition database of Australian foods NUTTAB95 [21], using software developed by the Cancer Council of Victoria. The validation of the FFQ against a 7-day weighted food record showed Pearson correlation coefficient = 0.40 for dietary zinc and 0.44 for dietary iron. The FFQ validation study deemed the correlation coefficient acceptable as it is of similar magnitude to those previously reported [20].

6.1.3.3 Ascertainment of cardiovascular disease

History of CVD was collected at both baseline and follow-up. Participants were classified as having CVD if they had been diagnosed/treated by a doctor for heart attack and/or stoke which was self-reported. In this paper, incident cases of CVD at follow-up were classified as those with a diagnosis/treatment of heart attack and/or stroke, after excluding the prevalent cases: who self–reported a diagnosis/treatment of the condition at Survey 1, 2, or Survey 3.

6.1.3.4 Measurement of non-dietary factors:

Social and behavioral characteristics were based on information collected at Survey 3 in 2001. Participants were asked to report frequency of engaging in vigorous (e.g., aerobics, jogging) and less vigorous (e.g., walking and swimming) exercise lasting for <20 min in a normal week. Responses were scored using approximate weekly frequencies of exercise. The resulting physical activity scores ranged from 0 to 80 and were categorized as "nil/sedentary (<5)," "low (5 to 15)," "moderate (16 to 25)," or "high (>25)." A score of 15 is commensurate with the current recommendation of moderate intensity activity on most days of the week. This measure is described in more detail elsewhere [22] and has previously been shown to have acceptable test-retest reliability [23]. Standard questions were used to categorize respondents as never-smoker, ex-smoker, or current smoker; Body mass index (BMI) was calculated as self-reported weight in kg, divided by height in m^2. Medical history of hypertension and diabetes as diagnosed by a physician along with use of hormone replacement therapy (HRT) (coded as either yes or no) were all self-reported. The participants were also asked to report the number of supplements being used and categorized as taking multivitamin & mineral supplements (yes or no). Alcohol intake was quantified and classified according to Australian National Health & Medical Research (NHMRC) guidelines [24]. Annual household income and level of education was sub-classified into different categories.

6.1.3.5 Statistical analysis

Zinc and iron were adjusted for total energy intake, using the residual method (Willett 1998). The natural log of zinc and iron were each regressed on the natural log of energy using linear regression and extracting the standardized residuals. All micronutrients were energy adjusted in the same manner. The macronutrient variables are adjusted for total energy intake by calculating their component of total energy (as a %). Alcohol was not adjusted for total energy intake.

Associations between baseline characteristics and quintiles of energy-adjusted zinc were tested using chi-square for categorical variables and analysis of variance (ANOVA) for continuous variables. Quintiles of energy-adjusted zinc were obtained using the *xtile*

command in Stata. Predictors of 6-year incidence of CVD were examined using forward stepwise multivariable logistic regression in stepped approach, with the main predictor being energy adjusted zinc and zinc/iron ratio measured at S3 used to predict incidence of CVD by S5.

The multivariable analysis controlled for dietary factors (energy adjusted fiber, fat and iron), non-dietary factors (BMI, smoking status, education, marital status, HRT, exercise group, history (yes/no) of hypertension and diabetes) along with alcohol intake and use of multivitamins/minerals. P values for trends were conducted by treating quintile of energy-adjusted zinc as a continuous variable. Statistical significance was considered when 2 sided $p<0.05$. STATA software version 11 was used for all statistical analyses.

6.1.4 Results

Table 1 shows the baseline characteristics at survey 3 of the participants by quintile of energy-adjusted dietary zinc (quintile 1 = lowest intake; quintile 5 = highest intake). At baseline (Table 1), the participant characteristics found to be significantly different across quintiles included age, smoking status, alcohol intake, exercise, education, household income, BMI, HRT and hypertension. Besides total energy intake, energy-adjusted dietary factors found both macronutrients and micronutrient including iron to be significantly different across quintiles. Those in the highest quintile of zinc intake were more likely to be current smokers, low-risk drinkers, and have a history of hypertension. Interestingly those with higher intake of zinc had higher BMI though women in the lower quintiles were found to have a more sedentary life with little or no exercise. Tables 2 and 3 shows the output of the stepwise approach that examined energy adjusted zinc and zinc to iron ratio as an independent predictors of incident CVD respectively.

At the end of the 6 years follow-up 320 (3.6%) incident cases of CVD were identified out of 9264 participants. The dietary zinc intakes of the lowest and highest quintiles were 5.94 mg/day (95% CI 5.90, 5.99) and 17.35 mg/day (95% CI 17.12, 17.59) respectively, and the mean intake was 10.66 mg/day. At baseline (Table 1) the participant characteristics found to be significantly different across quintiles included age, BMI, HRT, smoking status, alcohol

intake, exercise, education, household income, marital status and self-reported hypertension. Energy-adjusted dietary factors found to be significantly different across quintiles includes carbohydrates, total protein, total fat (including cholesterol and saturated, polyunsaturated, monounsaturated fat), dietary fiber, minerals (iron, calcium, magnesium, sodium and potassium) and vitamins, which includes retinol, vitamin C and E.

Those in the highest quintile of zinc intake were more likely to be a smoker, a low-risk drinker and have a history of hypertension. Women in the higher quintiles were found to have a higher income, education and interestingly also had higher BMI and the highest quintile of zinc intake also had the highest intake of dietary iron. Women in the lower quintiles were found to have a more sedentary life with nil or little exercise. Age was similar across all the quintiles.

In an age adjusted analysis there was no significant association across quintiles 2,3 and 4 but quintile 5 showed a significant association (OR=1.43, 95% C.I 1.01, 2.04) between energy-adjusted zinc and risk of CVD (Table 2). After adjustment for non-dietary factors including age, the findings did not change and quintile 5 remained statistically significant with p=0.029 (0R=144, 95% C.I 1.01, 2.10). Further adjustment for dietary factors lead to an overall increase in odds of developing CVD and strengthened the association (p=0.011). Additional adjustment for alcohol intake and use of supplements (multivitamins/minerals) also showed a statistically significant increase in the odds of developing CVD across quintiles (P_{trend}=0.007). Compared with the lowest quintile of energy-adjusted zinc those in the highest quintile had almost twice the odds of developing CVD (OR=1.67, 95% C.I. 1.08 – 0.77).

The association between the energy-adjusted zinc/iron ratio and odds of developing CVD (Table 3) was significant (p=0.002) after adjusting for age with a consistent increase in the odds of developing CVD across the quintiles. A borderline statistically significant association was observed (P_{trend}= 0.057) after adjusting for age and non-dietary factors. A statistically significant increase in the odds of developing CVD across quintiles of energy-adjusted zinc to iron ratio was observed with further adjustment for energy-adjusted dietary factors (p=0.015) and alcohol intake and use of supplements (p=0.007). Those in quintile 4 (OR=1.78, 95% C.I 1.13-2.79) and 5 (OR=1.72, 95% C.I 1.05-

2.81) had almost twice the odds of developing CVD compared with the lowest quintile of energy-adjusted zinc to iron ratio.

Table 1: Characteristics of subjects at baseline (survey 3) by quintile of energy-adjusted zinc

		Quintile of Energy-adjusted Zinc			
Characteristic	**Sub group /or mean(Std)**	**Q1 Lowest (n=2125)**	**Q3 Middle (n=2125)**	**Q5 Highest (n=2124)**	**p-value**
Smoking status	Never smoker	948 (53%)	1033 (58%)	922 (52%)	<0.001
	Former smoker	568 (32%)	531 (30%)	569 (32%)	
	Current smoker	261 (15%)	215 (12%)	287 (16%)	
Alcohol Intake	Abstainer/rarely drinks	960 (49.84%)	762 (39.08%)	748 (37.72%)	<0.001
	Low risk/moderate drinker	902 (46.83%)	1088 (55.79%)	1049 (52.90%)	
	Binge/risky drinker	53 (2.75%)	93 (4.77%)	156 (7.87%)	
	Chronic/ High risk drinker	11 (0.57%)	7 (0.36%)	30 (1.51%)	
Exercise group	Nil/sedentary	321 (19%)	271 (16%)	323 (19%)	0.003
	Low	621 (37%)	683 (40%)	622 (36%)	
	Moderate	318 (19%)	355 (21%)	338 (20%)	
	High	437 (26%)	408 (24%)	433 (25%)	
Education	No formal qualification	417 (19.80%)	300 (14.23%)	365 (17.29%)	<0.001
	School/intermediate certificate	597 (28.35%)	658 (31.21%)	764 (36.19%)	
	Secondary schooling completed	343 (16.29%)	372 (17.65%)	342 (16.20%)	
	Trade qualification/TAFE	415 (19.71)	435 (20.64%)	397 (18.81%)	
	University/other tertiary study	334 (15.86%)	343 (16.27%)	243 (11.51%)	
Annual household income	$1-15,999	127 (9.25%)	82 (5.27%)	86 (5.66%)	<0.001
	$16,000-$36,999	415 (30.23%)	398 (25.56%)	428 (28.18%)	
	$37,000-$77,999	472 (34.38%)	589 (37.83%)	545 (35.88%)	
	$78,000 or more	189 (13.77%)	276 (17.73%)	253 (16.66%)	
	Don't know or missing	170 (12.38%)	212 (13.62%)	2017 (13.63%)	
Hormone Replacement Therapy	No	1250 (70%)	1193 (67%)	1160 (65%)	0.020
	Yes	535 (30%)	591 (33%)	624 (35%)	
Hypertension	No	1498 (85%)	1495 (85%)	1449 (82%)	0.038

		Quintile of Energy-adjusted Zinc			
Characteristic	**Sub group /or mean(Std)**	**Q1 Lowest (n=2125)**	**Q3 Middle (n=2125)**	**Q5 Highest (n=2124)**	**p-value**
	Yes	271 (15%)	274 (15%)	316 (18%)	
Diabetes	No	1724 (99.60%)	1790 (99.50%)	1719 (99.42%)	0.659
	Yes	7 (0.40%)	9 (0.50%)	10 (0.58%)	
Use of multivitamins/minerals	No	957 (45.38%)	918 (43.47%)	989 (46.78%)	0.260
	Yes	1152 (54.62%)	1194 (56.53%)	1125 (53.22%)	
Age	mean (SD)	52.6 (1.4)	52.6 (1.5)	52.4 (1.5)	0.004
Body Mass Index (kg/m^2)	mean (SD)	26.0 (5.4)	26.5 (5.1)	27.2 (5.4)	<0.001
Total energy intake (KJ)	mean (SD)	6676 (2415)	6604 (2275)	6687 (2790)	0.508
Carbohydrates (% of energy)	mean (SD)	48.2 (5.8)	45.6 (5.8)	41.0 (7.5)	<0.001
Dietary fibre (% of energy)	mean (SD)	2.4 (0.7)	2.5 (0.6)	2.5 (0.8)	<0.001
Total protein (% of energy)	mean (SD)	17.1 (2.1)	20.8 (1.7)	25.1 (2.8)	<0.001
Total fat (% of energy)	mean (SD)	35.5 (5.5)	34.3 (5.9)	34.5 (6.6)	<0.001
Saturated fat (energy adjusted)	mean (SD)	14.0 (3.8)	13.5 (3.4)	13.9 (3.3)	<0.001
Polyunsaturated fat (% of energy)	mean (SD)	6.3 (2.3)	5.5 (1.9)	4.7 (1.5)	<0.001
Monounsaturated fat (% of energy)	mean (SD)	12.1 (2.2)	12.1 (2.4)	12.6 (2.8)	<0.001
Iron (energy adjusted)	mean (SD)	-0.605 (0.984)	0.022 (0.905)	0.543 (0.897)	<0.001
Cholesterol (energy adjusted)	mean (SD)	-0.584 (1.170)	-0.043 (0.827)	0.607 (0.855)	<0.001
Retinol (energy adjusted)	mean (SD)	0.451 (0.947)	0.030 (0.932)	-0.525 (0.993)	<0.001
Vitamin C (energy adjusted)	mean (SD)	-0.092 (1.198)	0.024 (0.957)	0.028 (0.895)	0.001
Vitamin E (energy adjusted)	mean (SD)	0.346 (1.094)	0.035 (0.923)	-0.407 (0.939)	<0.001
Calcium (energy adjusted)	mean (SD)	-0.374 (0.901)	0.126 (0.940)	0.089 (1.166)	<0.001
Magnesium (energy adjusted)	mean (SD)	-0.453 (1.001)	0.064 (0.901)	0.268 (1.069)	<0.001
Sodium (energy adjusted)	mean (SD)	-0.432 (1.030)	0.026 (0.930)	0.345 (0.999)	<0.001
Potassium (energy adjusted)	mean (SD)	-0.594 (1.072)	0.067 (0.891)	0.454 (0.915)	<0.001

Table 2: Association between energy-adjusted zinc and incidence of cardiovascular disease at 6 year follow up

	Quintile of Energy-adjusted Zinc					
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**P**
Energy-adjusted zinc [median (min, max)]	-1.25 (-4.80, -0.79)	-0.48 (-0.79, -0.23)	0.01 (-0.23, 0.26)	0.50 (0.26, 0.79)	1.24 (0.79, 4.45)	
Number of cardiovascular disease	54	55	62	72	77	
Odds ratio						
• Age adjusted	1.00	0.99 (0.68 to 1.46)	1.10 (0.76 to 1.60)	1.28 (0.90 to 1.84)	1.43 (1.01 to 2.04)	0.015
• Age & non-dietary† factors adjusted	1.00	0.92 (0.63 to 1.48)	1.19 (0.84 to 1.88)	1.16 (0.82 to 1.83)	1.44 (1.01 to 2.10)	0.029 0.011
• Age, non-dietary† and dietary‡ factors adjusted	1.00	0.96 (0.62 to 1.47)	1.26 (0.84 to 1.90)	1.25 (0.82 to 1.90)	1.63 (1.07 to 1.90)	0.007
• Age, non-dietary† and dietary‡ factors adjusted plus alcohol intake and use of supplements	1.00	0.94 (0.60 to 1.48)	1.18 (0.76 to 1.82)	1.36 (0.84 to 2.00)	1.67 (1.08 to 2.62)	

† Non-dietary factors were BMI; smoking status; HRT; exercise group; education level and history of diabetes and hypertension.
‡ Dietary factors were energy-adjusted fiber, iron and fat
Adjustment for family income in the models resulted in a loss of 1300 observations but education was adjusted for.

Table 3: Association between dietary zinc/iron ratio and incidence of cardiovascular disease at 6 year follow up

	Quintile of Zinc to Iron Ratio					
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**P**
Zinc/Iron ratio [median(min, max)]	0.69 (0.28, 0.77)	0.84 (0.77, 0.90)	0.95 (0.90, 1.00)	1.06 (1.00, 1.12)	1.21 (1.12, 1.75)	
Number of cardiovascular disease	51	52	69	74	74	
Odds ratio						
• Age adjusted	1.00	1.02 (0.69 to 1.51)	1.38 (0.96 to 1.99)	1.51 (1.05 to 2.17)	1.55 (1.08 to 2.23)	0.002
• Age & non-dietary† factors adjusted	1.00	0.95 (0.61 to 1.47)	1.38 (0.92 to 2.06)	1.47 (0.99 to 2.09)	1.40 (0.93 to 2.09)	0.057
• Age, non-dietary† and dietary‡ factors adjusted	1.00	1.03 (0.66 to 1.59)	1.45 (0.95 to 2.20)	1.57 (1.02 to 2.42)	1.54 (0.97 to 2.45)	0.015
• Age, non-dietary† and dietary‡ factors adjusted plus alcohol intake and use of supplements	1.00	1.15 (0.73 to 1.83)	1.52 (0.97 to 2.36)	1.78 (1.13 to 2.79)	1.72 (1.05 to 2.81)	0.007

† Non-dietary factors were BMI; smoking status; HRT; exercise group; education level and history of diabetes and hypertension.
‡ Dietary factors were energy-adjusted fiber and fat.

6.1.5 Discussion

Findings from this longitudinal study showed that dietary zinc intake was associated with a higher incidence of CVD in women aged 50 years and older, even after adjusting for potential dietary and non-dietary confounders. The same association was also observed between zinc to iron ratio and risk of CVD in the study. To our knowledge this is the only longitudinal study that has looked at the relationship between dietary zinc, and zinc to iron ratio, and CVD in an adult women population.

The association between dietary zinc and CVD suggests that high dietary zinc increases the risk of CVD. The only other prospective study that looked at dietary intake of zinc and iron and CVD was carried out by the Iowa women's health study [26]. The study found that dietary zinc and iron was not associated with risk of CVD mortality but the study might have lacked power. The end point of the study was CVD mortality; the study did not have CVD incidence data and hence, may have missed cases of CVD with a different cause of death. The study however reported that the benefit of higher zinc intake in CVD mortality occurred only in the presence of a trigger such as alcohol (≥10g/d) that can disturb iron homeostasis although another study reported that alcohol consumption (>40 mL/d) was negatively linked to serum zinc[27]. Moreover, this association reported by Lee at al, 2005 was not statistically significant. They also observed that women with higher dietary zinc intake were less likely to smoke and engaged in more physical activity [26], which is in contrast with the characteristics observed in this study. It is well known that smoking and BMI are one of the main modifiable risk factors for CVD and could be a possible explanation for the observed benefit of higher zinc intake in CVD mortality despite the presence of alcohol intake (≥10g/d). The study also does not appear to have controlled for type 2 diabetes though it considered certain dietary and non-dietary factors as independent variables.

The relationship between type 2 diabetes and CVD is well known; CVD events related to type 2 diabetes and the high incidence of other macrovascular complications, are a major cause of chronic disease burden [28]. It has been shown that the age-adjusted prevalence of coronary heart disease is twice as high among those with type 2 diabetes

as among those without diabetes [29, 30]. Hence, zinc status in patients with type 2 diabetes and CVD events have been assessed [31, 32], which showed a low serum zinc and high concentration of urinary zinc excretion in the patients with diabetes mellitus and all CVD events [31, 32]. Zinc status was also assessed for older population of men and women in Australia [33] and in the same cohort of women with type 2 diabetes [34]. These studies showed that a higher zinc intake was inversely associated with risk of insulin resistance and type 2 diabetes.

In contrast to the observed association between type 2 diabetes and dietary zinc intake in the same cohort, the finding from this study indicates that dietary zinc intake after a certain level increases the risk of CVD and that those in the highest quintiles are above the threshold and was zinc replete. A possible explanation for this could be the fact that the participants are residents in Australia, a mainly affluent Western society, with reasonably good nutrition and minimal zinc deficiency. It was observed that those in the highest quintile of zinc also had the highest quintile of dietary iron intake. The most commonly recorded dietary source of zinc and iron was meat as the highest contributor, which could explain the increase in iron as zinc intake increases, though fish, poultry, cereals and dairy products were other substantial source. We cannot be sure whether the zinc intake is directly increasing the incidence of CVD, as zinc intake tends to increase as other micronutrients intakes increase (for example, iron in this study), or diet quality more generally. However, recent studies have shown that high intake of red meat in multivariable analyses including age, smoking, and other risk factors [35] and specifically, zinc and heme iron from red meat and not other sources[3] was associated with increased risk of CVD. Findings from these studies lend support to the association observed in our study, as the main source of zinc and iron in the majority of the cohort was meat.

Another goal of this study was also to look at dietary zinc to iron ratio as part of the analysis as iron is known to interact with the absorption of zinc [15]. Zinc causes a dose-dependent inhibition of iron binding to phospholipids and competition of zinc for iron binding site is particularly relevant as zinc deficiency promotes intracellular iron accumulation[36]. We observed that risk of CVD was positively associated with higher

quintiles of zinc to iron ratio. This suggests that the proportion of zinc intake in relation to iron is an important determinant of CVD risk. The association of zinc to iron ratio has also been observed in type 2 diabetes[34] in the same cohort however, the direction of association was different.

The strengths of this study are the prospective design, where dietary assessment preceded the outcome, and the generalizability, this being a population-based cohort rather than a clinic sample. The main advantage of the prospective design is that it reduces selection bias and potential recall bias. It is also the only prospective study so far that looks at incident CVD with both dietary zinc and zinc to iron ratio. The large sample size also means that it is possible to obtain reasonably stable parameter estimates. Despite the good generalizability of this study there are some limitations. Use of FFQ to collect dietary information (especially micronutrients) has limitations due to the lack of uniformity in food composition tables[37] and over or under reporting of certain foods/food groups. However, the use of FFQs to collect dietary information in large population-based samples is the most cost-effective and feasible method available. The use of an FFQ to assess dietary zinc intake is mostly likely to have undervalued the amount of zinc consumed by study participants and biased the effect size towards the null in this study [38]. Additional limitation is that dietary assessment was carried out at only one time. However, the analysis controlled for participants who developed comorbidities during the follow-up period instead of excluding them in order to account for any modification in dietary habit, which may have resulted from the development of comorbid conditions. Adjustments were made for all known potential confounders but residual confounding cannot be entirely excluded. Despite controlling for type 2 diabetes and alcohol intake, stratifying analysis by alcohol status to duplicate findings by Lee at al, 2005 [26] will be worthwhile in the future.

This study also considered the use of supplementary multivitamins/minerals and analysis showed that the proportion of people taking supplements were not significantly different between quintiles of zinc intake. Although multiple dietary assessments are useful to reduce random measurement error, given that single dietary

assessment is likely to bias results toward the null, the fact that we still observed a significant association is an indication that the association is likely to be robust and even stronger than that detected in this study.

In conclusion, this prospective studies showed that higher total dietary zinc intake and zinc to iron ratio is associated with a higher incidence of CVD in women aged 50 and above. This study finding is preliminary and needs to be investigated further. Future research is necessary to confirm this association in both men and women across different age groups. A positive finding should prompt further research to investigate the association of zinc and iron from meat and other major sources as the dietary aspects of it have high potential to create or change dietary guidelines which will affect the CVD burden.

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SECTION 3 Conclusions and research implications

Chapter 7 Conclusion

7.1 Main study findings

7.1.1 Serum Zinc is Associated with Pancreatic Beta Cell Function and Insulin Sensitivity in Pre-Diabetic Individuals.

This population-based cross-sectional study of older community-dwelling adults examined the concentration of serum zinc in diabetic, prediabetic and normoglycaemic group of participants and whether the level of serum zinc was associated with the HOMA parameters, steady state beta cell function (%B), insulin sensitivity (%S) and insulin resistance (IR) for normoglycaemic and pre-diabetic groups. We observed that there was no statistical significant difference in serum zinc concentration across all three groups, however insulin concentration was significantly different between normoglycaemic and prediabetic groups ($p<0.001$) and all the HOMA parameters were found to be statistically significantly different between groups ($p=0.001$). After adjusting for multiple possible confounders, a significant association between serum zinc concentration and insulin sensitivity ($p=0.01$) in the prediabetic group was observed while no significant association was seen in the normoglycaemic group.

7.1.2 Dietary zinc intake and zinc to iron ratio is associated with incident type 2 diabetes

In this longitudinal study of mid aged Australian women we examined whether higher dietary zinc intake and zinc to iron ratio was associated with lower incident of type 2 diabetes after a follow-up period of 6 years. The findings from the study suggest that higher dietary zinc intake and zinc to iron ratio was inversely associated with developing type 2 diabetes. After controlling for dietary and non-dietary factors, we observed that the highest quintile of dietary zinc intake had almost half the odds of

developing type 2 diabetes (OR = 0.50, 95% C.I. 0.32–0.77) and similarly, the highest quintile for zinc to iron ratio had half the odds of developing type 2 diabetes (OR = 0.50, 95% C.I 0.30-0.83) compared with the lowest quintile.

7.1.3 Higher dietary zinc intakes, but not zinc to iron ratio is associated with a lower incidence of depression.

In this study two large cohorts were used to examine the association of dietary zinc intake measured using a validated FFQ and incidence of depression using CESD score. Findings from both cohorts showed that higher dietary zinc intake was associated with a lower incidence of depression at the end of the 6 years follow-up, even after adjusting for potential confounders in both the cohorts of men and women aged 50 years and above. We observed that those with the highest zinc intake had significantly lower odds of developing depression with a reduction of about 30–50% compared to those with the lowest zinc intake. However, even after adjusting for potentially known confounders, no significant association between zinc to iron ratio and developing depression was observed in either of the cohorts.

7.1.4 Higher zinc intake and zinc to iron ratio increases cardiovascular risk

This large prospective cohort in mid aged Australian women showed that high dietary zinc intake and zinc to iron ratio is associated with a greater incidence of CVD in women at the end of follow up for 6 years. After adjusting for multiple dietary and non-dietary factors, those in the highest quintile of zinc had almost twice the odds of developing CVD (OR=1.67, 95% C.I. 1.08 – 0.77). A similar trend was also observed with zinc to iron ratio and incidence of cardiovascular disease with those in quintile 4 (OR=1.78, 95% C.I 1.13-2.79) and 5 (OR=1.72, 95% C.I 1.05-2.81) having almost twice the odds of developing cardiovascular disease compared with the lowest quintile of energy-adjusted zinc to iron ratio.

7.2 Research strength and limitations

Every observational research faces the possibility of being influenced by systematic error (bias). Therefore, the findings and conclusions reached in this thesis need to be interpreted with caution despite significant efforts to avoid such error. However, in spite of this potential bias this research has several strengths that support the conclusions mentioned above.

1. The studies were conducted in a population-based cohort of men and women rather than a clinic sample and hence the external validity of the findings is sound (McEvoy MA, Schofield P et al. 2013, Lee C, Dobson AJ et al. 2005 and Brown WJ, Bryson L et al. 1999).
2. The prospective design of the study in chapter 4, 5 & 6, where dietary assessment preceded the development of chronic disease reducing selection bias and potential recall bias.
3. The study conducted in chapter 4 and 5 are the only prospective study that looks at dietary zinc intake and zinc to iron ratio association with incident depression and cardiovascular disease to the best of our knowledge.
4. The large sample size indicating that it is possible to achieve reasonably stable parameter estimates.
5. Serum zinc concentration was measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Perkin Elmer Sciex) as ICP-MS has greater precision and sensitivity compared to the older classic atomic absorption spectroscopy (AAS) technique often used to measure zinc.
6. All outcome measures used in this research have been validated beforehand and are well accepted. For example:
    a. Fasting blood glucose was measured and participants were categorized into three groups according to American Diabetic Association (ADA) guidelines.
    b. Dietary intake was assessed using an FFQ known as the Dietary Questionnaire for Epidemiological Studies (DQES) Version 2 and its validation in mid-aged Australian women has been previously reported (Hodge A, Patterson AJ et al. 2000).

c. Depression was determined by using a well validated self-reported depressive symptoms questionnaire (Radloff 1977).

Despite the good generalizability of the studies and its various strength, this research also has some limitations like most observational research.

1. The cross-sectional design employed to examine the association of serum zinc concentrations with pancreatic beta cell function and insulin sensitivity does not allow the assessment of cause-effect relationships.

2. The use of FFQ to collect dietary information (especially micronutrients) as one of the known limitations is the lack of homogeneity in food composition tables (Liu L, Wang PP et al. 2013) and over or under reporting of certain foods/food groups.
3. Dietary assessment for ALSWH showed that Pearson correlation coefficient was 0.40 for dietary zinc and 0.44 for dietary iron and although it was deemed acceptable as other studies provided a similar estimate, it is still below 0.5 and the interpretation of findings needs to consider this limitation.
4. Dietary assessment was carried out one time only and was unable to account for any changes that might have occurred in the dietary intake of zinc.
5. Follow-up period of 6 years may be too short in order to see the full impact of diet on chronic disease.
6. Finally, although literature review was carried out to determine possible confounders and adjustments were made for all known potential confounders, residual confounding cannot be entirely excluded.

7.3 Recommendations and conclusions

According to Bradford Hill's criteria, a strong association is more likely to have a causal component than is a modest association (Hill 1965). In our prospective studies, we observed that those in the highest zinc intake had significantly lower odds with a reduction of about 30–50% in developing depression, type 2 diabetes and almost twice the odds of developing CVD compared to those with the lowest zinc intake. However, the absence of a strong association does not rule out a causal effect

as Hill demonstrated with two counter-examples (Hill 1965). As dietary assessment preceded the development of chronic disease, it also fulfills another of Hills's criteria that the factor must precede the outcome it is assumed to affect. In order to demonstrate consistency more prospective cohort studies are urgently needed in support of a causal relationship between higher dietary zinc and the development of depressive symptoms, depression in older adults and cardiovascular disease. There is also an equal need for more longitudinal studies in type 2 diabetes and zinc intake to support the findings in this study and for cohesiveness.

The relation of HOMA parameter with different glycemic status also needs to be further explored in other settings and populations. Future research should also include investigation of the association of zinc and iron from meat and other major sources especially in regards to cardiovascular disease. Determining the difference in the effects of elevated intakes of zinc and other nutrients from dietary sources in comparison to supplementations needs be emphasized. Furthermore, population based randomized controlled trials are needed to determine if zinc has efficacy for preventing or improving pharmacotherapy as an adjuvant for chronic disease. The mechanism behind the possible predisposing or protective effects of zinc needs to be explored further as diet may affect multiple pathways in the pathogenesis of chronic disease.

This PhD research provided formative assessment of the potential role of dietary zinc in chronic disease such as type 2 diabetes, depression, and cardiovascular disease. These chronic diseases are of great public health interest due to the significant proportion of the total global burden of disease attributable to these conditions. Given that plasma zinc levels decreases with age and that there is no specific storage system for zinc in the body, it is important to rigorously assess zinc status, dietary habits and food intake as the main sources is through our diet and dietary aspects of it have high potential to create or change dietary guidelines. Differences in the effects of dietary zinc may occur depending on age and gender therefore, studies with stratified age groups and gender groups should be emphasized. Individual difference and background behind the sensitivity to the effects of zinc should also be studied more in detail. Given that there is scarcity of good quality study and large longitudinal study,

further research designs that employ longitudinal analyses and rigorous randomized controlled trials aimed at determining the effects of zinc in disease state and chronic disease are needed to support a causal relationship before the global burden of these disease is beyond control. Thus, there is a need for increased investment in further research for prevention and early detection aimed at prevention and control of chronic disease and other health conditions.

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APPENDIX

1. Medline search strategy

Name of host: OVID

Search date: 21st September 2015

#	Search strings	Results
1	zinc.mp.	105781
2	zn.mp.	41481
3	dietary zinc.mp.	1136
4	micronutrient*.mp.	10184
5	diabetes*.mp.	431925
6	type 2 diabetes.mp.	69791
7	type II diabetes.mp.	5886
8	non-insulin dependent diabetes mellitus.mp.	6697
9	noninsulin dependent diabetes mellitus.mp.	862
10	noninsulin-dependent diabetes mellitus.mp.	862
11	niddm.mp.	6803
12	NIDDM.mp.	6803
13	maturity onset diabetes.mp.	1448
14	depression.mp.	285061
15	depressive disord*.mp.	89043
16	depressive sympto*.mp.	29759
17	CESD.mp.	313
18	CES-D.mp.	2525
19	Centre for Epidemiological Studies Depression.mp.	107
20	CESD-R.mp.	13
21	dysthymia.mp.	1799
22	cardiovascular disea*.mp.	174150
23	stroke.mp.	191201
24	CVD.mp.	17931
25	CAD.mp.	24000
26	coronary diseas*.mp.	135161
27	coronary artery disea*.mp.	86847
28	cardiovascular disord*.mp.	4098
29	heart attack.mp.	3205
30	heart failure.mp.	143735
31	myocardial infarction.mp.	196107
32	heart diseas*.mp.	188875
33	MI.mp.	31405
34	chronic diseas*.mp.	258886
35	non communicable disea*.mp.	2262
36	non-communicable disea*.mp.	2262
37	noncommunicable disea*.mp.	954
38	chronic conditio*.mp.	9473
39	1 or 2 or 3 or 4	134313
40	5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13	432073
41	14 or 15 or 16 or 17 or 18 or 19 or 20 or 21	318716
42	22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33	884470
43	34 or 35 or 36 or 37 or 38	266892
44	40 or 41 or 42 or 43	1749155
45	39 and 44	6027