Magnesium and Diabetes:

It's Implication for the Health of

Indigenous Australians

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Declaration

This work 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.

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Dedication

This thesis is dedicated to the memory of my parents

Professor James Rubert Longstreet and Wilda Graul Longstreet

They led by example, and their faith in me was without measure.

I just wish they were here to see it finished.

PUBLICATIONS AND PRESENTATIONS

The following articles have been published or accepted for publication or presentation during the period of PhD candidature, and sections of these articles have been included in the present thesis.

Published Journal Papers:

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Longstreet DA, Heath DL, Savage, I, Vink R., Panaretto KS. Estimated nutrient intake of urban Indigenous participants enrolled in a lifestyle intervention program. *Australian Indigenous HealthInfoNet* 2008 ;8 (3) electronic journal http://healthbulletin.org.au

Submitted Journal Papers:

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"Magnesium and diabetes: Implications for the Australian Indigenous people" Presented 21 August 2006 Townsville Hospital Professional Journal Club, Townsville, Queensland, Australia

"The impact of magnesium on diabetes and its implication for the health of Indigenous Australians" Presented 16 January 2008 to the Townsville Aboriginal and Islander Health Service Board of Directors, Townsville, Queensland, Australia; *in completion of the ethical obligation to return research to the Aboriginal and Torres Strait Islander peoples*.

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ABBREVIATIONS

ABS	Australian Bureau of Statistics
ACR	Albumin to creatinine ratio
ADA	American Diabetes Association
AI	Adequate Intake
ATP	Adenosine triphosphate
BMI	Body Mass Index
BMR	Basal Metabolic Rate
BP	Blood pressure
BSL	Blood glucose
Ca_i	Ionic Calcium
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
EAR	Estimated Average Requirement
ESRD	End stage renal disease
GP	General Practitioner
HbA1c	Glycosylated haemoglobin
HDL	High density lipoprotein
HOMA	Homeostasis model assessment
LDL	Low density lipoprotein
LGA	Local Government Area
MgATP	Magnesium- Adenosine triphosphate complex
Mg_i	Ionic or free serum magnesium
Mg_s	Total serum magnesium
NHLIBI	National Heart Lung and Blood Institute

NNS	1995 National Nutrition Survey
NRV	Nutrient Reference Value
RDA	Recommended Daily Allowance
RDI	Recommended Dietary Intake
RNA	Ribonucleic acid
SD	Statistical Division
sd	standard deviation
sem	standard error of the mean
TAIHS	Townsville Aboriginal and Islander Health Service, Ltd
TCA cycle	Tricarboxylic acid cycle
UL	Upper Level of Intake
WAT	Walkabout Together Program

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ABSTRACT

Diabetes in Indigenous Australians occurs at a younger age and at almost four times the rate of non-Indigenous Australians. While the cause for this health disparity is multi-factorial, recent studies suggest that nutrition, and particularly magnesium intake, may play a role in onset of diabetes and related pathologies. No study has ever examined whether there is any relationship between diabetes and magnesium intake in Indigenous Australians, and the present study therefore sought to establish whether any such interrelationship existed. As part of this study, dietary magnesium intake was estimated in an urban cohort of Aboriginal and Torres Strait Islander subjects and compared to the average Australian dietary intake. An ecological study then explored environmental correlates, and specifically the magnesium level in drinking water, to diabetes mortality. Finally, total and free serum magnesium concentrations were determined to identify any differences in magnesium status between diabetic and non-diabetic Indigenous and non-Indigenous Australians, and also to compare which of the two parameters was a more sensitive measure of magnesium status and diabetic risk.

All Aboriginal and Torres Strait Islander people that were recruited for this study were patients of the Townsville Aboriginal and Islander Health Services, Townsville, North Queensland, who presented for health monitoring and subsequently required fasting blood tests as part of that routine care. Additional non-Indigenous people were recruited from five GP practices in the Townsville area. Inclusion criteria included persons over the age of 15 (Tanner Stage 5) who had lived in the Townsville area for at least ten days. Exclusion criteria included chronic diarrhoea, alcoholism or binge drinking in the past two weeks, use of diuretics, consumption of magnesium supplements, reduced renal function (urinary albumin to creatinine ratio exceeding > 2.5 mg/mmol in men and > 3.5 mg/mmol in women), severe mental illness, pregnancy, or breastfeeding. Our results indicated that 60% of the Indigenous people assessed in this study had a dietary intake of magnesium that

was below the estimated average magnesium requirement for half the national population. Additionally, the average magnesium intake in Indigenous Australians was significantly less than the intake of non-Indigenous Australians (p<0 .001). A significant negative correlation was found between the incidence of diabetes related mortality and the concentration of magnesium in drinking water in Queensland, confirming previous reports from the USA that drinking water magnesium may be an important factor in development of diabetes. The needs assessment study confirmed that diabetes in both Indigenous and non-Indigenous Australians was associated with reduced levels of total serum magnesium, and more importantly, that total serum magnesium was lower in Indigenous Australians who did not have diabetes compared with their non-Indigenous counterparts (p=< 0.001). In the absence of diabetes, the prevalence of hypomagnesaemia was 17.2% for the non-Indigenous but 36.9% for the Indigenous subjects. Finally, the ionic serum magnesium analysis confirmed the results of the total serum magnesium study, and demonstrated that ionic magnesium was strongly correlated to the total magnesium concentration (r: 0.75. p < 0.001), with the relationship being apparent irrespective of either diabetic (r: 0.66 to 0.81. p<0.001) or ethnicity (r = 0.71 to 0.81. p<0.001)." We conclude that although not causal, the evidence suggests that magnesium may be a significant contributing factor to diabetes in Australia, especially for Aboriginal and Torres Strait Islander peoples, and that further investigation of the potential relationship between magnesium and diabetes in the Australian Indigenous populations, and possible corrective interventions, is highly warranted.

CHAPTER 1:

INTRODUCTION

Diabetes in Indigenous Australians occurs at a younger age and at almost four times the rate of non-Indigenous Australians. The age-adjusted prevalence of diabetes amongst Indigenous people is 16% in remote areas and 9% in non-remote areas, with the actual prevalence estimated between 20% - 25% and possibly higher than 30% in some remote areas (2004). In a cross-country comparison, Australian Aboriginals and Torres Strait Islanders had the highest mortality rates amongst all population groups for cerebrovascular disease and diabetes (Bramley, Hebert et al. 2004). While the cause for this disparity in diabetes morbidity and mortality is multifactorial, recent evidence suggests that nutrition, and particularly magnesium intake, may play a role.

While central obesity remains a major risk factor, magnesium deficit has been posited to be an underlying common mechanism for the insulin resistance found in type 2 diabetes, as well as in metabolic "syndrome X", hypertension, and impaired glucose tolerance (Barbagallo *et al* 2003; Barbagallo and Dominguez 2007). A clinical correlation between low plasma magnesium and diabetes has been investigated extensively over the past two decades (Saris, Mervaala et al. 2000; Fox, Ramsoomair et al. 2001; Walti, Zimmermann et al. 2003), with evidence of low serum magnesium associated with both type 1 and type 2 diabetes (Djurhuus *et al* 2001; Takaya *et al* 2003; Walti *et al* 2003a). Serum magnesium deficits have been reported in 25 to 39% of diabetic outpatients in the USA and Switzerland, and up to 73% of diabetic outpatients in Mexico. With magnesium deficits being observed in diabetes, the studies examining the effects of magnesium-rich foods on diabetes become relevant. The Nurses Health Study and the Health Professionals' Follow-up Study, which included 85,060 women (18 years follow-up) and 42,872 men (12 years follow-up), demonstrated that after adjusting for confounding variables, a magnesium-rich diet reduced the relative risk of developing diabetes by 34% in women and 33% in men

(Lopez-Ridaura, Willett et al. 2004). A similar inverse correlation between magnesium intake and diabetes risk was shown in the Iowa Women's Health Study with a cohort of 35,988 older women (Meyer, Kushi et al. 2000), in the Honolulu Heart Program with a cohort of 8,006 men (Abbott, Ando et al. 2003) and in the Women's Health Study with a cohort of 39,345 female health professionals (Song, Li et al. 2007). Epidemiological studies have also identified that diets replete in magnesium protect against the development of type 2 diabetes (Lopez-Ridaura, Willett et al. 2004; Song, Manson et al. 2004). Clinically, a graded inverse relationship has been observed between serum magnesium levels and type 2 diabetes (Kao, Folsom et al. 1999). Additionally, the co-morbidities associated with both types of diabetes appear to be influenced by magnesium deficit (De Leeuw *et al* 2004; de Valk 1999; Rodriguez-Moran and Guerrero-Romero 2001), with low serum magnesium significantly increasing the prediction of all-cause mortality in diabetic patients (Haglin, Tornkvist et al. 2007). Clearly, there is a role for magnesium in the metabolic pathology of diabetes.

Despite this growing body of evidence supporting the involvement of magnesium in diabetes, consideration of magnesium status has not been integrated into Australian medical care for diabetes, and more specifically, for Indigenous Australians. It is known that the traditional diet of hunter-gathers such as Indigenous Australians was much more nutrient and magnesium rich than the current estimated Australian intake (Eaton and Eaton 2000). Even so, there remains a significant research gap regarding the actual nutrient intake of Aboriginal and Torres Strait Islander populations despite an extensive history of research on Australian Indigenous populations. Of particular concern is the lack of dietary data available on those Aboriginal and Torres Strait Islander populations who have integrated into urban lifestyles (National Health and Medical Research Council 2000;

National Public Health Partnership 2001), especially considering that the largest communities of Indigenous peoples are located in urban areas (2000; 2002). Additional environmental factors such as low levels of magnesium in municipal water supplies, and ambient temperatures affecting magnesium loss in sweat, may also contribute to the potential development of low magnesium status. It is therefore possible that the total dietary magnesium intake may be too low to maintain normal serum magnesium homeostasis, and that this might contribute to the development of type 2 diabetes. Accordingly, the purpose of this investigation was to examine the interrelationship between magnesium and diabetes and it's implication for the Australian Indigenous population.

1.1 Magnesium

The medicinal utility of magnesium dates back to ancient times, although its precise role in health and disease has only been described within the past 100 years. Magnesium was identified as an essential nutrient for animals in 1926, and for humans in 1934 (Vormann 2003). As the fourth most abundant cation and second most abundant intracellular cation, the total magnesium content of the adult human body is 20-28 grams (Shils 1999). As a divalent cation, magnesium shares many characteristics with calcium, a close relative on the periodic table of elements. However magnesium is unique in that it has a high binding affinity and a small ion radius. As a solute, it coordinates with six molecules of water creating the largest hydrated radius amongst biological cations. These characteristics allow for both flexibility and stability in biologic systems (Wolf and Cittadini 2003; Wolf *et al* 2003).

1.1.1 Function and homeostatic balance

The abundance of magnesium and its high binding energies make it extremely versatile. Magnesium can function as an essential cofactor to mediate enzyme-substrate interactions, stabilize intermediate metabolites, bridge reactive species, directly bind to enzymes, or form part of active substrates (Gunther *et al* 1984). Magnesium is used in over 300 enzymatic reactions in the human body, particularly all reactions requiring adenosine triphosphate (ATP). In fact, about 40% - 60% of total magnesium in muscle cells is bound to ATP (Heaton 1993). Magnesium is involved in gluconeogenesis, lipid metabolism, amino acid activations via RNA and DNA, cellular membrane stabilization, calcium channel activity, and many other essential metabolic reactions (Shils 1999; Whitmire 2001). Magnesium activates seven of ten enzymes in the glycolytic pathway including phosphofructokinase and pyruvate kinase, which are the two main regulatory points of glycolysis. Similarly, magnesium activates four enzymes involved in the tricarboxylic acid (TCA) cycle, including the rate controlling enzymes pyruvate dehydrogenase and isocitrate dehydrogenase. Magnesium is crucial for glucose metabolism, yet acts primarily as a chronic, rather than acute, glycolytic regulatory agent (Heaton 1993). In an early study where chicken erythrocytes and rat thymocytes were gradually depleted of magnesium, Gunther et al (1984) described the impact of magnesium deficiency on metabolic activity. Protein syntheses, followed by respiration, were the processes most sensitive to depletion. DNA and RNA synthesis were not affected until more than 50% of the magnesium was reduced, whereas glycolysis was inhibited only when the cellular magnesium was reduced to 10% of its initial value (Gunther, Vormann et al. 1984). This was later confirmed by Heaton (1993) who demonstrated that mitochondrial changes and a partial uncoupling of oxidative phosphorylation appear to occur at relatively early stages of magnesium

deficiency, but only profound magnesium depletion directly impacts glycolysis. Thus the glycolytic pathway appears to be well protected from magnesium deficiency.

Other aspects of magnesium biochemistry are also pertinent to diabetes pathology. Membrane dynamics associated with insulin resistance, post-receptor functioning and intercellular communication appear to be highly vulnerable to magnesium deficit. There is evidence that an inherited structural membrane defect in magnesium binding may be related to both type 2 diabetes and salt-sensitive hypertension (Wells 2008). Additionally, an emerging area of study that may be related is epigenetics, where gene expression alterations occur in the absence of changes in the underlying genetic material, primarily through environmental influences. A crucial mechanism by which animals and humans adapt to changing environments is phenotypic plasticity; the ability of the genotype to be expressed as different phenotypes. Since magnesium plays a crucial role in stabilizing DNA transcription, changes in magnesium status might be an additional mechanism by which a deficit may influence phenotypic plasticity, subsequently modifying diabetes risk.

Hypomagnesaemia has been defined as a total serum magnesium concentration of less than 0.74 mmol/l (<1.8 mg/dl), although in clinical practice, it is often asymptomatic (Whitmire 2001), and its lower cut-off value has been questioned(Spatling, Classen et al. 2000; Elin 2001). Clinical hypomagnesaemia may result from renal dysfunction or drug interactions, as well as from gastrointestinal related decreased intake or increased losses. These gastrointestinal induced fluctuations are frequently associated with alcoholism and conditions involving chronic diarrhoea. Endocrine balance can also influence magnesium status, predominantly through changes in the balance of other minerals such as calcium or

potassium. Hereditary disorders of magnesium homeostasis are relatively rare (Schlingmann, Konrad et al. 2004).

Symptoms of hypomagnesaemia are thought to become evident as total magnesium levels fall below 0.5 mmol/L. Initially symptoms may be non-specific, typically associated with other electrolyte imbalances. Clinical signs and symptoms of hypomagnesaemia include arrhythmia, hypertension, congestive heart failure, dysphagia, tremulousness, disorientation, ataxia, nystagmus, hyperactive deep tendon reflexes, convulsions and /or coma (Whang 1993). Latent or subclinical hypomagnesaemia has a prevalence of around 14% in the general population. Many disease conditions such as hypertension and cardiovascular disease are associated with subclinical hypomagnesaemia. Subclinical hypomagnesaemia has also been widely associated with insulin resistance and diabetes mellitus (Barbagallo *et al* 2003; Corica *et al* 2006; Fox *et al* 2001; Huerta *et al* 2005; Ma *et al* 1995; Paolisso and Barbagallo 1997; Seyoum *et al* 2008; Shils 1999). A 10-year follow-up study identified those with hypomagnesaemia had a 2.54 relative risk of developing type 2 diabetes (95% confidence interval, 1.1-4.1) and was independently associated with the development of impaired glucose tolerance(Guerrero-Romero, Rascon-Pacheco et al. 2008). Magnesium deficiency leads to a proinflammatory state that has been implicated in the onset of diabetes (Weglicki, Quamme et al. 2005). Consistent with this, in a study of obese children, serum magnesium was significantly lower and inversely correlated to fasting insulin levels (Huerta, Roemmich et al. 2005). Magnesium deficiency has accordingly been described as the most evident disturbance of mineral metabolism in diabetes (Bonnefont-Rousselot 2004; Elamin and Tuvemo 1990).

In terms of homeostatic balance, approximately 60% of the body's magnesium is adsorbed to hydroxyapatite in the bone, 38% is in muscle, organs, and other soft tissues, and less

than 1% is found in the blood. Magnesium is further compartmentalized in cells with varying amounts found in cell organelles and the cytosol. Magnesium may be bound to proteins in complex with anions such as phosphate, bicarbonate, and citrate, or be in a free ionised form (Padgham *et al* 1993; Sanders *et al* 1999) with the distribution between the free and bound forms regulating the activity of many magnesium activated enzymes. Magnesium content appears to be relatively stable and compartmentalized within the cell, however, it is still uncertain what controls the flux of magnesium between different cellular compartments (Wolf, Torsello et al. 2003).

Three systems – the gastrointestinal tract, kidneys, and bone – work in concert to maintain human magnesium homeostasis (Whitmire 2001). Absorption of ingested magnesium is a function of intake and total body magnesium status. Healthy persons normally absorb about 30-40% of intake, although absorption can increase to 70% during times of dietary depletion (Shils 1999; Whitmire 2001). Some magnesium absorption may occur along the entire gastrointestinal tract, however the primary sites of absorption are the jejunum and ileum. There are no age-related changes in intestinal absorption of magnesium (Morley 2007). A dietary ratio of 2:1 for calcium to magnesium is considered optimal for magnesium absorption (Seelig and Franz 1999), and only very high levels of calcium intake interfere with this absorption. Phytate, the principle storage form of phosphorus in plants (Bohn, Davidsson et al. 2004), and fatty acids may bind magnesium, thereby decreasing its bioavailability (Sanders, Huijgen et al. 1999). Intestinal resection or chronic diarrhoea adversely also affects magnesium absorption. While both type 1 and type 2 diabetes are associated with increased incidence of gastroparesis (Hasler 2007; Intagliata and Koch 2007), diabetes has limited further direct impact on gastrointestinal function.

Indeed, there is no evidence of diabetes altering absorption of magnesium (Walti, Zimmermann et al. 2003).

Figure 1.1: Magnesium Homeostasis (Shils 1999; Martini and Wood 2001)

Magnesium Homeostasis

INGESTION 350 mg/day 14.4 mmol/day	**Skeleton & soft tissue reserves**
Digestive juices 30 mg/day 1.2 mmol/day	
INTESTINE 380 mg/day 15.6 mmol/day	**MAGNESIUM POOL**
Absorb 180 mg/day 7.4 mmol/day	
FAECES 200 mg/ day 8.2 mmol/day	**SWEAT** 50 mg/day 2.1 mmol/day **URINE** 130 mg/day 5.3 mmol/L
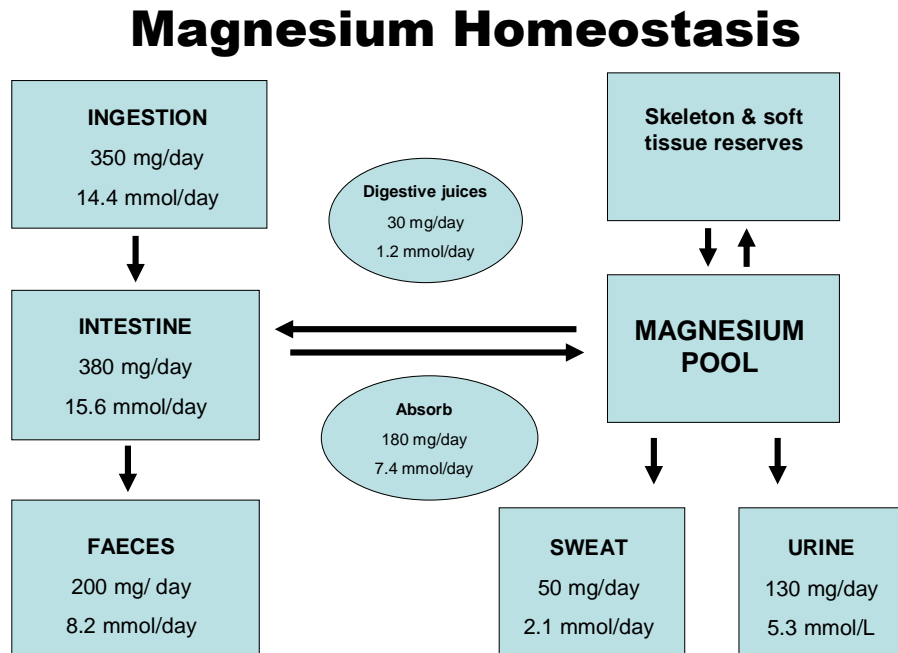
In healthy individuals, homeostasis is well regulated, primarily through the kidneys. Normal urine magnesium is about 5 mmol/day (Martini and Wood 2001) and the kidney begins to respond within hours of hypo- or hyper-magnesaemia (Quamme and de Rouffignac 1993). 80% of plasma magnesium is filtered through the glomerular membrane, with 70-80% of the filtered magnesium being free; the remainder is bound to filterable anions such as oxalate, citrate, or phosphate. Minimal absorption occurs in the descending loop, while 50-60% is absorbed in the ascending loop of Henle. It is within the ascending loop of Henle that predominantly non-hormonal factors modulate the rate of magnesium excretion. No specific hormone influences magnesium uptake or excretion, although parathyroid hormone (PTH), antidiuretic hormone (ADH), calcitonin, glucagon, and insulin have all been identified as having some impact on magnesium retention, albeit

that the findings are inconsistent (Quamme and de Rouffignac 1993; Resnick *et al* 1993; Sanders *et al* 1999; Shils 1999). Excess supplementation with calcium or intakes over 2600 mg per day when combined with high sodium intakes can also increase urinary magnesium excretion (NHMRC 2006). Normally less than 5 mmol (about 121.5 mg) of magnesium should be excreted per 24 hours, however hypomagnesaemia can result in urinary excretion being decreased to less than 0.5 mmol/ day (12 mg/day)(Sanders, Huijgen et al. 1999). There is a circadian excretory rhythm with the maximum magnesium urinary excretion occurring during the night (Fox, Ramsoomair et al. 2001). In healthy humans, this circadian pattern is not affected by glucose loading (Jacomella, Sauser et al. 1997). Nephropathy, a common complication of diabetes, alters magnesium excretion (Shils 1999), but hypermagnesaemia does not present until the urinary albumin to creatinine ratio (ACR) exceeds > 2.5 mg/mmol in men and > 3.5 mg/mmol in women. Acute diabetic ketoacidosis, as with any acidotic condition, increases magnesium excretion (Shils 1999).

Figure 1.2: Factors influencing urinary magnesium excretion

(Cole and Quamme 2000; Quamme and de Rouffignac 1993; Resnick *et al* 1993; Shils 1999)

Decreasing reabsorption or increasing excretion	Increasing reabsorption or decreasing excretion
Hypermagnesaemia	Hypomagnesaemia
Hypercalcaemia	Hypocalcaemia
Extracellular fluid volume expansion	
Loop and thiazide diuretics (e.g. lasix, bumex, edecrin, and hydrochlorthiazide)	
Anti-neoplastic drugs (e.g. cisplatin)	
Antibiotics (e.g. gentamicin and amphotericin)	
Phosphate depletion	
Acute metabolic acidosis	
Inherited disorders	
Alcoholism	

Lower serum magnesium levels in diabetes are typically attributed to increased urinary excretion secondary to osmotic diureses (Quamme and de Rouffignac 1993; Sanders *et al* 1999; Takita *et al* 2004). Elevated concentrations of circulating insulin in the absence of diabetes have also been found to increase renal excretion of magnesium (Djurhuus, Skott et al. 1995). However, Djurhuss et al (2000) subsequently found blood glucose excursions influence magnesium homeostasis independently of circulating insulin levels, and has consequently suggested hyperinsulinemia has only a minor impact (Djurhuus 2001). In type 1 diabetes, magnesium absorption and retention does not appear to be impaired in patients with relatively well-controlled blood sugar (Walti, Zimmermann et al. 2003), and urinary magnesium loss has been attenuated by improving glycaemic control (Djurhuus, Klitgaard et al. 2001). Improving metabolic control also helps to decrease magnesium loss by 38% in patients with type 1 diabetes (Djurhuus, Henriksen et al. 1999) and there is no evidence of increased urinary loss of magnesium in the absence of glycosuria (Walti, Zimmermann et al. 2003). Type 1 diabetes presents as a deficit of insulin with subsequent profound glycosuria; the onset may also involve diabetic ketoacidosis. However, treatment with exogenous insulin does not necessitate a sustained increase in magnesium loss. Thus with type 1 diabetes, hypomagnesaemia may be expected only at the onset and should improve once blood glucose levels are normalized. In contrast, the onset of type 2 diabetes may be preceded by years of insulin resistance and hyperinsulinaemia, with some studies indicating that alterations in magnesium balance may occur even in early stages of this condition (Takita, Wakamoto et al. 2004). The minor impact of insulin on urinary magnesium excretion might result in a deficit when experienced as a chronic condition, although urinary magnesium loss is only one contributor to altered magnesium homeostasis in diabetes.

Bone acts as a magnesium reservoir. As serum levels decline, bone magnesium helps maintain extracellular magnesium levels (Laires, Monteiro et al. 2004). However only about one-third of the magnesium in bone is in an exchangeable form (Sanders, Huijgen et al. 1999). There are no clear mechanisms controlling skeletal magnesium exchange, and both bone and extracellular magnesium may be sacrificed to maintain intracellular magnesium (Whitmire 2001). In humans, the magnesium buffering capacity of bone is reduced with increasing age, in part as a consequence of the 50% reduction in magnesium content of bone lost over a lifetime (Vormann 2003). Magnesium deficiency has even been suggested as a risk factor for postmenopausal osteoporosis (1997) although this has not been supported by all studies (NHMRC 2006). There is however, a correlation between aging, bone loss, and increased prevalence of insulin resistance and diabetes (Barbagallo, Resnick et al. 1997). Magnesium metabolism may well be an underlying link between these conditions.

1.1.2 Assessment of magnesium status

In general, methods to assess magnesium status may be grouped into tissue magnesium tests, for which both ionic and bound magnesium may be tested, and physiological assessments. Various methods of measurement include atomic absorption spectrometry, inductively coupled plasma mass spectrometry, fluorescent probes, magnesium selective electrodes, nuclear magnetic resonance (NMR) spectroscopy, electron probe micro-analysis, radiotracers, stable isotopes, metallochromic dyes, null point titration, and energy dispersive X-ray microanalysis (Kimura *et al* 2004; Silver 2004; Wolf *et al* 2003). The concentration of ionic magnesium can be directly measured with magnesium ion electrodes or with a mellochromic dye, while phosphorus NMR spectroscopy can be used as an indirect method to estimate ionic magnesium concentration (Martini and Wood 2001).

With respect to the ion selective electrodes, Zoppi et al (1996) confirmed the utility of the AVL 988/4, an instrument that determines ionised magnesium with an ion-selective electrode based on the ionophore ETH 7025. Heath and Vink (1998) subsequently demonstrated that the ion selective electrode based measurements obtained with this instrument correlated well with phosphorus NMR based measurements, as well as outcome, following traumatic brain injury. In such comparisons, care must be taken to address potential confounders such as sampling tube and variations in lactate, citrate, and pH (Ritter, Ghahramani et al. 1996; Zoppi, De Gasperi et al. 1996).

1.1.2.1 Tissue magnesium

Less than 1% of the total body magnesium content is in blood. Even though it is only a small fraction of total body magnesium, total serum magnesium is commonly used as an indicative measure of magnesium status because it is readily available and affordable. The reference range for serum magnesium varies (Liebscher and Liebscher 2004), with a range of 0.75 -0.95 mmol/L nominated based on the first National Health and Nutrition Examination Survey in the USA which included 15,820 healthy subjects aged 1-74 years (Lowenstein and Stanton 1986). Other researchers have suggested the correct reference range should be higher, especially given the outcomes of the past two decades of research in to magnesium in chronic disease (Liebscher and Liebscher 2004). Vormann's 2003 review cited normal serum magnesium concentration as $0.8 - 1.2$ mmol/L ($1.9 - 2.9$ mg/dL or $1.6 - 2.4$ mEq/L)(Vormann 2003), while more recently, the Society for Magnesium Research recommended that the cut-off for normal serum magnesium be set at 0.8 mmol/L (Spatling, Classen et al. 2000). However, total serum hypomagnesaemia is widely regarded as a late and potentially insensitive indicator of magnesium status. For example, when total serum magnesium level is low, intracellular magnesium has been widely shown

to be low. However, when intracellular magnesium is low, the serum total magnesium level may not be (Fox, Ramsoomair et al. 2001). Thus, decreases in tissue magnesium concentration have been found in the absence of lower serum magnesium levels (Takita, Wakamoto et al. 2004). Nonetheless, despite the apparent shortcomings, no other measure has yet been readily accepted for routine clinical evaluation of magnesium status.

As a predominantly intracellular ion, the magnesium content in various types of tissues have been investigated including blood cells, sublingual epithelial cells, bone, and skeletal muscle. Because of ease of access, various components of blood have been particularly well studied. No good correlation between erythrocyte magnesium and other tissue pools of magnesium has been demonstrated (Martini and Wood 2001; Gunther 2007), despite compensating for the fact that the age of stored erythrocytes affects their magnesium content (Gunther 2007). Corica et al (1996) has used platelet assays because of the similarities between platelets and vascular smooth muscle. In a study in normotensive and hypertensive patients with type 2 diabetes, both groups of diabetic patients showed significantly lower platelet magnesium levels than controls. Moreover, platelet magnesium was significantly lower in the hypertensive diabetics in comparison to normotensive diabetics (Corica, Ientile et al. 1996). Subsequently, Takaya et al (2003) confirmed that platelet ionic magnesium was lower in children with type 1 or type 2 diabetes, or obesity, than in controls. Finally, lymphocytes have been used to elucidate interrelationships between glucose and magnesium homeostasis, with the choice of lymphocytes being based on the fact that it replicates a nucleated cellular model. Indeed, a direct correlation has been described between the concentration of magnesium in lymphocytes and that of striated muscle (Delva, Degan et al. 2002). Interestingly, the effect of glucose on ionic lymphocyte magnesium concentrations appears to be present at physiologic concentrations

equivalent to 5 mmol/L (100 mg/dl), well below the diabetes diagnostic level of ≥ 7.0 mmol/L. It has been suggested that a likely cause for the decrease in intracellular ionised magnesium brought about by glucose may be the increase in glycolytic output of MgATP (Delva, Degan et al. 2002).

Reflecting on the various functions of magnesium, the appropriate compartment to measure magnesium may vary depending on the disease condition being evaluated. For example, the magnesium content of sublingual epithelial cells has been correlated to the content of atrial muscle cells (Silver 2004) and may thus be well applied to studies of heart disease. For hypertension, platelet assays may be of interest because of similarities between platelets and vascular smooth muscle (Corica, Ientile et al. 1996). For type 2 diabetes, insulin resistance occurs predominantly in muscle tissue and liver, and the use of lymphocytes to approximate muscle magnesium concentration has been suggested (Delva, Degan et al. 2002). Skeletal muscle comprises 40% of the body mass and 27% of the total body magnesium. Accordingly, a readily assessable muscle magnesium test might help clarify the effect of magnesium deficit on insulin resistance. Phosphorus MR spectroscopy with a surface coil can be used to non-invasively determine skeletal muscle ionised magnesium, and such studies have described a significant negative correlation between muscle intracellular ionised magnesium and serum magnesium (Ryschon, Rosenstein et al. 1996). The effort to develop and validate alternative methods for clinical evaluation of magnesium status is an on-going endeavour.

1.1.2.2 Total or ionised magnesium

In serum, approximately 40% of magnesium is bound to anions, and accordingly biologically unavailable (Mikhail and Ehsanipoor 1999; Padgham *et al* 1993). The

remainder is free and biologically available, representing the largest fraction of magnesium in serum. Many consider the size of the free pool the most critical and several attempts at setting a reference range have followed (Ising *et al* 1995; Mikhail and Ehsanipoor 1999; Newhouse *et al* 2002; Saris *et al* 2000). Normal range varies between 0.39 and 0.64 mmol/ L depending on the blood collection method, storage method and ion selective analyser used (Greenway *et al* 1996; Hristova *et al* 1995; Huijgen *et al* 1999; Thode *et al* 1998), although most studies cite normal values of between 0.51 and 0.57 mmol/L. Issing et al (1995) recommended a cut-off for hypomagnesaemia at 0.46 mmol/L, although whether this is suitable to detect sub-clinical magnesium deficiency and its associated pathologies remains to be determined. Given the pronounced effects of circadian rhythm on ionic magnesium (Ising, Bertschat et al. 1995), and that they may introduce individual variation (Newhouse, Johnson et al. 2002), it has been suggested that taking a morning sample may decrease this variability, although subject based reference intervals have also been recommended (Newhouse, Johnson et al. 2002).

In an early evaluation of the use of magnesium specific ion electrodes, Resnick et al (1993) found that type 2 diabetes was uniformly associated with a significant suppression of ionic magnesium (Resnick, Altura et al. 1993). Maj-Zurawska (1997) noted that ionic magnesium concentration was significantly lower in diabetes compared to healthy subjects, even though total magnesium concentration was normal (Maj-Zurawska 1997). In contrast, Mikhail and Ehsanipoor (1999) found higher levels of ionic magnesium among 32 diabetic patients than in matched controls, though no details were given regarding screening the control population to rule out undiagnosed diabetes (Mikhail and Ehsanipoor 1999). One small cross-sectional study in chronic renal failure patients found that the prevalence of hypomagnesaemia was somewhat higher when ionic as opposed to total magnesium was

determined (Dewitte, Dhondt et al. 2004), although it should be noted that evaluating hyper or hypomagnesaemia in chronic renal failure may be confounded by both catabolic magnesium release and alterations in urinary magnesium excretion. Nonetheless, several studies have since suggested that total serum magnesium concentration is a poor estimate of ionic magnesium concentration (Kulpmann and Gerlach 1996; Maj-Zurawska 1997; Sasaki *et al* 2000). To quote Sasaki (2000), "The measurement of serum ionised magnesium may be an earlier indicator of magnesium deficiency in patients than measurement of serum total magnesium" (Sasaki, Oshima et al. 2000). Unfortunately, the equipment to perform ionic measurement of magnesium is expensive and not always readily available.

1.1.2.3 Physiologic assessments of magnesium status

Magnesium balance studies have been used to assess magnesium status, although whole-body retention studies are difficult to perform and interpret properly (Martini and Wood 2001). An alternative approach is to measure retention after an intravenous magnesium load. Costello et al (1997) used a magnesium load retention test in their study of magnesium supplementation in congestive heart failure patients. They concluded that the magnesium load retention test was a more accurate measure than serum magnesium determinations (Costello, Moser-Veillon et al. 1997). Others have shown that a short-term magnesium loading test was as good as the standard load retention test (Rob, Dick et al. 1999), but this method would still be difficult to utilize in many populations as it requires careful 24-hour urine collection. It has been suggested that using functional biomarkers would be a more accessible assessment methodology (Franz 2004). Biomarkers are typically by-products of biochemical processes that act as a proxy measure for the nutrient or compound being evaluated. Although several magnesium-related biomarkers have been

investigated, none have been validated (Franz 2004). It has also been noted that a distinction should be made between physiologic deficit and deficiency, and that magnesium deficit in diabetes appears to derive from complex dysregulation rather than insufficient intake (Durlach, Durlach et al. 1992), thus further confounding efforts to accurately assess magnesium status.

Thus far, there is no consensus as to the best method to evaluate magnesium status in humans (2000). Measuring static total tissue magnesium, such as in serum, remains the most convenient, accessible and economic method of magnesium assessment, though there is clear controversy as to whether it is reflects the dynamic changes in magnesium status (Martini and Wood 2001; Ranade and Somberg 2001). The challenge remains to clarify how magnesium metabolism influences pathological processes, how disease pathologies affect magnesium metabolism, and how these different aspects can be effectively and accurately measured.

1.1.3 Assessment of dietary adequacy

Magnesium status is also a function of magnesium intake. Healthy persons normally absorb about 30-40% of intake, although absorption can increase to 70% during times of dietary depletion (Shils 1999; Whitmire 2001). The quantity of magnesium in the digestive tract is a major factor in how much of the mineral is absorbed (Fine *et al* 1991; Shils 1999)

1.1.3.1 Nutrient reference values

In recent years, the UK, USA, Canada, Australia and New Zealand have moved to a system of nutrient reference values to improve accurate utilization of recommended dietary intakes. The following definitions are those established for Australia (NHMRC 2006).

Estimated Average Requirement (EAR*)* - A daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group.

Recommended Dietary Intake (RDI) - The average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97–98 percent) healthy individuals in a particular life stage and gender group.

Adequate Intake (AI, used when an RDI cannot be determined) -The average daily nutrient intake level based on observed or experimentally-determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate.

Upper Level of Intake (UL) - The highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population.

Different countries choose their own scientific review processes and inclusion criteria and, accordingly, nutrient reference values (NRV) vary between countries. Like the Australian NRV, the USA RDA for magnesium was set at 310 – 320 mg (12.7 – 13.2 mmol) / day for adult women and 400 – 420 mg (16.7 -17.3 mmol) / day for adult men (Yates, Schlicker et al. 1998; 2000). A ratio of calcium to magnesium of 2/1 has long been considered optimum(Seelig and Franz 1999). Dr. Mildred Seelig raised serious concern regarding the 1997 revision of the USA RDA and UL for calcium (Seelig 1998) which ranges from 2.5 /1 to 4.1/1. The Australian and New Zealand scientific review included a careful consideration of the USA deliberations which is reflected in the similarity of the two

Figure 1.3: Australian Nutrient Reference Values (NRV) for Magnesium (mg per day). (NHMRC 2006)

NOTE:
This figure is included on page 20
of the print copy of the thesis held in
the University of Adelaide Library.

country's nutrient reference values. The Australian NRV calcium to magnesium ratio ranges from 2.5 /1 to 3.8/1 (NHMRC 2006).

Assuming a nutrient-rich diet, the magnesium content should be proportional to energy intake. However, USA dietary surveys indicate magnesium intake is frequently below the RDA (Humphries, Kushner et al. 1999; Shils 1999; Saris, Mervaala et al. 2000). In the UK, the mean daily intake of magnesium from food sources was 308 mg for men and 229 mg for women, respectively, which is also below their established NRV for women in all age groups and in the youngest age group for men (Henderson, Irving et al. 2003). The Australian National Nutrition Survey of 1995 (McLennan and Podger 1995), which is the most recent national evaluation of nutrient intake in Australia, found the mean daily intake for magnesium was 381 mg for adult males and 283 mg for adult females, also below both the EAR and RDI. Notably, dietary intake of magnesium was lower in those at greater socio-economic disadvantage, and lowest amongst obese persons. This is clear evidence that a high-energy (kJ) intake does not necessarily result in a magnesium replete diet. Nonetheless, not all assessment studies have identified poor magnesium intake. The usual daily median intake of magnesium for New Zealanders was 365 mg for males and 265 mg for females (Russell, Parnell et al. 1999), within the reference values accepted by that country. Of interest to the present thesis was the observation by Walti et al (2002) who found the dietary intake of magnesium in type 2 diabetic patients in Switzerland was adequate and was not a contributor to any magnesium deficit (Walti, Zimmermann et al. 2002).

There are no known assessments of dietary magnesium intake of Australian Aboriginal and Torres Strait Islander people prior to the current investigation. Since the Australian

National Nutrition Survey demonstrated that low dietary intake of magnesium was positively correlated to socio-economic disadvantage, and there are higher rates of socio-economic disadvantage amongst the Australian Indigenous population (2002), it is reasonable to assume that low magnesium intake might be found in this population. Indeed, indications of critically low magnesium intake have been found in other Indigenous populations. Studies in both the Inuit and Navajo Indians have identified very low intakes of dietary magnesium (Ballew *et al* 1997; Food Mail Project 2002; Kuhnlein *et al* 1996). In contrast, a study of Pima Indians has shown that mean magnesium intake was a generous 416 mg per day (Reid, Fullmer et al. 1971), even though their serum magnesium has been found to be lower than that of Caucasians (Paolisso and Ravussin 1995).

1.1.3.2 Food sources

The essential nature of magnesium in biologic systems is evidenced by it being widely distributed in the plants and animals we use as food. However, the fact that magnesium is present in many different foods does not guarantee dietary adequacy (Henderson *et al* 2003; Humphries *et al* 1999; McLennan and Podger 1995b). The Australia New Zealand Food Standards Code defines a good food source of a vitamin or mineral as containing no less than 25% of the RDI for that nutrient (2007). By this definition, a good food source for magnesium would contain at least 80 mg per normal serving, and few foods would qualify. Most lists of magnesium rich food sources include whole grains, green vegetables, nuts and seeds, legumes, seafood, dairy, and a few fruits such as avocado (Elamin and Tuvemo 1990; English and Lewis 2002; Pennington 1998). Highly refined flours, tubers, fruits, oils and fats contribute only minor amounts of magnesium. Refining or processing food,

including the use of boiling as a cooking method, may deplete the magnesium content of food by up to 85% (Swaminathan 2003).

Several of the best food sources of magnesium (whole grains, legumes, oilseeds) are also rich in phytic acid, and phytate can decrease magnesium absorption (Shils 1999; Saris, Mervaala et al. 2000; Whitmire 2001). The fermentable carbohydrates in these same foods may counteract the inhibitory effect of phytic acids. Physiological levels of phytate do not appear to be a major inhibitor of magnesium absorption. Of note, higher cereal fibre intake has been found to be inversely associated with diabetes risk (Bo *et al* 2006; Juntunen *et al* 2003; Pereira *et al* 2002; Schulze *et al* 2007). Moderate intakes of calcium, iron, or manganese do not affect magnesium absorption (NHMRC 2006), although protein intakes exceeding 94g per day may increase renal excretion of magnesium(NHMRC 2006).

Beverages also contribute to our dietary magnesium intake. Specifically, as magnesium salts in water may be highly bio-available (Sabatier *et al* 2003; Verhas *et al* 2002; Yokota *et al* 2004), they can make a significant contribution to dietary magnesium adequacy (Kiss *et al* 2004; Rubenowitz *et al* 1998; Sabatier *et al* 2002; Verhas *et al* 2002). In a review of mineral content of tap water from 21 major USA cities, half the tap water sources examined contained 6% to 31% of the magnesium RDA in two litres of drinking water per day (Azoulay, Garzon et al. 2001). It has been noted that drinkers of magnesium-rich mineral water have significantly higher magnesium intake than those drinking low-mineralised or tap water (Galan, Arnaud et al. 2002).

Figure 1.4: Food sources of magnesium

FOOD SOURCE	Magnesium / 100g	Magnesium / usual serve size
Fruits:		
Avocado	23	104
Banana	27	32
Kiwi Fruit	30	23
Whole grains:		
Brown rice	43	43
Oats, rolled, uncooked	148	44
Whole wheat flour, plain	138	41
Seafood:		
Crab	41	41
Mackerel / Jack	97	97
Oysters	109	109
Prawns	34	34
Salmon	34	34
Tuna	29	29
Vegetables:		
Artichoke	60	72
Baked beans	32	41
Kidney beans	45	40
Lima beans	55	50
Lentils	36	36
Silver beet, beet greens	67	48
Soybeans	86	74
Spinach	87	80
Tofu	94	118
Nuts & Seeds:		
Almonds	295	89
Brazil	260	78
Cashew	250	75
Peanut	168	50
Pecan	110	33
Walnut	150	45
Milk/ Dairy		
Milk, non-fat (skim)	11	27
Yoghurt	19	47
Cheese, cheddar	28	8
Miscellaneous		
Coconut meat, raw	32	14
Coconut milk	37	37
Cocoa, dry powder	499	27
Dark chocolate	116	33

Several studies have also identified a correlation between coffee drinking and decreased risk of type 2 diabetes (Agardh *et al* 2004; Salazar-Martinez *et al* 2004; Tuomilehto *et al* 2004; van Dam *et al* 2006). Coffee contains 11 mg magnesium per 100 gram dry. A 250-ml cup of brewed coffee made with filtered water has 13 mg magnesium (Pennington 1998). The magnesium content of coffee could be of significance at the higher consumption rates at which a risk reduction of diabetes has been found. However coffee also contains caffeine, which has been suggested by some (Salazar-Martinez, Willett et al. 2004; Iso, Date et al. 2006), but not all studies (Pereira, Parker et al. 2006), to enhance insulin secretion and sensitivity. Caffeine has been also has been found to have a variable effect on pancreatic beta cell response and insulin sensitivity (van Dam, Willett et al. 2006), which may reflect an adaptive response. Chlorogenic acid, another component of coffee, might also have an impact through reducing glucose absorption and inhibiting glucose output by the liver (Agardh *et al* 2004; van Dam *et al* 2006). Finally, coffee contains several antioxidant compounds which may serve a protective role (Svilaas, Sakhi et al. 2004). Thus the magnesium content of coffee may be only one of several components contributing to a decreased diabetes risk ratio associated with coffee consumption.

1.1.3.3 Magnesium containing drugs and supplements

Over-the-counter drugs and supplements containing magnesium may contribute to daily intake. Some anti-inflammatory medications are bound to magnesium e.g. magnesium salicylate tetrahydrate, choline magnesium trisalicylate, and esomeprazole magnesium. Antacids may also be formulated as a magnesium salt, be it an oxide, hydroxide, carbonate, phosphate, trisilicate or citrate salt (Swain and Kaplan-Machlis 1999), with the form of salt influencing the onset and duration of action. The use of magnesium salts as cathartics is also well known, with magnesium salt that is sold as an antacid or cathartic at one dose

being useful as a nutritional supplement at a reduced dose. Magnesium salts without chloride are converted to magnesium chloride in the stomach, with half the chloride then being excreted in the faeces, often resulting in diarrhoea (Innerarity 2000).

The effect of nutrient supplementation is greatly influenced by the bioavailability of the magnesium compound chosen. Most nutrient bioavailability studies use indirect measures of either 24-hour urinary excretion or changes in plasma concentration. In a review of the bioavailability and pharmacokinetics of magnesium salts (Ranade and Somberg 2001), magnesium L-lactate and aspartate were found to have the highest bioavailability, with the chloride, citrate, fumarate, gluconate, glycinate, and potassium salts all having good bioavailability (see Figure 1.5). Magnesium oxide was ranked as having extremely low bioavailability. Magnesium sulfate was noted as having a limited and variable bioavailability; its use is predominantly as an intravenous solution.

Figure 1.5: Bioavailability of magnesium salts (Ranade and Somberg 2001)

NOTE:
This figure is included on page 26
of the print copy of the thesis held in
the University of Adelaide Library.

Firoz and Graber measured excess urinary excretion after ingestion of a magnesium supplement to determine bioavailability (Firoz and Graber 2001). As with previous studies (Lindberg *et al* 1990; Muhlbauer *et al* 1991), magnesium oxide remained the poorest choice, while the aspartate, lactate and chloride salts were better absorbed. In a study utilizing serum, urinary, and salivary magnesium to assess apparent bioavailability, Walker et al (2003) found magnesium citrate had the highest bioavailability (Walker, Marakis et al. 2003). Clearly, different methodologies generate variable outcomes, although magnesium oxide seems to consistently have the lowest bioavailability. Enteric coatings do not seem to improve magnesium bioavailability (Fine, Santa Ana et al. 1991).

Water solubility has been linked with greater bioavailability and oral absorption (Ranade and Somberg 2001). Despite this finding, Classen (2004) has suggested that magnesium orotate may be a more useful supplement since it is poorly soluble in water, does not bind gastric acid, and does not cause diarrhoea (Classen 2004). The author claims that orotate has beneficial effects on pyrimidine metabolism and thereby favours magnesium transport. However, few studies have critically examined this salt in comparative studies. An international supplement company claims magnesium diglycinate (their product) is the most absorbable magnesium supplement preparation (2003). Their claims are based on one published study of 12 patients with ileal resection (Schuette, Lashner et al. 1994) which showed that four patients who had the poorest response to magnesium oxide had an elevated response to magnesium diglycinate. The authors postulated some portion of magnesium diglycinate was absorbed intact (not as a salt) therefore would be better absorbed and utilized. A second unpublished study found significantly better absorption of magnesium diglycinate in comparison to magnesium chloride (2003). Interestingly, a rat study using a robust methodology to compare the bioavailability of ten organic and

inorganic magnesium supplements favoured magnesium gluconate (Coudray, Rambeau et al. 2005), but did not evaluate magnesium diglycinate. No further evidence favouring magnesium diglycinate, nor the postulated alternative absorption pathway, has been published.

Interpreting research on magnesium bioavailability is difficult because the methodology has not been standardized, and all of the factors influencing the metabolic availability of the nutrient have not been controlled. At best, it is clear that organic salts tend to be better absorbed than inorganic salts. The quantity of magnesium in the digestive tract is a major factor in how much is absorbed (Fine *et al* 1991; Shils 1999), with fractional absorption decreasing at doses above 10 mEq (120 mg) (Fine, Santa Ana et al. 1991). Magnesium oxide, despite being a poorly absorbed supplement, continues to be used because 60% of the compound is available as elemental magnesium allowing for easy dosage and economic use. Lactate and citrate that have greater bioavailability require larger tablets to achieve the same dose, although their powdered forms are highly soluble and conveniently taken in water.

Figure 1.6: Percent elemental magnesium content compared to bioavailability of common oral supplements.
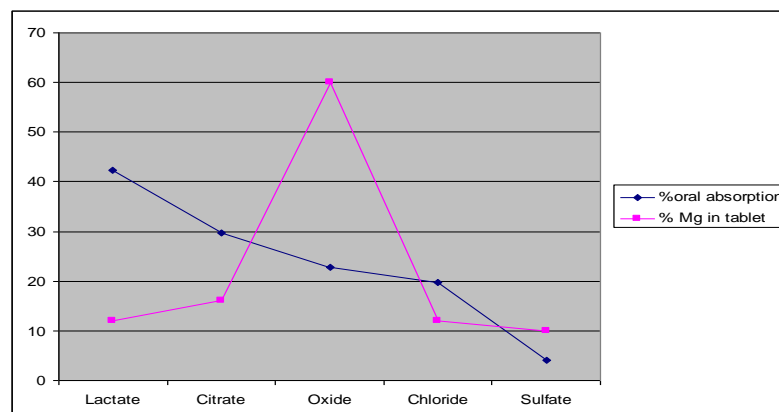
In a study evaluating the use of alternative medicines, Canadians spent almost as much money on over-the-counter supplements as they did on prescribed medications, with 2.2% using magnesium as an complementary therapy for diabetes (Ryan, Pick et al. 2001). In the USA, an average of 18.6% of those surveyed indicated they used herbs or supplements in the previous 12 months (Kennedy 2005). Of interest, the usage of supplements was higher in Native populations (21.9%) than in Caucasians (19.1%). In 1995, 30% of Australians had recently used a vitamin and mineral supplement (1998), a much higher percentage than reported in the USA. Surveys specifically assessing Australian Indigenous dietary supplementation were not found. Given that traditional Aboriginal "bush" medicine is based on plant pharmacopoeia (Devanesen 2002), the modern use of herbal supplements might be well accepted in this population.

1.1.3.4 Magnesium in drinking water

The health benefits of mineral waters were documented as early as 1697, and as noted above, magnesium salts in water can be highly bio-available (Sabatier *et al* 2003; Verhas *et al* 2002; Yokota *et al* 2004). Magnesium content of water affects total body magnesium status (Rubenowitz *et al* 1998; Sabatier *et al* 2002; Verhas *et al* 2002) and makes a significant contribution to dietary adequacy (Kiss *et al* 2004; Sabatier *et al* 2002; Verhas *et al* 2002). Modern urban drinking water has been shown to supply between 6% and 31% of the recommended dietary intake of magnesium (Azoulay, Garzon et al. 2001). In the context of dietary magnesium intake influencing diabetes, an association between magnesium content of drinking water and diabetes may thus be feasible. Water hardness (due to calcium and magnesium salts) has previously been associated with reduced incidence of cardiovascular and other chronic diseases (Ferrandiz *et al* 2004; Rubenowitz *et al* 1996; Rubenowitz *et al* 2000; Sauvant and Pepin 2002; Seelig 2002; Yang *et al*

1999), though not all studies have supported such an association (Maheswaran *et al* 1999; Nerbrand *et al* 2003; Rosenlund *et al* 2005). Indeed, different studies within the same country have described conflicting results. For example, a Swedish study (Rubenowitz, Axelsson et al. 1996) reported an inverse association between death due to acute myocardial infarct and water hardness (food intake was not assessed). In contrast, Nerbrand et al (2003) subsequently evaluated the influence of calcium and magnesium in drinking water and diet on cardiovascular risk factors in individuals living in hard and soft water areas in 76 municipalities in Sweden. Both diet and water supply was considered, and inclusion criteria included at least 5 years receiving the same water supply. After determining calcium and magnesium content of whole blood, serum, muscle and urine, no significant association was found between magnesium and cardiovascular risk (Nerbrand, Agreus et al. 2003). Nonetheless, the magnesium content in the water of Sweden has been reported to be lower than in most other countries, leading to a recommendation that drinking water contain at least 30 mg magnesium per litre to provide a competitive inhibitor against the potentially toxic effects of cadmium and lead (Durlach, Durlach et al. 1992). While there may be a threshold effect at which only higher levels of magnesium will consistently produce a measurable ecologic effect, the existing evidence seems to confirm that there is a link between water hardness and cardiovascular risk.

Few ecological studies have specifically examined the association between magnesium in drinking water and diabetes (Mahaba 1998; Rosenlund *et al* 2005; Yang *et al* 1999; Zhao *et al* 2001). Zhao et al (2001) has correlated drinking water composition to Type 1 diabetes in Devon and Cornwall, England, reporting that children living in areas with magnesium levels greater than 2.61 mg/L in their drinking water were at 28% less risk of developing type 1 diabetes compared with children in areas with <1.90 mg/L magnesium

in the water supply (Zhao, Mold et al. 2001). These estimates of exposure were however crude given that no dietary assessment of magnesium intake was included as part of the study. In contrast, a Taiwanese study used a design that included a control group made up of cases that died from other causes, pair-matched by gender, year of birth and age at death, and found a significant correlation between water concentration of magnesium and diabetes mortality rates (Yang, Chiu et al. 1999). In contrast, an earlier study in Iowa, USA, did not find any correlation between magnesium and diabetes (Joslyn, Lynch et al. 1990), although it did not have the stringent design of the Taiwanese study. Of course, ecological studies do not provide causal evidence, but are nonetheless useful for establishing a case for further research.

1.1.3.5 Methodological issues with dietary assessments

When evaluating dietary intake of magnesium, accounting for the use of supplements, drugs containing magnesium, and magnesium in the drinking water significantly improves the quality of the assessment. Even so, the difficulties of accurate dietary reporting have been well documented (Maurer, Taren et al. 2006). The 24-hour recall method has been extensively used in population studies, but is limited in its effectiveness to measure individual intake. Seven day dietary records require a considerable degree of participant education and compliance. In some populations an interviewer-administered food frequency questionnaire has been found to be more feasible than multiple 24-hour dietary recalls (Yanek, Moy et al. 2001). The use of doubly-labelled water (where the hydrogen and oxygen have been partially or totally replaced for tracking purposes) to validate 24-hour recalls, food records, or food frequency tools consistently indicate all traditional methods are subject to misreporting (Willett 1998). Recent trends in dietary intake studies include assessment of biomarkers to validate self-reported intake (Bingham *et al* 2007;

Franz 2004). While several magnesium-related biomarkers have been suggested, none have been validated (Franz 2004).

There are relatively few published quantitative dietary studies of the Australian Indigenous populations, primarily due to concerns regarding reliable assessment methods (Gracey 2000). A comprehensive review of dietary intake methodology used with the Australian Indigenous people was included in the 2000 Nutrition in Aboriginal and Torres Strait Islander People information paper by the National Health and Medical Research Council (2000). Neither diet history or food frequency methods were found to obtain valid quantitative data in remote Australian Aboriginal communities, and a 'store-turnover' method was developed to determine the apparent dietary intake for the community as a whole (Lee, O'Dea et al. 1994). The store-turnover method, which approximates the intake of a community based on grocery sales, was recommended as having the least potential for bias (Lee, Bailey et al. 1994). Most dietary research amongst Aboriginal and Torres Strait Islander people has focused on remote communities, and only rarely includes estimates of nutrient intake (Smith and Smith 2003). One ethnographic study was conducted in Melbourne providing a cultural context of food and activity, yet did not quantify food or nutrient intake (Thompson, Gifford et al. 2000). No other dietary studies with the urban Indigenous were identified, and the use of traditional dietary assessment methods in urban Aboriginal and Torres Strait Islander people has not been evaluated.

1.1.4 Environmental factors affecting magnesium status

The most commonly cited environmental factor which impacts on incidence on type 2 diabetes is urbanization, which is accompanied by a subsequent change in diet and physical activity (Trevisan, Vedovato et al. 1998). Research into the Palaeolithic era has highlighted the changes of both diet and lifestyle that began approximately 10,000 years ago as a result

of the introduction of agriculture and animal husbandry (Cordain, Eaton et al. 2005). The rate of conversion to a Western food regime has dramatically increased in the past century, contributing to a world-wide epidemic of obesity. A diet comprised of highly refined grains, processed foods, and limited green vegetables are significantly lower in magnesium content. For Indigenous Australians, this dietary change has been even more profound. With the exception of fish and shellfish, almost all foods now used in Australia are exotic to the continent. Furthermore, even among theses exotic plants, changes in farming methods have altered vitamin and mineral content (Worthington 2001). Several studies highlight decreasing nutrient content of produce (Davis *et al* 2004; Mayer 1997; Thomas 2003), and there continues to be robust debate regarding soil depletion of minerals (Lyne and Barak 2000). Potassium fertilizers, commonly used in modern farming practice, have been found to reduce magnesium content in some crops (Lopez, Leenhardt et al. 2002). Acid rain with subsequent acidification of the soil may leach magnesium from the soil and enhance aluminium uptake rather than magnesium uptake by plants (Sanders, Huijgen et al. 1999). Additionally cross-breeding and the development of hybrids to improve yield and pest resistance may not favourably affect nutrient content (Davis, Epp et al. 2004). Thus, not only have there been changes in the types of foods available, the actual composition of raw food has changed. Lastly, the shift to urban centres also potentially changes the water supply. Bore water is rich in minerals, including magnesium; municipal water treatment typically removes calcium and magnesium as part of the water softening process. The environment has significantly changed, affecting both our food and water supply.

Franz and Bailey (2004) have previously identified a significant correlation between climate and diabetes prevalence, with a hot climate and high precipitation being associated

with a higher prevalence of diabetes. The authors attributed their findings to the potential for increased sweat loss of calcium and magnesium that might not be compensated by diet or water intake (Franz and Bailey 2004). The case of the Pima Indians may be used to illustrate this effect. Although the Pima population remains in the Gila River Indian Community (USA), located in a hot climate, their diet has changed with rapid urbanization. When compared to the Mexico-based Pima Indians, who have retained their traditional lifestyle and diet, age-adjusted and sex–adjusted prevalence rate for type 2 diabetes is only 6.9% amongst Mexican Pima Indians, in comparison to a 38% prevalence rate amongst the USA Pima Indians (Schulz, Bennett et al. 2006). The mean fasting plasma magnesium content is lower in the USA Pima Indians (Paolisso and Ravussin 1995) despite their magnesium intake being estimated at 416 mg per day which would appear to be adequate (Reid, Fullmer et al. 1971). What is unclear is whether there is increased magnesium need specifically due to sweat loss in these communities, which was normally provided for in a traditional diet, or whether there is a genetic factor involved that requires higher magnesium intake.

Major differences in erythrocyte magnesium content have been identified amongst different ethnic groups. Mean erythrocyte magnesium values are 20-30% lower in black African men than Amerindian Quechuas, with European Caucasians exhibiting an intermediate value. The differences are relatively stable and largely independent of the local climate. Twin and family studies support the existence of genetic regulation of magnesium level, not only in red cells, but also in plasma, soft tissues and bone (Henrotte 1993). Additionally there has been emerging evidence of an inheritable defect in magnesium binding to the plasma membrane of somatic cells which may be part of the etiology of type 2 diabetes in certain populations (Wells 2008). For the Pima Indians, what

may have previously been an appropriate diet for their genetics and environment has changed, resulting in the rapid rise to the highest rate of diabetes world-wide.

It is well known that heat acclimation increases sweat loss, and that sweat contains magnesium. Magnesium losses in sweat are approximately 0.8 mmol/L (range 0.2-1.5 mmol/LMg or 19-365 mg/day) (Sawka and Montain 2000). Daily fluid requirements for sedentary to very active persons range from 2-4 L/day in temperate climates to 4-10L/day in hot climates. Residents of desert climates often have sweat rates of $0.3 - 1.2$ L/hour while performing occupational activities, while athletes performing high-intensity exercise in the heat have sweating rates of 1.0 -2.5 L/h. Surprisingly, a recent study in men performing controlled work for 8 hours on ergocycles in the heat (100F) showed that they lost only 15.2-17.8 mg magnesium per day in sweat, accounting for 4-5% of daily magnesium and 10-15% of total magnesium excretion (faces, urine and sweat) (Lukaski 2000). The only known studies on mineral sweat loss amongst Aboriginal people concluded differences in sweat concentrations were not attributable to genetic differences, but due to diet and lifestyle (2000). With consideration of the Australian climate, there may be potential for significant magnesium loss through sweat and further studies on sweat loss might therefore be considered useful for Aboriginal and Torres Strait Islander peoples. However, accurate measurement of sweat loss is an invasive procedure and a compelling need must first be established prior to further research on sweat loss in the Aboriginal or Torres Strait Islander people.

1.2 DIABETES

1.2.1 Prevalence

There is an emerging global epidemic of diabetes with estimates predicting that 221 million people world-wide will be affected with diabetes by 2010 (Schrier, Estacio et al. 2007), 333 million by 2025 (2003) and 366 million by 2030 (Wild, Roglic et al. 2004). In Australia, diabetes mellitus accounted for 3.0% of disease burden and injury in 1996, as measured in disability-adjusted life years (Queensland Health 2001). Diabetes-related deaths accounted for 7.4% of all Australian deaths in the two years 1997 and 1998, however for Indigenous Australians for the same time period, 16.4% of deaths were diabetes related (2001).

1.2.1.1 Australian Indigenous – a population at risk

The health disparity experienced by the Aboriginal and Torres Strait Islander people is profound. In 2000, the death rate for Indigenous males was 2.7 times that of the total male Australian population; the death rate for Indigenous females was 2.4 times the rate of the total female population. These figures are based on the number of actual registered deaths and is thought to be potentially underreported by as much as 59-90% (2002). Aboriginal and Torres Strait Islander people are the least healthy sub-population in Australia (2004). Internationally, a comparison of Australian Indigenous mortality with New Zealand Maori and Native Americans dramatically (Ring and Firman 1998) illustrated that the Australian Indigenous population is more disadvantaged than native populations in other countries (see Figure 1.7). At the time of the study, mortality rates for Australian Indigenous people in the mid 1990s were comparable with the Maori rates of the early 1970s. The Maori and Native American populations have made rapid gains in health and life expectancy over the past two decades, whilst the Australian Indigenous mortality trends have shown little evidence of improvement. A later study by Bramley et al (2004) again compared the disparity, this time including Indigenous and Non-Indigenous populations in New Zealand,

Australia, Canada, and the United States (Bramley, Hebert et al. 2004). While overall life expectancy was highest in Australia, the lowest life expectancy for Indigenous people was

Figure 1.7: Trends in annual directly standardised mortality rates for persons: US indigenes, New Zealand Maoris, Australian indigenes, and all Australians. (Ian Ring, Health Information Unit, Queensland Health; unpublished data; reprinted with permission).

NOTE:
This figure is included on page 37
of the print copy of the thesis held in
the University of Adelaide Library.

also in Australia. In other words, the health disparity described by Ring and Firman in 1998 had remained a profound issue. The pattern of mortality is distinct from the profiles of most other countries worldwide, with a relatively low infant and childhood mortality rate followed by a much higher mortality in the young and middle adult years (Paradies, Montoya et al. 2007). Four groups of conditions accounted for almost 70% of all of the excessive Indigenous deaths: circulatory diseases, including predominantly ischemic heart

disease, cerebrovascular disease and hypertension, accounted for 26%; respiratory diseases accounted for 16%; injury and poisonings including suicide accounted for 15%; and finally endocrine conditions, largely diabetes, causes a further 10% mortality (Hetzel 2000). In contrast, one smaller study evaluated five years of health care usage in an Aboriginal community of 1900 residents and concluded nutrition and health had improved considerably in the last 40 years, with a sick minority (14%) accounting for half of the ambulatory health service visits (Dugdale and Watlemaro 2001). Unfortunately, this scenario does not seem to hold true for the majority of Aboriginal and Torres Strait Islander peoples.   A 5-year follow-up study of young Australian Aborigines indicated abnormalities of carbohydrate and lipid metabolism were well established by late in the second decade of life (Braun, Zimmermann et al. 1996). For Australian Indigenous children born between 1998-2000, life expectancy was estimated at 19 -21 years less than that of the general population (2002; Bramley, Hebert et al. 2004). In Queensland, the life expectancy of Aboriginal and Torres Strait Islander people is 22.5 – 24.6 years less than other Queenslanders (2006). The heath disparity is even more striking when you limit the comparison to diabetes as a cause of death. Indigenous people who have type 2 diabetes develop the disease earlier than other Australians and often die at younger ages (2004). Diabetes-related deaths accounted for 7.4% of all Australian deaths in 1997 and 1998. However, for Indigenous Australians for the same time period, 16.4% of the deaths were diabetes related (2001). In 1999-2002, deaths due to endocrine, nutritional and metabolic disorders (predominantly diabetes) were 12 times more common than expected for Indigenous females living in Queensland, West Australia, South Australia, and Northern Territories, and eight times more likely for Indigenous males (2004). Among the four countries previously compared by Bramley et al (2004), the age standardized mortality

rates (per 100,000 population) for deaths due to diabetes was highest for the Australian Indigenous (see Figure 1.8).

Figure 1.8. Diabetes related mortality in Indigenous and non-Indigenous populations in Australia, Canada, New Zealand and the USA (data extracted from (Bramley, Hebert et al. 2004).
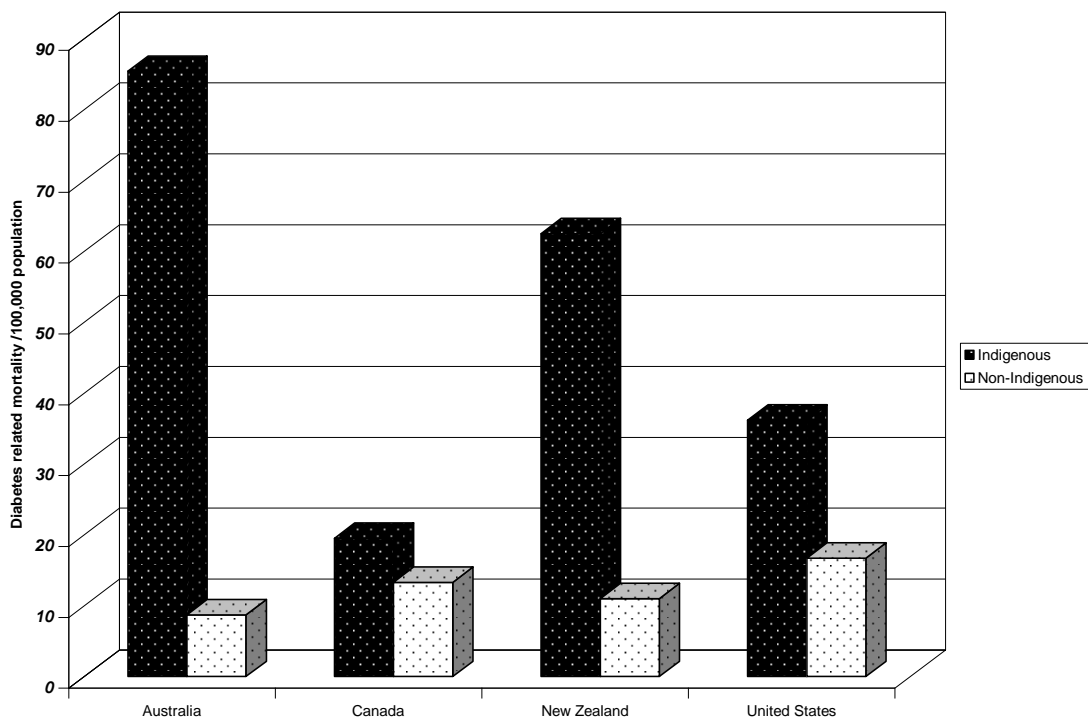
It should be noted that the Australian Indigenous population as described above is not homogeneous. Due to the small numbers frequently involved, it is considered reasonable to statistically group together both Aboriginal and Torres Strait Islander people, despite the fact the two groups have significantly different cultures, diet and lifestyle. In 2001, Torres Strait Islanders comprised 11% of the Indigenous population in Australia (2004). Nonetheless, both the Aboriginal and Torres Strait Islander populations independently experience a severe health disparity associated with diabetes. Community surveys of Indigenous populations in North Queensland report a diabetes prevalence of 24% among

Torres Strait Islanders aged 15 years and over, and 13% among Aboriginal people aged over 15 years (Health 2001). The overall prevalence amongst Indigenous people is estimated to be between 10-30%, which is two to four times that of non-Indigenous people (2002). In an 8-year follow-up study of two central Australian Aboriginal communities, the age- and sex-adjusted, BMI-specific diabetes incidence rates were 11-47 cases per 1,000 person-years which was "among the highest in the world, corresponding closely with the age-and sex-adjusted BMI-specific rates for Pima Indians" (Daniel, Rowley et al. 1999).

To put these prevalence rates into perspective, the highest rate of diabetes world-wide is amongst the Pima Indians (35%), followed by Nauru at 24% (King, Aubert et al. 1998; 2003), which is the same prevalence rate that was identified for the Torres Strait Islanders. The Australian Aboriginal people were identified as having a higher prevalence of diabetes (13%) than Fiji (10.6%) or the Maori in New Zealand (8.3%) (King *et al* 1998; Moore and Lunt 2000). Interestingly, for the same year, the diabetes prevalence rate was 7.4% for Papua New Guinea and 4.3% for Indonesia (King, Aubert et al. 1998). Clearly, this geographic pattern cannot be attributed to genetic differences. The genetic origins of the Australian Indigenous are from an ancient wave of migration out of Africa through Malaysia and Papua New Guinea (Mellars 2006). The people located in the Torres Strait experienced more genetic mixing than the more isolated Aboriginal people. If we were to assume a genetic factor, the Torres Strait Islanders should have diabetes rates close to those in Papua New Guinea. Unfortunately, comparing the diabetes prevalence rates in different countries is confounded by differences in study design, methodology and problems in adjusting for age distribution in the population. Additionally, type 2 diabetes is a complex disease resulting from the interaction of a genetically susceptible individual in a

disease-producing environment. Ethnic differences do exist, however they are clearly a blend of nurture as well as nature. One outcome of mapping the human genome has been the realization that there is no genetic code for race, thus no evidence to support the use of race in medical genetics (Garte 2002; Cooper, Kaufman et al. 2003). "Race is a social construct, not a scientific classification"(Schwartz 2001), however, geography does influence genetic variation (Burchard, Ziv et al. 2003). For the Australian Aboriginal population, 40,000 years of isolation may have been sufficient to result in genetic variation. It has been noted that "the gene pool of Australian Aborigines has been reported to bear the least resemblance to other ethnic groups" (Heitmann, Swinburn et al. 1997). However the Australian Indigenous people are no longer isolated and significant genetic mixing has occurred since colonization. The Aboriginal and Torres Strait Islander populations have some of the highest diabetes prevalence rates in the world, but it's clearly not attributable to genetic variation by geographic distribution. The same basis of diabetes pathology seems to apply to all humans.

1.2.2 Diabetes disease aetiology

Diabetes mellitus is characterized by high blood glucose due to the absence (type 1) or abnormal secretion and function (type 2) of insulin. Though the presenting symptom of hyperglycaemia may be the same, the aetiology and treatment protocol differs between type 1 and type 2 diabetes. Type 1 diabetes is immune-mediated, although genetic, environmental factors and possibly viral triggers are involved. Type 2 diabetes is a result of overlapping mechanisms that are dependent on insults at both the peripheral and beta-cell level (Pirola, Johnston et al. 2004) and accounts for about 90% of all cases of diabetes.

Insulin resistance and beta cell dysfunction precede overt type 2 diabetes by many years. Insulin resistance is a major contributor to the underlying pathology of type 2 diabetes, yet typically a diagnosis only occurs after beta-cell function has decreased sufficiently to allow abnormal glucose excursions. By this time, a substantial percentage of beta cell mass has been lost and the remaining cells are dysfunctional (Caveaghan 2004). Postprandial hyperglycaemia may be the earliest measurable abnormality in the continuum of glucose intolerance (Blonde, Gavin et al. 2004) and failure of first-phase insulin secretion, which blunts postprandial glucose, is an early marker of beta-cell dysfunction (Caveaghan 2004). The homeostasis model assessment (HOMA) for beta cell function has been found to be inversely correlated to serum magnesium levels (Randell, Mathews et al. 2008).

Low serum magnesium has been identified in both type 1 and type 2 diabetes (Djurhuus *et al* 2000; Elamin and Tuvemo 1990; Saggese *et al* 1991; Sharma *et al* 2007; Walti *et al* 2003a), although most of the magnesium research has focussed on type 2 diabetes. In the USA, 25 – 39% of outpatients with diabetes had low serum magnesium (Nadler, Buchanan et al. 1993), while in Switzerland 37.6% of patients with diabetes recorded low serum magnesium levels (Walti, Zimmermann et al. 2003). In another study of type 2 diabetes, where patients did not have good glycaemic control, 47.7% were found to have low plasma magnesium (Lima, Cruz et al. 1998). Magnesium deficiency has even been associated with insulin resistance in obese children (Huerta, Roemmich et al. 2005). It also seems that some populations are more prone than others to low magnesium levels. For example, Rodriguez-Moran and Guererro-Romero have published a number of well-designed studies on magnesium and diabetes from Durango, Mexico (2000, 2002a, 2002b, 2005, 2006). Using a random selection of households from a randomly selected geographic area, they reported that 52.5% of their subjects with impaired glucose tolerance had low serum

magnesium (Guerrero-Romero and Rodriguez-Moran 2000). They have also reported that 65.6% of their patients with metabolic syndrome had low serum magnesium (Guerrero-Romero and Rodriguez-Moran 2002). In a study of foot ulcers, 93.9% of diabetics with foot ulcers had low serum magnesium levels compared to only 73.1% of non-diabetics with foot ulcers. Ethnicity has been suggested as one possible reason why low magnesium levels are so prevalent in this Mexican population although inadequate dietary intake has not been ruled out. As discussed earlier, the Pima Indians with their high rates of type 2 diabetes, also have low serum magnesium levels (Paolisso and Ravussin 1995) despite an apparent adequate magnesium intake (Reid, Fullmer et al. 1971). Differences in erythrocyte magnesium content have also been shown to exist between different ethnic groups (Paolisso and Ravussin 1995), with twin and family studies supporting the concept of genetic regulation of magnesium level, not only in red cells, but also in plasma, soft tissues and bone (Henrotte 1993). Furthermore, an inheritable defect in magnesium binding has recently been suggested (Wells 2008). Clearly, genetics has the potential to influence magnesium homeostasis and intermediate metabolism, and subsequently disease pathology.

Even prior to diabetes onset, there is significant evidence supporting a relationship between magnesium and whole-body glucose homeostasis and insulin sensitivity (Barbagallo, Dominguez et al. 1999). As early as 1988, intercellular magnesium metabolism was linked to both hypertension and diabetes(Resnick, Gupta et al. 1988). A growing body of studies suggest suppressed intracellular free magnesium and concomitant increased intracellular free calcium produces insulin resistance and modulates insulin-mediated glucose uptake (Nadler, Buchanan et al. 1993; Paolisso and Barbagallo 1997). The action of magnesium as a mild calcium antagonist has indeed been proposed as the

basis for some or all of the favourable impact of magnesium on insulin function (McCarty 2003). Barbagello et al (2003) have further suggested that magnesium depletion is the probable underlying connection between hypertensive states and metabolic diseases, including type 2 diabetes (Barbagallo, Dominguez et al. 2003). In terms of the mechanisms by which insulin and magnesium interact, it is thought that insulin regulates intracellular magnesium concentrations by stimulating the plasma membrane ATP pump (Takaya, Higashino et al. 2004). Insulin binding activates tyrosine phosphorylation at the intracellular part of the receptor. Hypomagnesaemia may result in abnormal tyrosine-kinase activity at the insulin receptor, resulting in insulin resistance. Several studies have also implicated protein kinase pathways in the insulin-mediated shift of magnesium (Delva *et al* 2002; Ferreira *et al* 2004; Laires *et al* 2004b). In a potentially vicious circle, high levels of insulin and excess blood sugar increase the loss of magnesium, while a deficiency of magnesium aggravates insulin resistance. While the specific mechanisms may be uncertain, it is clear from the accumulating evidence that insulin resistance and magnesium deficit are clearly interrelated.

Obesity, especially central obesity, is an identified risk factor for developing insulin resistance and type 2 diabetes (Burke, Zhao et al. 2007). Low serum magnesium is correlated with being overweight and obese in some (Huerta *et al* 2005; Laires *et al* 2004b; Randell *et al* 2008) but not all studies (Mataix, Aranda et al. 2006), although such an association is most likely due to poor dietary choices resulting in a low magnesium intake (Huerta *et al* 2005; Laires *et al* 2004b; Mataix *et al* 2006; McLennan and Podger 1995b). Of relevance is the fact that magnesium may have an alternate pathway to mediate insulin sensitivity through the adipose-secreted cytokine adiponectin. There is a significant association between magnesium intake and adiponectin, which both improves insulin

sensitivity as well as promoting an anti-atherosclerotic effect (Qi, Rimm et al. 2005). For Aboriginal people, there is a strong linear age-adjusted correlation between body mass index (BMI) and diabetes risk (Daniel, Rowley et al. 1999), as well as impaired glucose tolerance (Daniel, Rowley et al. 2002). In a study of nine Torres Strait Islander communities, obesity rates were identified as being three times higher than in other Australians while diabetes was six times higher than in other Australians (Leonard, McDermott et al. 2002). The only Indigenous studies that have not linked BMI with insulin resistance were in the context of lifestyle intervention programs promoting physical activity (McDermott *et al* 2000; Rowley *et al* 2000). Ethnicity also appears to affect the significance of certain risk factors such as obesity. For example, it has been suggested that an appropriate BMI for Aboriginal people is less than for those of European descent. Specifically, an appropriate BMI has been estimated as between 17-22 kg/m^2 (instead of 18-24.9 kg/m^2) because metabolic abnormalities develop in this population as BMI rises above 22 kg/m^2 (Daniel *et al* 2002; Piers *et al* 2003; Rowley *et al* 1997).

1.2.3 Co-morbidities and complications

Chronic hyperglycaemia can wreak havoc throughout the body. Vascular damage is a hallmark of diabetes, and microvascular complications including retinopathy, neuropathy, and nephropathy often lead to amputations (due to neuropathy), loss of vision (retinopathy), and dialysis (nephropathy) resulting in a lifetime of disability and diminished quality of life. Macrovascular complications are also associated with diabetes and include cerebrovascular disease, peripheral vascular disease, and coronary heart disease, all of which account for 75-80% of diabetes mortality (Davies, Tringham et al. 2004; Schrier, Estacio et al. 2007).

In terms of microvascular complications, diabetes is the leading cause of new adult blindness in industrialized countries (Schrier, Estacio et al. 2007). According to the findings of the Katherine Region Diabetic Retinopathy Study (Jaross, Ryan et al. 2003), the overall prevalence of diabetic retinopathy was less in Aboriginal people than that found in the general Australian diabetic community. However, prevalence of clinically significant macular oedema and vision-threatening retinopathy was much higher. The prevalence of retinopathy among the Aboriginal population was 18% in 1993 and 21% in 1996 (Jaross, Ryan et al. 2003). Two studies have identified a strong association between low serum magnesium and diabetic retinopathy (Mahaba *et al* 2000; Sharma *et al* 2007).

In the Western world, diabetic foot problems secondary to neuropathy, have been reported as the most common complication of diabetes, and involve half of all amputations in the United States. Although there is no comparable data available on amputations in Australia, it has been estimated that there are 3,000 diabetes-related amputations in Australia per year (Watson, Obersteller et al. 2001). Diabetes is also the leading cause of end-stage renal disease in industrialized nations (Schrier, Estacio et al. 2007). Among Aboriginal people, nephropathy is multi-determinant (Hoy, Vanbunynder et al. 2001) having other root causes besides diabetes. Nonetheless, the incidence of end stage renal disease (ESRD) was 74 per 1,000,000 for the non-Indigenous population compared to 322 cases per 1,000,000 in the Indigenous people Australia-wide from 1994-1998 (2002). Hospitalization for dialysis is the most common hospital treatment for Indigenous people in Australia (2004). The average age at onset of dialysis is approximately 10 years less for the Indigenous than the non-Indigenous, and mortality rates are higher even when adjusted for traditional co-morbidities (McDonald and Russ 2003). In a remote Aboriginal community study in Arnhemland (Western Australia), microalbuminuria was found in 42% of those with

diabetes compared to 28% expected from the AUSDIAB (Australian Diabetes) study (Maple-Brown, Brimblecombe et al. 2004). In animal models, the kidney appears to be an early target for damage from diabetes. Specifically renal mitochondrial function appears to be impaired from the onset and does not recover when insulin treatment restores euglycemia (Katyare and Satav 2005). This might potentially involve magnesium as mitochondrial changes and a partial uncoupling of oxidative phosphorylation appears to occur at relatively early stages of magnesium deficiency (Heaton 1993). This association is supported by a retrospective clinical study of 550 type 2 diabetic patients that showed lower serum magnesium was associated with more rapid renal function deterioration (Pham, Pham et al. 2005), an observation that has since been confirmed in later studies (Kisters, Gremmler et al. 2006). Finally, dialysis patients with higher levels of serum magnesium have better rates of survival (Hwang, Lin et al. 2001).

While strict control of blood sugar decreases microvascular complications, it requires intensive control of blood pressure to decrease the macrovascular complications and deaths associated with diabetes (Schrier, Estacio et al. 2007). Macrovascular complications include cerebrovascular disease, peripheral vascular disease and coronary heart disease (Davies, Tringham et al. 2004; Schrier, Estacio et al. 2007). Approximately 40% of patients with diabetes have hypertension, which significantly increases the prevalence of nephropathy, retinopathy, and cardiovascular disease (Schrier, Estacio et al. 2007). Research with the Australian Indigenous suggests that there is a cross-sectional relationship between hypertension and albuminuria, hyperglycaemia, and cardiovascular disease risk (Hoy and McDonald 2004). Many studies have linked low serum magnesium to hypertension and cardiovascular disease independent of diabetes (Abbott *et al* 2003; Gums 2004; Ma *et al* 1995; Paolisso and Barbagallo 1997; Resnick *et al* 2001; Song *et al*

2006; Touyz 2003) and hypomagnesaemia has been linked to low serum HDL cholesterol irrespective of glycaemic control (Guerrero-Romero and Rodriguez-Moran 2000). In the presence of diabetes, magnesium intake appears to reduce or prevent hypercholesterolemia, hypertriglyceridemia, high blood pressure and other risks for cardiovascular disease (Guerrero-Romero and Rodriguez-Moran 2000; Olatunji and Soladoye 2007; Soltani *et al* 2005a; Soltani *et al* 2005b; Soltani *et al* 2007), supporting the concept that magnesium has a role in both hypertension and cardiovascular disease (Al-Delaimy *et al* 2004; Delva 2003; Maier 2003; Touyz 2003). It is therefore likely that magnesium deficit which is fostered by diabetes pathology is a significant contributor to the macrovascular complications of diabetes (Haglin, Tornkvist et al. 2007).

Hyperglycaemia is the primary risk factor for the development of both macro- and microvascular complications of diabetes. Elevated blood glucose with subsequent protein glycation and advanced glycation end-product formation results in oxidative stress which further contributes to diabetic complications (Bonnefont-Rousselot 2004). Magnesium deficiency triggering a low-grade inflammatory response has also been implicated as a contributor, especially if there is insufficient antioxidant capacity (Guerrero-Romero and Rodriguez-Moran 2005). C-reactive protein (CRP) is an acute-phase reactant produced by the liver that is widely accepted as a biomarker for inflammation. Magnesium intake has been found to be inversely associated with CRP (Song *et al* 2005; Song *et al* 2007), and magnesium supplementation has been found to significantly decrease CRP (Almoznino-Sarafian *et al* 2007; King *et al* 2006; Xu and Whitmer 2006). Both CRP and diabetes independently predict cardiovascular disease and appear to have a synergistic effect on its development (Corrado *et al* 2006; Xu and Whitmer 2006). Notably, CRP levels have been found to be much higher in Aboriginal Australians than in other populations (Wang and

Hoy 2006), and is independently associated with the risk of developing diabetes amongst Aboriginal people (Wang and Hoy 2007). CRP levels have also been positively associated with insulin resistance (Mita *et al* 2006; Syrenicz *et al* 2006) and BMI (Kahn *et al* 2006; Mita *et al* 2006). It should be noted that magnesium deficiency has been identified as leading to a pro-inflammatory state implicated in the onset of diabetes (Bo *et al* 2006; Schulze and Hu 2005; Weglicki *et al* 2005) as well as in the development of subsequent complications (Bonnefont-Rousselot 2004). Even in the absence of diabetes, low serum magnesium levels are independently related to elevated CRP concentration (Guerrero-Romero and Rodriguez-Moran 2002a; Guerrero-Romero and Rodriguez-Moran 2006). As an anti-inflammatory agent and natural calcium channel blocker, magnesium may well be a factor linking both microvascular and macrovascular complications of diabetes (Mazur, Maier et al. 2006).

1.2.4 Magnesium and diabetes risk prevention

It is well known that diet and exercise can have a preventative impact on development of diabetes. In Queensland (Australia), it is estimated that 77 percent of the health risk for type 2 diabetes can be attributed to excess body fat and physical inactivity (2006). Several studies have examined specific foods associated with reduced risk of developing diabetes, most of which incidentally are also significant sources of magnesium. Whole grain intake, especially in overweight subjects, is inversely associated with fasting plasma insulin, and this association was independently attenuated by both fibre and magnesium (McKeown, Meigs et al. 2002). In a Framingham Offspring Cohort (McKeown, Meigs et al. 2004), dietary fibre, cereal fibre, fruit fibre, and whole grains were associated with lower homeostasis model assessment insulin resistance (HOMA-IR). Fibre from cereal was inversely associated with metabolic syndrome, whilst fibre from fruit, vegetables and

legumes were not (McKeown, Meigs et al. 2004). Thus whole grain intake was inversely associated with HOMA-IR and a lower prevalence of metabolic syndrome. Unfortunately, the independent effect of magnesium was not evaluated. One additional small randomized control trial has shown oat fibre will improve insulin sensitivity by 8% (Weickert, Mohlig et al. 2006). What should be borne in mind is that fibre may have an independent affect on glycemia and insulin response via changes in the rate of digestion and absorption.

Nut and peanut butter consumption, also magnesium-rich, has also been identified as having and inverse association with type 2 diabetes (Jiang, Manson et al. 2002; Parker, Harnack et al. 2003). Nuts and peanuts are also high in fibre, monounsaturated fat and protein, all factors that affect digestion, absorption, and insulin response. High levels of coffee consumption, as a source of both magnesium and caffeine, has been correlated to higher serum magnesium levels (Svilaas, Sakhi et al. 2004) and lower risk of diabetes (Agardh *et al* 2004; Pereira *et al* 2006; Salazar-Martinez *et al* 2004; Tuomilehto *et al* 2004; van Dam *et al* 2006). While it may be unclear as to the relative contributions of caffeine versus magnesium, it is clear that all of the various components of any food, and their effects, must be considered as potential confounders to the effect of magnesium.

There is a growing body of evidence that implicates dietary magnesium as a preventative component independent of being a marker for healthy food intake (Larsson and Wolk 2007). In a cohort of 39,345 U.S. women followed for an average of 6 years (Song, Li et al. 2007), there was a significant inverse association between magnesium intake and the risk of type 2 diabetes (p=.001). The effect was appreciably modified by BMI, although the association was only significant in women with BMI ≥ 25 kg/m^2. As noted before, poor dietary choices resulting in obesity are also associated with low magnesium intake (Huerta

et al 2005; Laires *et al* 2004b; Mataix *et al* 2006; McLennan and Podger 1995b), and low serum magnesium has been correlated with being overweight and obese in some studies (Laires, Moreira et al. 2004; Huerta, Roemmich et al. 2005). A cross-sectional study of healthy non-diabetic women showed a relationship between median plasma fasting insulin levels and magnesium intake (Song, Manson et al. 2004). After adjusting for all potentially confounding variables, the linear trend was borderline significant and only statistically significant for the overweight women (p=.003). It was hypothesized that the extent to which magnesium influences insulin sensitivity may differ among women with different body weight (Song, Manson et al. 2004), and may be mediated by adiponectin (Qi, Rimm et al. 2005). In the Melbourne Collaborative Cohort Study of 41,528 people between 1990 and 1994, total carbohydrates, sugars, and magnesium were inversely associated with diabetes incidence (Hodge, English et al. 2004). Two similar studies using a cohort of 121,700 female registered nurses in the Nurses' Health Study, and a cohort of 51,529 U.S. health professionals in the Health Professionals' Follow up Study, both reported a significant inverse association between magnesium intake and risk of type 2 diabetes (Lopez-Ridaura, Willett et al. 2004). The Iowa Women's Health Study also found a strong inverse association between the incidence of diabetes and dietary magnesium intake, as well as intakes of total grains, whole grains, dietary fibre and cereal fibre (Meyer, Kushi et al. 2000). This six year prospective cohort study included 35,988 older women and 1141 incident cases of diabetes. In a subset analysis of the Nurses Health Study (Fung, Manson et al. 2003), it was reported that magnesium intake was inversely associated with fasting insulin concentration; additional adjustment for glycaemic load and cereal fibre did not alter the association. Humphries et al (1999) also found a significant negative correlation between total dietary magnesium and the sum of insulin levels measured in a sample of young black Americans without diabetes during an oral glucose tolerance test. This

association suggests that magnesium may be associated with insulin resistance prior to the development of diabetes in a black population. Overall there is substantial epidemiologic evidence of diabetes risk reduction associated with a diet replete in magnesium.

In contrast, the relative risk of diabetes increases with diets high in trans fatty acids, processed meats, heme iron and high glycaemic index as opposed to a diet high in polyunsaturated fats or vegetable fats, fibre, whole grains, caffeine, nuts and magnesium (Murakami, Okubo et al. 2005). In critically analysing the available data (Agardh *et al* 2004; Burke *et al* 2007; Larsson and Wolk 2007; Murakami *et al* 2005; Salazar-Martinez *et al* 2004; Schulze and Hu 2005; Tuomilehto *et al* 2004; van Dam *et al* 2006; Yates *et al* 1998), the following factors have been identified as protective against developing diabetes:

> BMI < 25 kg/m^2

> Waist circumference for men under 102cm (40inches) or for women under 88cm (35inches)

> Dietary cereal fibre, lower glycaemic load, and lower glycaemic index carbohydrates

> Moderate to intense physical activity 30 minutes or more on most days

> No smoking

> Moderate alcohol consumption (1-2 drinks per day), especially wine

> Coffee drinking

> Consumption of cruciferous vegetables

> Substitute saturated fat for unsaturated fat

> Avoid processed meats (<four times per month)

> Avoid sugar-sweetened soft drinks

➢ Assess and avoid body iron overload

➢ Higher magnesium intakes

To summarize, there is clear evidence that type 2 diabetes may be prevented through lifestyle modification of diet and exercise, especially when it is well-implemented and maintained (Davies, Tringham et al. 2004). There is also clear evidence supporting an independent role for dietary magnesium in the prevention of diabetes irrespective of it being a marker for a healthy diet. However for the Australian Aboriginal and Torres Strait Islander populations, dietary modification alone may be insufficient (McDermott, Rowley et al. 2000). Prevention strategies must address a complex interaction between genetic-susceptibility, diet, food accessibility, exercise, obesity, and psychological stress, all within a context of self-determination and community-direction (Rowley and O'Dea 2001). Even so, if magnesium does contribute to the health disparity experienced by the Australian Indigenous, it may be more amenable to change than other dietary factors. This may be especially applicable in the context of limited healthy food sources, since high bioavailability of magnesium in water itself might lend itself to a different, possibly more effective, intervention strategy.

1.2.5 Magnesium and clinical management of diabetes

The case for magnesium supplementation in diabetes clinical management has not been well established. With type 2 diabetes there is both a dietary deficiency and a metabolic deficit that must be addressed. There are issues with bioavailability, concentration of the supplement, and the duration of supplementation necessary to effect diabetic magnesium status. Additionally, how to effectively measure magnesium status continues to be problematic.

When analysing some of these factors in experimental research, the complexity becomes even more apparent. For example, magnesium sulphate was effective in ameliorating changes in plasma glucose levels and aortic and pancreatic abnormalities in diabetic rats only within the range of 10 -30 g/L in the drinking water (Soltani, Keshavarz et al. 2005). A second study, however, demonstrated that 10g/L supplementation in diabetic rats resulted in blood pressure, aortic elasticity, and blood glucose levels not significantly different from non-diabetic controls despite the fact plasma magnesium was not normalized by supplementation (Soltani, Keshavarz et al. 2005). In other words, supplementation improved health outcomes in spite of continued low plasma magnesium levels. Another study of type 1 diabetes found decreased insulin-stimulated uptake after 24 weeks of supplementation with magnesium oxide (Djurhuus, Klitgaard et al. 2001). Despite its poor bioavailability, the study demonstrated an increase in muscle magnesium content and serum magnesium indicating that the magnesium oxide supplementation regimen in this particular case was apparently sufficient to increase magnesium status. However, most studies using magnesium oxide have not demonstrated such positive results (Lindberg *et al* 1990; Muhlbauer *et al* 1991; Ranade and Somberg 2001; Schuette *et al* 1994).

In a study examining the effects of combined nutrient supplementation on the lipid profiles of type 2 diabetes (Farvid, Siassi et al. 2004), after 3 months of supplementation, a significant improvement in serum HDL-c and apoA1 was shown for the combined magnesium, zinc, vitamin C and vitamin E supplement. No significant impact was noted for the mineral-only supplementation, although as indicated above, this study may have been flawed by the use of the less bioavailable magnesium oxide supplement. Nonetheless,

the positive effect of the combined supplement is evidence supporting the inflammatory pathways involved in lipid metabolism. A second double-blind random control trial by the same group, still using magnesium oxide, found three months of supplementation with a blend of antioxidant nutrients including magnesium showed significantly lower urinary albumin excretion, a marker for glomerular renal function. Again, these results were independent of changes in blood pressure or glucose (Farvid, Jalali et al. 2005). Finally, long term supplementation with 300 mg of magnesium gluconate per day has been shown to decrease the stage of polyneuropathy in type 1 diabetic patients (De Leeuw, Engelen et al. 2004).

Using a meta-analysis of randomized clinical control trials investigating the effect of magnesium supplementation on glycaemic control in patients with diabetes mellitus, Sheth et al (2002) cautioned that all studies to date were of low to moderate quality and evidenced significant heterogeneity. They did, however, conclude that magnesium supplementation caused a significant reduction in fasting blood glucose (Sheth, Kamanger et al. 2002). Subsequent studies have addressed many of the quality issues noted in the aforementioned meta-analysis, and using the more absorbable magnesium chloride solution, demonstrated improvement in both serum Mg and metabolic control as measured by HOMA-IR index during a 16-week randomized clinical control trial that (Rodriguez-Moran and Guerrero-Romero 2003). Using a salt-lake water source of magnesium chloride, another small study found improvements in insulin sensitivity after only 30 days of supplementation (Yokota, Kato et al. 2004). Although there is mixed evidence supporting improvement of blood glucose control, magnesium supplementation has demonstrated benefits in improving insulin resistance (Guerrero-Romero *et al* 2004; Rodriguez-Moran and Guerrero-Romero 2003) and significantly delaying the onset of neuropathic (De Leeuw, Engelen et al. 2004), renal (Farvid, Jalali et al. 2005), and

cardiovascular complications (Djurhuus *et al* 2001; Yokota *et al* 2004). As such, it would be reasonable to consider assessment and possible supplementation for this patient population.

In 1992, an American Diabetes Association (ADA) consensus panel acknowledged the potential pathological relationship between hypomagnesaemia and diabetes(1992). The ADA consensus statement recommended clinicians screen patients with diabetes who had one or more additional risk factors for hypomagnesaemia. Four years later, in evaluating physician implementation of those guidelines, neither magnesium dosage nor duration of supplementation was implemented according to the guidelines; neither was matched to the degree of magnesium depletion (Garber 1996). In a subsequent review, Swain and Kaplan-Machlis (1999) stated that "empiric magnesium replacement may be considered in patients taking diuretics (especially loop), patients who are malnourished or alcoholic, patients with low potassium, *or those who are diabetic*" (italics added). Despite that specific reference to diabetic patients, the Diabetes Management in General Practice guidelines established by the Royal Australian College of General Practice made no mention of the association of hypomagnesaemia and diabetes, nor included any recommendation to screen patients with one or more risk factors of hypomagnesaemia (Harris, Joyner et al. 2003; Harris, Mann et al. 2007). Despite a growing body of evidence supporting the involvement of magnesium in diabetes, the need to consider magnesium status has not been integrated in Australian medical care.

1.3 Factors contributing to Indigenous health disparity

In any study involving the Aboriginal and Torres Strait Islander people, the history of dispossession, alienation and the social, political and economic disadvantage that continues

to be experienced, provides a context that must be acknowledged and respectfully considered (2001). For over 40,000+ years the Australian Aboriginal people successfully lived as hunter-gathers, living in harmony with a frequently harsh and hostile environment. When first seen, Europeans were impressed by the athletic stature of the Aborigines. In an environment in which the white settlers barely survived, the Aborigines thrived as an apparently healthy population. The subsequent deterioration observed in Indigenous health after white settlement was so remarkable that it was once believed that the Aboriginal population would die out, the speed of the process depending largely on the extent to which they had contact with Western civilization (McArthur *et al* 2000; Shannon 2002). As late as 1900, the devastation of infectious diseases was still compounded by widespread murder (Hetzel 2000). Despite the harsh impact of white colonization, Aboriginal Australians survived. Robust fertility rates disproved earlier predictions of extinction. In 2002, total fertility rates were 2,193 births per 1,000 Indigenous mothers compared to 1,752 per 1,000 for all mothers. Indigenous women tended to have more babies and have them at a younger age than did non-Indigenous women (2004). The census counts for Indigenous people were 16% higher in 1996, with the birth rate contributing to 12% of that increase and 4% being attributed to better reporting.

In the past two centuries, the native diet of Indigenous Australians has been drastically changed by Western colonization (2000). Traditional Aboriginal food, now often called "bush food", was secured by hunting and fishing by the men, and gathering of plants, eggs, insects, and small game (e.g. snakes, goanna) by the women. It took the knowledge and skill of the whole tribe to safely collect and prepare food since many Australian plants are highly toxic and require special treatment to be edible. A wide variety of food would be available in different regions during different seasons. Local custom and belief also

influenced what was hunted or gathered (Hiddens 1999). For example, the Torres Strait Islander people were marine hunting, supplementing their diet with subsistence agriculture and trading. Turtle and dugong still holds a special place in their cultural life. Many bush foods are rich sources of nutrients. The billy goat plum, Terminalia ferdinandiana, is famous as perhaps the richest source of vitamin C in the world (Brand-Miller, James et al. 1993). Other bush fruits which have subsequently gained some commercial uses have 3.5 - 5.4 fold higher antioxidant activity than blueberries (Netzel, Netzel et al. 2006). These are just a few examples of the potential nutritional contribution native foods can provide. Nomadic people move frequently, allowing the plants and animals time to regenerate, thus limiting the impact of humans on the environment. Once Aborigines settled on stations, their lifestyle and diet changed dramatically, especially as the available bush foods were depleted in the surrounding areas.

An expedition to Arnhem Land in 1948 measured the health and nutritional status of Aborigines in settlements and indicated that signs of malnutrition were already evident in the infants and children (McArthur, Billington et al. 2000). In part, this may have been due to the fact the Aborigines would eat foods a European would not eat; an inferior diet was considered good enough. The standard ration consisted of white flour, white rice, tea, sugar, salted buffalo and beef. Where possible, cassava, sweet potato, and other fruits and vegetables were raised. Unless supplemented by bush foods, the diet described in 1948 was marginal in protein, energy, calcium, vitamins A, C, B-complex, and iron. It was interesting to note that even in 1948, it was acknowledged that the original native diet was potentially better nutritionally than that of many Europeans (McArthur, Billington et al. 2000). There was certainly no refinement of food, no food storage, no overcooking and no opportunity for vitamins and minerals to leech into the cooking water in the traditional

diet. Moreover, there was little waste. This changed with the reliance on the Western style of diet. One specific example of dietary change is damper, a type of bread used as a dietary staple. Damper is now made from refined white flour, but traditionally damper was made from the ground seeds of mulga, acacia, cycad, and a variety of grasses (Gracey 2000). Seeds and grasses are typically rich sources of magnesium. Today's version is a poor nutritional substitute for the original recipe. There are only limited records of pre-colonial diet patterns, with most assessments being based on remote populations who had retained their traditional lifestyle. These studies are subject to even more error than most dietary studies. How do you quantify foods eaten in the process of gathering, foods shared without portion control, or assess the nutrient value of bush foods that have not been analysed for content? Despite these problems, there is some indication that the native diet was substantially richer in mineral content than the Western diet. For example, using 1993 nutrient composition tables to assess the 1969 dietary records of Aboriginal hunter-gather groups, one study (Smith and Smith 2003) derived an average intake of 713 mg magnesium per day, more than double the modern estimated average requirement (EAR). Nutrient estimates extrapolated from a study of the Palaeolithic diet of hunter/ gatherers indicated magnesium intake, based on 3000 kcal per day, to be 1223 mg per day (Eaton and Eaton 2000), three and half times the EAR. These two studies suggest that the Australian Aboriginal people may have been adapted to a higher magnesium intake than they are currently consuming.

It is unrealistic to suggest Aboriginal people simply return to their traditional lifestyle. Most efforts to continue using bush foods meet substantial barriers. Traditional Aboriginal law prohibited taking from the land without permission of the traditional owners who may no longer be present. Knowledge of safe collection and handling of bush foods has been

largely lost in the disruption of tribal and family ties. Settling near or in urban centres has limited the availability of bush foods either by the loss of habitat or by over-harvesting. Whilst bush foods are still popular and culturally important, the actual total intake is generally low due to lack of access, dislocation, living in larger settlements, decreased transfer of cultural knowledge to youth, and decreased time due to employment (Riley 2000). Likewise, for the Torres Strait Islanders, life has changed. The subsistence gardening, a tradition of the past, meets many obstacles today such as the lack of available land, lack of water, quarantine restrictions, and the relative ease of purchasing food from the island store (Leonard, Beilin et al. 1995).

Rapid urbanization also distances people from the origin of their food as well as the understanding of the required commodities for a healthy diet (Wahlqvist 2005). Diets have changed from nutrient-dense to energy-dense foods, and are high in fat, especially saturated fat, and refined carbohydrates while being deficient in fibre, vitamins A, E, folate, and other nutrients (Gracey 2000; Kouris-Blazos and Wahlqvist 2000; Lee *et al* 1994a; O'Dea 1991a; Riley 2000; Shannon 2002). Little is published about the nutrient intake of Indigenous persons living in large urban centres. One study used a non-validated questionnaire to examine the food habits of Aborigines and persons of European decent in two country towns and one urban centre (Guest and O'Dea 1993). These authors reported that Aborigines ate take-away food and fried foods more frequently than Europeans, and that they reported a higher use of salt (Guest and O'Dea 1993). An evaluation of apparent dietary intake, as measured by store turnover, in six remote Aboriginal communities identified excessive intake of energy, sugars, and saturated fats, and inadequate intake of fibre, and several vitamins and minerals (Lee, O'Dea et al. 1994). Another study in rural Australia noted that the closer the Aboriginal community was to the store, the higher the

prevalence of impaired glucose tolerance (Gault, O'Dea et al. 1996). Magnesium was not assessed in either study. Finally, a small ethnographic study of Aborigines in Melbourne noted food habits were steeped with meaning and provided strong symbolic links to family, past, and home country. Foods that were seen as linking generations together, "traditional family food", included sugar, salt, meat and fat (Thompson, Gifford et al. 2000). It is interesting to note that foods dating from the transitional era of station and Mission living were now considered "traditional."

As with other native populations, the loss of their original native diet and adoption of the Western "diabetogenic" foods, has been detrimental to the Australian Indigenous population (Baschetti 2000). Food preferences and eating behaviours that once supported survival as hunter-gatherers now promote the development of obesity (O'Dea 1991). High rates of obesity, type 2 diabetes, hypertension, cardiovascular disease, and renal disease all tend to cluster as part of insulin resistance syndrome (Rowley, Best et al. 1997). However, nutrition is not the only determinant of the health disparity affecting Indigenous Australians. Because Indigenous people are no longer as physically active securing and preparing food, their lifestyle is much more sedentary (Shannon 2002). Moreover, it has been recently estimated that about one-fifth of total death and disability burden is attributable to socioeconomic inequality (2006). The Indigenous are more likely to be exposed to unemployment, lack of education, poor living conditions, poor sanitation or hygiene, smoking, drug and alcohol abuse, and violence (Australian Bureau of Statistics 2004; Australian Indigenous Health InfoNet 2002; Hetzel 2000; National Public Health Partnership 2001). Specific to the Aboriginal and Torres Strait Islander peoples are the following issues that have been identified as social determinants of health:

➢ Lower socioeconomic status (unemployment, lower incomes, poor educational attainment)

➢ Specific health behaviours such as higher rates of tobacco and alcohol use

➢ Remote locations with subsequent barriers to health care and access to fresh fruits and vegetables

➢ Poor housing and subsequent over-crowding

➢ Poverty in general

Living in remote communities regardless of ethnicity or socioeconomic status can challenge access to medical care. Indeed, in comparison to metropolitan areas, the burden of disease is estimated to be ten percent higher in regional areas and 21 percent higher in remote areas(2006). However, it is a misconception that the health disadvantage of Aboriginal people is due to living in remote locations; the urban Indigenous peoples also have poorer health which is partly due to socioeconomic disadvantage (2006). Moreover, while health determinants may be potent contributors to the disparity experienced by the Aboriginal and Torres Strait Islander people, they do not in themselves explain why the Australian Indigenous are so disproportionably affected. Native populations of the USA, Canada and New Zealand have very similar problems with the social determinants of health, and yet the health disparity experienced with regards to diabetes is much worse in Australia.

The "thrifty genotype" theory suggests humans evolved to maximize metabolic efficiency and that in time of abundance these genes predispose their carriers to diseases such as obesity and diabetes (O'Dea 1991). By incorporating an efficient system of converting excess protein to glucose and fat, in which gluconeogenesis is not suppressed by insulin,

plus a high capacity for hepatic lipogenesis, the author (O'Dea 1991) proposes that the feast would be maximally stored for the famine. Of interest to the present discussion is that the theory was presented in an interesting schematic that identified a metabolic advantage for insulin resistance in traditional hunter-gatherers such as the Australian Aboriginal people. It should be noted that the thrifty genotype hypothesis has not been substantiated by experimental findings despite the widespread use of the theory (Chukwuma and Tuomilehto 1998). In support of this point of view, the thrifty genotype would presumably also exist within the American Indian, Inuit, and Maori. Yet this theory, however popular it may be, does not adequately address the differing cross-county rates of diabetes mortality.

We can also consider genetic predisposition. The USA Pima Indians have, as noted earlier, an apparent disconnect between once instance of assessed sufficient magnesium intake (Reid, Fullmer et al. 1971) and a separate determination of low fasting plasma magnesium level (Paolisso and Ravussin 1995). It has been suggested, based upon the lower erythrocyte magnesium content found among Pima Indians, that there might be a pathological relationship between magnesium and insulin resistance that is independent of the race studied (Paolisso and Ravussin 1995). There have been several studies that draw similarities between the Pima Indians and the Australian Aborigines. As may be the case for the Pima Indians, two studies imply that the Australian Aboriginal people may have been adapted to a higher magnesium intake than they are currently consuming (Eaton and Eaton 2000; Smith and Smith 2003). In further support of the concept that there may be genetic (not racial) differences in the aetiology of diabetes, Torres Strait Islanders with two or more coexisting risk factors are almost all insulin resistant while approximately half of Aboriginal people with two or more coexisting risk factors exhibit insulin résistance (O'Dea and Rowley 2002). Recently, a mutation in a mitochondrial tRNA has linked the

presence of hypomagnesemia to hypertension and hypercholesterolemia (Wilson, Hariri et al. 2004). Since mitochondrial changes and a partial uncoupling of oxidative phosphorylation appear to occur at relatively early stages of magnesium deficiency (Heaton 1993), it has suggested that mitochondrial dysfunction may be responsible for the low magnesium concentration and the subsequent development of a constellation of symptoms similar to metabolic syndrome (Wilson, Hariri et al. 2004). A crucial mechanism by which animals and humans adapt to changing environments is phenotypic plasticity, the ability of the genotype to be expressed as different phenotypes. The role of magnesium in stabilizing DNA transcription might be an additional mechanism by which a deficit may influence phenotypic plasticity, subsequently modifying diabetes risk. Clearly, genetic or ethnic differences contribute to the negative effects we see from Westernization on native populations. However, research into the genetic basis for racial disparities has the potential to be confounded by issues of socioeconomic status and access for health care. As Baschetti (2000) eloquently argued, instead of looking for genetic markers of poor adaptation amongst the native populations, we could instead be looking for that rare mechanism by which Europeans seem better adapted to a diabetogenic diet.

1.4 Synopsis

This thesis will examine the case for magnesium as a contributor to the health disparity experienced by the Aboriginal and Torres Strait Islander people. The connection between magnesium and diabetes is becoming increasingly apparent and increasingly accepted (Saris, Mervaala et al. 2000; Fox, Ramsoomair et al. 2001; Walti, Zimmermann et al. 2003). Magnesium deficit has been posited to be the underlying common mechanism for the insulin resistance found in metabolic syndrome, hypertension, impaired glucose tolerance, and type 2 diabetes (Barbagallo, Dominguez et al. 2003; Barbagallo and

Dominguez 2007), all conditions experienced by the Australian Aboriginal and Torres Strait Islander people. Clinically, a graded inverse relationship between serum magnesium levels and type 2 diabetes has been described (Kao, Folsom et al. 1999), and diabetes co-morbidities appear to be influenced by magnesium deficit (De Leeuw *et al* 2004; de Valk 1999; Rodriguez-Moran and Guerrero-Romero 2001). Low serum magnesium levels significantly increase the prediction of all-cause mortality in diabetic patients (Haglin, Tornkvist et al. 2007) while diets replete in magnesium protect against the development of type 2 diabetes (Lopez-Ridaura, Willett et al. 2004; Song, Manson et al. 2004). Clearly there is a role for magnesium in the metabolic pathology of diabetes, which may, in part, help address the health disparity experienced by Indigenous Australians.

The focus of this thesis will be to establish a case for magnesium affecting the Australian Indigenous people, who are disproportionately affected by diabetes morbidity and mortality. First, the nutrient intake of a cohort of Aboriginal and Torres Strait Islander subjects will be examined and compared to the average Australian dietary intake. Then an ecological study will examine environmental correlates, including the magnesium level in drinking water, to diabetes mortality. A needs assessment study will establish if total serum magnesium is lower in Indigenous Australians with and without diabetes. Finally, total and free serum magnesium concentrations will be determined to identify any differences in magnesium status between diabetic and non-diabetic Indigenous and non-Indigenous Australians, and also to compare which of the two parameters is a more sensitive measure of magnesium status and diabetic risk.

A brief introduction will precede each experimental investigation, along with a summary of methodological protocol, which will be fully outlined in Chapter Two. Although each

chapter will report results specific to the chapter, many results will have implications to other aspects of the thesis. This will result in some overlap across the chapters in both interpretation and discussion. Finally a concluding general discussion will integrate the major conclusions drawn from each chapter.

CHAPTER 2:

STUDY METHODS

Ethics approval for the studies described herein was obtained from the Townsville Health Service District Ethics Committee and the Townsville Aboriginal and Islander Health Services Board of Directors ethics sub-committee. Additional community consultation was obtained at community diabetes events and from small focus groups held at TAIHS. All subjects gave informed consent for participation in the study.

2.1 Selection of study location

The current study was undertaken using the facilities at the Townsville Aboriginal and Islander Health Service (TAIHS), a community-controlled Aboriginal Medical Service located in a suburban area of Townsville, Queensland, Australia. This study location was selected on the following basis. In Australia, the Indigenous population is estimated at only 2.2-2.5% of the total. Amongst the Australian states in 2002, the Northern Territory had the highest representation with approximately 29% of all residents being Indigenous. However, only 13% of all Australian Indigenous people reside in the Northern Territory. More than half of all Indigenous people lived in New South Wales (29%) and Queensland (28%) (2002). Comparing diabetes mortality amongst the Australian States, the 1995 figures demonstrate the Northern Territory was the most severely affected with 31 / 1000 population dying due to diabetes, reflecting the heavy representation of Indigenous people. Focusing on the two States with the largest populations of Indigenous peoples, Queensland had a higher mortality rate of 14 / 1000 deaths due to diabetes compared to New South Wales with 11/ 1000 deaths due to diabetes (ABS 1998). Queensland was therefore chosen on the basis of the higher mortality in a state with the larger Indigenous population.

Within Queensland, the diabetes related mortality in the Northern Zone was 60-70% higher for both sexes compared to all of Queensland, despite the diabetes prevalence rate being

6.9% compared to 6.6% in all of Queensland (Queensland Health 2001). Prevalence of self-reported diabetes amongst those aged 18 years or older in Queensland was 6.6% in 2000, with a slightly higher rate of 6.9% for the Northern Zone among the non-Indigenous. Community surveys in Indigenous populations in North Queensland report a prevalence of 24% among Torres Strait Islanders aged 15 years and over, and 13% among Aboriginal people aged over 15 years (Health 2001). The Northern Zone is subdivided into four health reporting areas: the North West, Far North, Northern, and Mackay. Again focusing on the largest number of Indigenous people in an area, the Far North has the largest Indigenous population of 27,280, followed by the Northern reporting area with 11,117 Indigenous (Figure 2.1). Between these two areas, the Northern reporting area had the higher rate of deaths due to diabetes in 2002. In 2001, the area with the most Indigenous Australians, based on Census usual residence counts, was Townsville (4,369), which is located in the Northern reporting area (2002). In seeking to reach an adequate sized population of Indigenous people for the purpose of studying diabetes, Townsville, the largest population centre in Queensland outside of the state capital, was a logical choice.

Figure 2.1: Indigenous population and diabetes death rates for the Northern Zone of Queensland

Reporting Area	Indigenous population in 1999	% population Indigenous	2002 Diabetes deaths / 100,000
North West	8, 438	23.7	1136.4
Far North	27,280	12.3	194.9
Northern	11,117	5.6	374.7
Mackay	3845	3.1	525.9
Northern Zone	*51,122*	*8.8*	*381.4*
Queensland	*101,156*	*2.9*	*262.7*

The Townsville Aboriginal and Islander Health Service Ltd is a community-controlled Aboriginal Medical Service located in a suburban area of Townsville that serves a community of over 16,000 Indigenous residents. About 20% of the TAIHS patient population are non-Indigenous, mostly pensioners from the immediate neighbourhood. Diabetes is the number one reason for TAIHS general practitioner (GP) consultations (11.3 times per 100 doctor consults)(Larkins *et al* 2006). The capacity of TAIHS to support a research endeavour was evident from a recent history of successful investigations (Heath *et al* 2006; Heath and Panaretto 2005; Larkins *et al* 2006; Larkins *et al* 2007; Longstreet *et al* 2005; Panaretto *et al* 2006; Panaretto *et al* 2002; Panaretto *et al* 2005; Panaretto *et al* 2007). Therefore this study on magnesium and diabetes in the Aboriginal and Torres Strait Islander peoples was established at TAIHS.

2.2 Nutrient assessment study methods

Out of respect for the Aboriginal and Torres Strait Islander people, we strove to make the magnesium investigation as noninvasive as possible. In the context of Indigenous community consultation, it was suggested we use a existing program evaluation to obtain

data on magnesium intake. The program chosen was the Walkabout Together (WAT) program, which was part of a nutrient assessment study whose broader purpose was to evaluate the reported change in nutrient intake of adult urban Aboriginal and Torres Strait Islander people who participated in a medically-based lifestyle intervention program. Baseline nutrient intake was compared to an age and gender matched sample created from 1995 National Nutrition Survey data (McLennan and Podger 1995) to assess if there were differences between the Australian Indigenous and non-Indigenous nutrient intake. Observations about food intake were also recorded.

The subjects of the magnesium study were a subset of the Walkabout Together (WAT) Program, a prospective lifestyle intervention study located at TAIHS that followed a cohort of overweight (BMI>25) participants in an urban Indigenous community. About 20% of the TAIHS patient population are non-Indigenous. Medical staff referred WAT Program participants, and self-selection was actively encouraged. Comprehensive demographic and clinical data were recorded for all participants at baseline and 12 months. Weight was measured in light clothing, minus shoes, on a calibrated digital scale. Waist was measured as the horizontal circumference at the level of the umbilicus. Participants received nutrition and physical activity advice, and were provided a pedometer and log book. The dietary advice provided was based on the Australian Guide to Healthy Eating, modified as appropriate for identified co-morbidities. The cohort for this current study included all Indigenous WAT participants who completed (100 of 131) the 12-month assessment program. WAT received ethics approval from the Townsville Health Service District Ethics Committee and the TAIHS Board of Directors ethics sub-committee. All subjects gave informed consent at the onset of the WAT program. While not relevant to the present

study, further details on the specifics of the WAT program are available elsewhere to interested readers (Heath, Longstreet et al. 2006).

Analysis was based on multiple-pass 24-hour dietary recalls that were collected at baseline and at 12 months program completion. 10% of the Indigenous participants had an additional 24-hour dietary recall completed at 6 months. All dietary recalls were completed by two independent researchers (Diane Longstreet and Deanne Heath) using the multiple-pass 24 hour recall method (Johnson 2002). In the first pass, participants were asked to recall everything consumed the previous day. The second pass probed using a "forgotten food" list. The third pass used time, place, or event-related cues. A fourth pass used common household measures, plate, bowl and glass to clarify portion sizes. The final review simply asked the participant if anything was omitted. In an attempt to assess potential cultural issues that might impact accuracy of the diet interview process, a WAT participant satisfaction survey was performed by an Indigenous Health Worker. A short survey provided the framework for a casual discussion, creating a culturally safe environment in which individuals might freely express how they felt about the dietary interview process, how accurate they thought it might be, and which strategies they felt facilitated their recall.

To accommodate differences in the Australian food supply, nutrient analysis was completed using the Australian dietary software program FoodWorks Professional 2007, version 5.1 by Xyris (Queensland Australia) using the Nuttab 2006 and Nuttab Bush Foods 2006 Australian food databases. The day and month of interview date was tabulated.

Basal metabolic rate (BMR) and estimated energy expenditure was calculated using the Schofield equation (McCrory, McCrory et al. 2002) with the activity level being derived from WAT detailed physical activity assessment. Determining implausible intake was on the basis of baseline dietary histories and Goldberg's "cut-off 1" (McCrory, McCrory et al. 2002), which defines implausible intakes on a ratio of reported energy intake:BMR equalling a physical activity level of 1.35, which is consistent with the assessed sedentary WAT baseline activity level.

Baseline nutrient intake was evaluated against the 2006 Estimated Average Requirement (EAR). Consistent with the EAR cut-point method described by Murphy and Poos (Murphy and Poos 2002), if more than 2% of cases were below the EAR, the prevalence of inadequacy was interpreted as being high for that nutrient. A non- Indigenous "sample" was created from 1995 National Nutrition Survey (NNS) data (McLennan and Podger 1995) by extracting the mean NNS nutrient values from each age and gender survey group, matching case by case to each WAT participant. To control for energy intake, all nutrient intake was calculated per 1000 kJ. A non-parametric Mann-Whitney test was used to compare the nutrient means per 1000 kJ between the WAT participant intake and the matched NNS sample. A 95% confidence interval ($p=.05$) was accepted as significant. Changes in baseline and 12-month reported intake were evaluated using a paired-sample T test. A summary of general observations, including the most frequently reported foods, was noted. Food groups and portions were based on the Australian Guide to Healthy Eating, with mixed foods reflected as partial portions in appropriate groups. Where similar food groupings were reported, comparisons were made to the 1995 NNS data (McLennan and Podger 1995).

2.3 Environmental correlates study methods

The environmental correlates study examined the potential that magnesium might be a contributor to diabetes in Australia, especially for Aboriginal and Torres Strait Islander peoples. Specifically, we explored the associations between diabetes and the magnesium content of drinking water, as well as climatic and socioeconomic factors that may impact on magnesium status. Queensland age-standardized death rates due to diabetes were correlated with the magnesium content of drinking water, maximum average temperature, rainfall, unemployment rate, proportion of population with post-school qualification, weekly income, and the percent of population identified as Indigenous.

157 local government areas (LGA) and Aboriginal Councils in Queensland were identified and contacted regarding magnesium content of municipal water supplies (delivered product). Contact by telephone, email and /or facsimile was repeated a minimum of three times in an effort to obtain data from all areas. Due to difficulties that many LGA had in locating water test records, data collection was limited to only magnesium levels in the drinking water for 2002. All test results were derived from chemical analysis performed as part of routine water safety monitoring and met Queensland Health laboratory standards. Australian Drinking Water Guidelines specify both health-related and aesthetic guideline values (2004). Although operational monitoring guidelines include a recommended assessment of water hardness as $CaCO_3$, it does not require specific testing for magnesium. Therefore, not all LGA were able to provide data on the magnesium content of drinking water.

Age-standardized death rates for diabetes mellitus (International Classification of Diseases ICD-10 E10-E14), inclusive of diabetes as either underlying or associated cause of death in

2002, were obtained from the Australian Bureau of Statistics. All mortality data was derived from the Queensland Registrar of Births, Deaths, and Marriages, where cause of death and usual residential address is recorded on death certificates. Due to small populations in some LGA, available data was limited to ABS statistical divisions. Because of concerns regarding confidentiality, data was not available by Indigenous status. For additional points of comparison, the age-adjusted mortality data for the largest city in each statistical division and the balance of statistical division were obtained.

The Office of Economic and Statistical Research provided data on the proportion of each local government area population with post-school education qualifications (diploma, certificate, advanced diploma, graduate diploma, post-graduate and bachelors), median weekly household income, and percent unemployment for each LGA, with the original source being the ABS 2001 census. Annual rainfall and average maximum daily temperature were also obtained from OESR statistical division profiles, with original data from the Australian Bureau of Meteorology. A geographically dispersed selection of sample sites was obtained for each LGA to provide a representative sample of climate. The percentage of the population identified as Indigenous was also included in the analysis.

Available water data was evaluated at the local level with regard to mean, median, and range of magnesium content. Since the age-adjusted mortality data was only available for the largest city in each statistical division and the balance of statistical divisions, mean magnesium was aggregated to the same areas. All data was tested for normal distribution using the Kolmogorov-Smirnov test with Lilliefors correction. Where log-transformation successfully normalized distributions, Pearson's correlation was used. For non-normal distributions, nonparametric correlation of Spearman's rho was used to verify the bivariate

unadjusted linear associations. An independent sample t-test (two tailed) was used to examine differences between the cities and townships and the balance of the statistical divisions. Additionally, replicating the statistical approach of Franz and Bailey (Franz and Bailey 2004), we determined z-scores for rainfall and mean maximum daily temperature, and summed the z-scores to create a climate variable. A sum of z-scores for post-school qualifications, percent of population employed (inverse of unemployment), and mean weekly household income were used as a variable for socioeconomic environment. All statistical analyses were performed using SPSS v13.0 (www.spss.com). For spatial analysis, Map Info Professional v.7, a map software program used by the Australian Bureau of Statistics, was used to geo-code diabetes mortality and water magnesium levels (www.mapinfo.com/ location/integration).

2.4 Serum magnesium study methods

The purpose of this investigation was to investigate whether hypomagnesaemia was present in Indigenous and non-Indigenous people, in the presence and absence of diabetes. All Aboriginal and Torres Strait Islander people recruited for this study were TAIHS patients who presented for care and subsequently required fasting blood tests as part of routine care between August 2004 and February 2006. Additional non-Indigenous people were recruited from five GP practices in the Townsville area. Inclusion criteria included persons over the age of 15 (Tanner Stage 5) who had lived in the Townsville area for at least ten days. Exclusion criteria included chronic diarrhoea, alcoholism or binge drinking in the past two weeks, use of diuretics, consumption of magnesium supplements, reduced renal function (urinary albumin to creatinine ratio exceeding > 2.5 mg/mmol in men and > 3.5 mg/mmol in women), severe mental illness, pregnancy, or breastfeeding.

As part of ethics approval under Indigenous community consultation, this cross-sectional study was restricted to a convenience sample, integrated as part of on-going medical care, and included non-Indigenous subjects. The sample was divided into quarters comprising Indigenous people with type 2 diabetes, non-Indigenous with type 2 diabetes, Indigenous without diabetes, and non-Indigenous without diabetes. Power analysis determined that at least 92 subjects per group were required to achieve a power of 90% with a significance level of 5%, assuming a two-sided test.

A brief survey was administered to all subjects to verify the exclusion criteria and obtain a superficial measure of potential environmental factors affecting magnesium loss (e.g. sweat loss in the absence of air-conditioning). Medical records were reviewed for all TAIHS patients in the study. For subjects recruited from GPs, the most recent blood glucose level (BSL), glycosylated haemoglobin (HbA1c), blood pressure (BP), height, weight, and medications history were requested.   The diagnostic standards were confirmed symptoms of diabetes and a random (non-fasting) blood glucose > 11 mmol/L, or fasting plasma glucose $\geq$ 7.0 mmol/L, or a 2-hour plasma glucose > 11 mmol/L during an oral glucose tolerance test (Harris, Joyner et al. 2003). Venous blood samples were collected in sterile blood separation tubes (Becton-Dickinson 5ml vacutainer), serum separated, refrigerated immediately, and analysed for Mg_s in less than 24 hours using a colorimetric method with chlorphosphonazo III (COBAS INTEGRA 400®, Roche Diagnostics Australia Pty. Ltd., Castle Hill, NSW, Australia).

A multivariate analysis was designed to examine the effect of Indigenous status, diabetes, age, body mass index (BMI; kg/m^2), BSL, HbA1c, BP, use of BP medications, use of insulin, and exposure to air-conditioning / gross heat exposure. For BP analysis, in addition

to diastolic and systolic BP, degree of hypertension based on the National Heart Lung and Blood Institute (NHLBI) BP categories was examined. Gross heat exposure was based on six levels of exposure to air-conditioning: no air-conditioning at home or work; no air-conditioning at home but present at work; bedrooms only (night exposure) with none at work; night exposure and at work; most or all of the home air-conditioned but none at work; and full exposure to air-conditioning at both home and work. This was the first known attempt to evaluate heat exposure using this method.

Data from three or more groups was analyzed for significance using one-way ANOVA followed by Newman-Keuls posthoc tests. When comparing only two group means (e.g., diabetic Mg_s versus non-diabetic Mg_s), a two tailed t-test was used. Odds-ratios were calculated using the reference range established by the international Society for Magnesium Research (Spatling, Classen et al. 2000). A 95% confidence interval (p=0.05) was accepted as significant. Graph Pad Prism v. 5.01 and STATA v9.01 statistical software was used for all analyses (www.graphpad.com and www.stata.com).

2.5 Ionic magnesium study methods

This study was an extension of the serum magnesium study described in section 2.4 above. As above, this study was based at TAIHS where 20% of the patient population are non-Indigenous, mostly pensioners from the immediate neighbourhood. All Aboriginal and Torres Strait Islander subjects recruited for this study were TAIHS patients who presented for care and subsequently required fasting blood tests as part of routine care between August 2004 and February 2006. Additional non-Indigenous people were recruited from five GP practices in the Townsville area. Inclusion criteria included persons over the age of 15 (Tanner Stage 5) who had lived in the Townsville area for at least ten days. Exclusion

criteria included chronic diarrhoea, alcoholism or binge drinking in the past two weeks, use of diuretics, consumption of magnesium supplements, reduced renal function (urinary albumin to creatinine ratio exceeding > 2.5 mg/mmol in men and > 3.5 mg/mmol in women), severe mental illness, pregnancy, or breastfeeding.

A brief survey was administered to all subjects to verify the exclusion criteria and obtain a superficial measure of potential environmental factors affecting magnesium loss (e.g. sweat loss in the absence of air-conditioning). Medical records were reviewed for all TAIHS patients in the study. For subjects recruited from GPs, the most recent blood glucose level (BSL), glycosylated haemoglobin (HbA1c), blood pressure (BP), height, weight, and medications history were requested. The diagnostic standards were confirmed symptoms of diabetes and a random (non-fasting) blood glucose > 11 mmol/L, or fasting plasma glucose $\geq$ 7.0 mmol/L, or a 2-hour plasma glucose > 11 mmol/L during an oral glucose tolerance test. Venous blood samples were collected in sterile blood separation tubes (Becton-Dickinson 5ml vacutainer), refrigerated immediately, serum separated in less than 24 hours and stored at -80C before being analysed for ionic magnesium (Mg_i) concentration using a NOVA-8 STAT analyser equipped with ion selective electrodes (Nova Biomedical Canada Ltd., Mississauga, Ontario).

A multivariate analysis was designed to examine the effect of Indigenous status, diabetes, age, body mass index (BMI; kg/m^2), BSL, HbA1c, BP, use of BP medications, use of insulin, and exposure to air-conditioning / gross heat exposure. For BP analysis, in addition to diastolic and systolic BP, the degree of hypertension based on the National Heart Lung and Blood Institute (NHLBI) BP categories was examined. Gross heat exposure was based

on six levels of exposure to air-conditioning: no air-conditioning at home or work; no air-conditioning at home but present at work; bedrooms only (night exposure) with none at work; night exposure and at work; most or all of the home air-conditioned but none at work; and full exposure to air-conditioning at both home and work. This was the first known attempt to evaluate heat exposure using this method.

When comparing only two group means (e.g., diabetic Mg_i versus non-diabetic Mg_i), a two tailed t-test was used. Data from three or more groups was analyzed for significance using one-way ANOVA followed by Newman-Keuls posthoc tests. Linear regression analysis was used to examine correlations between total and ionic serum magnesium. A 95% confidence interval (p=0.05) was accepted as significant. Graph Pad Prism v. 5.01 and STATA v9.01 statistical software was used for all analysis (www.graphpad.com and www.stata.com).

CHAPTER 3:

DIETARY EVIDENCE

The health disparity experienced by the Australian Indigenous population is profound. In 2000-2002, the age-specific death rates for Aboriginal and Torres Strait Islander people were higher than the non-Indigenous for every age group, with the largest relative difference being five times higher for those aged 35 to 44 years old (2004). Overall, the death rates were 2.7 times higher for Indigenous males and 2.4 times higher for Indigenous females (2002). In a cross-country comparison, Australian Aboriginals and Torres Strait Islanders had the highest mortality rates amongst all population groups for cerebrovascular disease and diabetes (Bramley, Hebert et al. 2004). The cause of this health disparity is complex, and has its roots in the profound changes Westernisation has wrought on the Indigenous diet and lifestyle. Despite an extensive history of research on Australian Indigenous populations there remains a significant research gap regarding the nutritional issues pertaining to urban Indigenous communities, especially considering the largest concentrations of Indigenous people are located in urban areas (2000; 2002).

The purpose of this study was to evaluate the reported change in nutrient intake of adult urban Aboriginal and Torres Strait Islander people who participated in a medically-based lifestyle intervention program. Baseline intake was also compared to an age and gender matched sample created from 1995 National Nutrition Survey data (McLennan and Podger 1995) to assess if there were differences between the Australian Indigenous and non-Indigenous nutrient intake. Observations about food intake were also recorded. This is the first known study to report on the nutrient composition of foods consumed by an urban Indigenous population, and is therefore of broader potential use to the scientific community than just an assessment of only magnesium intake.

3.1 Methods

The subjects of this study were a subset of the Walkabout Together (WAT) Program, a prospective lifestyle intervention study that followed a cohort of overweight (BMI>25) participants in an urban Indigenous community that accessed the TAIHS located in Queensland, Australia (Heath, Longstreet et al. 2006). About 20% of the TAIHS patient population were non-Indigenous. Medical staff referred WAT Program participants and self-selection was actively encouraged. Comprehensive demographic and clinical data were recorded for all participants at baseline and 12 months. Weight was measured in light clothing, minus shoes, on a calibrated digital scale. Waist was measured as the horizontal circumference at the level of the umbilicus, or if the umbilicus had fallen then used the midpoint between the iliac crest and lowest rib. Participants received nutrition and physical activity advice, and were provided a pedometer and log book. The dietary advice provided was based on the Australian Guide to Healthy Eating, modified as appropriate for identified co-morbidities. The cohort for this current study included all Indigenous WAT participants who completed the 12-month assessment; 100 of 131 (76%) completed the program. WAT received ethics approval from the Townsville Health Service District Ethics Committee and the TAIHS Board of Directors ethics sub-committee. All subjects gave informed consent at the onset of the WAT program.

Analysis was based on multiple-pass 24-hour dietary recalls that were collected at baseline and at 12 months program completion. 10% of the Indigenous participants had an additional 24-hour dietary recall completed at 6 months. All dietary recalls were completed by two of independent researchers (Diane Longstreet and Deanne Heath) using the multiple-pass 24 hour recall method (Johnson 2002). In the first pass, participants were asked to recall everything consumed the previous day. The second pass probed using a "forgotten food" list. The third pass used time, place or event-related cues. A fourth pass

used common household measures, plate, bowl and glass to clarify portion sizes. The final review simply asked the participant if anything was omitted. In an attempt to assess potential cultural issues that might impact accuracy of the diet interview process, a WAT participant satisfaction survey was performed by an Indigenous Health Worker. A short survey provided the framework for a casual discussion, creating a culturally safe environment in which individuals might freely express how they felt about the dietary interview process, how accurate they thought it might be, and which strategies they felt facilitated their recall.

To accommodate differences in the Australian food supply, nutrient analysis was completed using the Australian dietary software program FoodWorks Professional 2007, version 5.1 by Xyris (Queensland Australia) using the Nuttab 2006 and Nuttab Bush Foods 2006 Australian food databases. The day and month of interview date was tabulated.

Basal metabolic rate (BMR) and estimated energy expenditure were calculated using the Schofield equation with the activity level being derived from WAT detailed physical activity assessment. Determining implausible intake was based on the baseline dietary histories using Goldberg's "cut-off 1", which defines implausible intakes based on a ratio of reported energy intake:BMR equalling a physical activity level of 1.35(McCrory, McCrory et al. 2002); consistent with the assessed sedentary WAT baseline activity level.

Basal metabolic rate (BMR) and estimated energy expenditure was calculated using the Schofield equation (McCrory, McCrory et al. 2002) with the activity level being derived from WAT detailed physical activity assessment. Determining implausible intake was on the basis of baseline dietary histories and Goldberg's "cut-off 1" (McCrory, McCrory et al.

2002), which defines implausible intakes on a ratio of reported energy intake:BMR equalling a physical activity level of 1.35, which is consistent with the assessed sedentary WAT baseline activity level.

Baseline nutrient intake was evaluated against the 2006 Estimated Average Requirement (EAR). Consistent with the EAR cut-point method described by Murphy and Poos (Murphy and Poos 2002), if more than 2% of cases were below the EAR, the prevalence of inadequacy was interpreted as being high for that nutrient. A non- Indigenous "sample" was created from 1995 National Nutrition Survey (NNS) data (McLennan and Podger 1995) by extracting the mean NNS nutrient values from each age and gender survey group, matching case by case to each WAT participant. To control for energy intake, all nutrient intake was calculated per 1000 kJ. A non-parametric Mann-Whitney test was used to compare the nutrient means per 1000 kJ between the WAT participant intake and the matched NNS sample. A 95% confidence interval (p=.05) was accepted as significant. Changes in pre-and post-program reported intake were evaluated using a paired-sample T test.

A summary of general observations, including the most frequently reported foods, was noted. Food groups and portions were based on the Australian Guide to Healthy Eating, with mixed foods reflected as partial portions in appropriate groups. Where similar food groupings were reported, comparisons were made to the 1995 NNS data (McLennan and Podger 1995).

3.2 Results

A total of 100 WAT Indigenous participants completed the twelve-month follow-up assessment with a total of 210 dietary recalls on file; 10 participants completed a six-month assessment. The participant ages ranged from 18 to 69 years old, with a mean age of 44.4 ± 1.3 SEM. There were 88 female and 12 male participants, with a mean BMI of 36.6 ± 0.65 SEM.

The distribution of months in which the recalls were collected was skewed toward February, the month in which the program began. The other 11 months of the year were evenly represented, allowing for good seasonal representation. The majority of recalls were performed on Tuesdays through Fridays; weekend dietary intakes were not adequately addressed. Distribution throughout the month allowed for adequate representation between weeks with and without pension payments.

Figure 3.1. Frequency of weekday attending for diet assessment
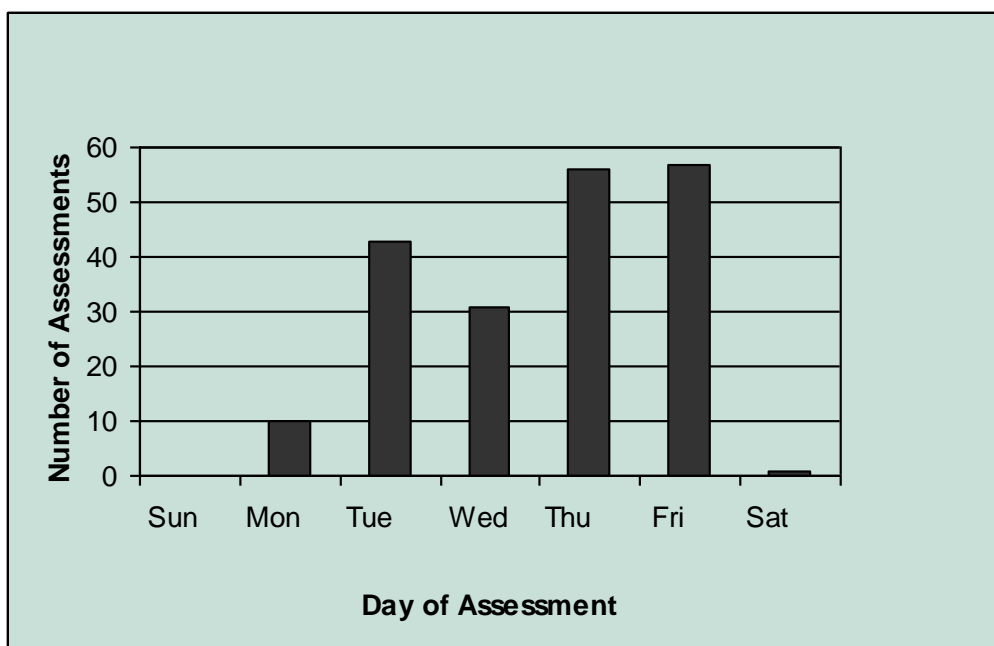
Estimated daily energy expenditure ranged from 7075 kJ – 14870 kJ (1692 – 3556 kcal). Before culling for implausible intakes, the estimated reported daily energy intake (EI) ranged from 2040 – 15155 kJ (487 – 3624 kcal). The mean EI was 7732 kJ for the baseline assessment and 6585 kJ for 12-month follow-up, which represented a significant decrease (p= 0.001). For the ten participants with three complete assessments, there was no significant difference between the EI at six-months and 12-months (p= 0.471). Thirty-five participants had implausible energy intakes at baseline and were removed from further nutrient analysis.

The WAT participants significantly decreased their energy, fat, and carbohydrate intake over the course of the program (Figure 3.2). Saturated fat dropped from a mean of 29 gm to 23 gm (p=0.02), although not to the level of the dietary guideline of 10% of energy. This is consistent with measured health outcomes of the program (Heath, Longstreet et al. 2006). After 12 months of enrolment in the WAT program, the mean weight decreased from 99.7 kg + 1.6 SEM to 97.8 kg + 1.6 SEM while waist circumference decreased from 113 cm + 1.2 SEM to 111 cm + 1.1 SEM.

Based on the percentage of cases with baseline nutrients below the EAR, there was a high prevalence of inadequacy for vitamin A, thiamine, riboflavin, niacin, folate, vitamin C, magnesium, calcium, phosphorus, iron and zinc (Figure 3.2). 1.54% of the cases were below the EAR for protein. The mean potassium level was above the Adequate Intake (AI) of 2,800 to 3,800 mg per day. The ratio of calcium to magnesium intake was 2.7 ± 1.08.

Figure 3.2: Reported nutrient intake comparing baseline to final assessment (n=65)

	Baseline intake mean ± sd	12-month intake mean ± sd	95% CI of the Difference	_p_	% baseline cases below EAR
Energy (kJ)	7548 ± 2700	6295 ± 2431	483.4 - 2022.7	0.002	
Protein (g)	84 ± 33	75 ± 28	-1.5 - 19.0	0.09	1.54%
Total fat (g)	70 ± 35	57 ± 33	2.5 - 24.0	0.02	
Saturated fat (g)	29 ± 16	23 ± 15	0.7 - 10.0	0.02	
Polyunsaturated fat (g)	9 ± 5	7 ± 4	0.3 - 3.8	0.02	
Monounsaturated fat (g)	26 ± 15	21 ± 13	0.2 - 9.6	0.04	
Cholesterol (g)	290 ± 185	219 ± 130	19.3 - 121.9	0.01	
Carbohydrate (g)	198 ± 78	164 ± 73	9.8 - 57.8	0.01	
Thiamine (mg)	1.3 ± .8	1.4 ± .9	-0.4 - 0.2	0.57	24.62%
Riboflavin (mg)	1.7 ± 1.0	1.4 ± .8	-0.1 - 0.5	0.13	29.23%
Niacin (mg)	19 ± 9.6	18 ± 7.8	-1.2 - 4.7	0.24	16.92%
Vitamin C (mg)	77 ± 70	90 ± 73	-37.2 - 10.0	0.25	21.54%
Folate (µg)	212 ± 89	210 ± 85	-25.5 - 30.8	0.85	47.69%
Vitamin A (µg)	665 ± 484	695 ± 580	-199.8 - 139.8	0.73	44.62%
Potassium (mg)	4604 ± 905	2263 ± 807	-4.7 - 549.4		*
Magnesium (mg)	242 ± 77	216 ± 74	4.8 - 46.4	0.02	60.00%
Calcium (mg)	690 ± 458	609 ± 395	-23.4 - 185.1	0.13	73.85%
Phosphorus (mg)	1339 ± 598	1130 ± 373	55.3 - 362.0	0.01	3.08%
Iron (mg)	10 ± 4	9 ± 4	-0.1 - 2.4	0.07	23.08%
Zinc (mg)	10 ± 5	10 ± 4	-1.0 - 1.8	0.58	29.23%

Paired sample T test on sample culled for implausible intake
EAR = Estimated Average Requirement
*Adequate Intake (AI) level for potassium is 2800mg for women and 3800mg for
men. Without an EAR, the same statistical handling is not valid.

Using the nutrient amount per 1000 kJ adjusts for variation in energy intake. The Mann-Whitney U test established that several of the mean nutrient levels (per 1000 kJ) were significantly different between the WAT participants and the sample derived from the 1995 Australian National Nutrition Survey (Figure 3.3). The estimated Indigenous intake of protein and cholesterol were significantly higher than the non-Indigenous. Polyunsaturated fat, fibre, vitamin A, vitamin C, niacin, potassium, magnesium, and iron were significantly lower. There was no significant difference for thiamine, riboflavin, calcium, phosphorus, or zinc.

Figure 3.3: WAT baseline intake compared to NNS, nutrients per 1000 kJ (n=65)

	Mean Difference ± SEM	95% CI of the Difference	*p*
Protein (g/MJ)	1.36 ± 0.37	0.61 - 2.11	0.001
Total fat (g/MJ)	0.04 ± 0.28	-0.53 - 0.60	0.90
Saturated fat (g/MJ)	0.15 ± 0.14	-0.14 - 0.43	0.30
Polyunsaturated fat (g/MJ)	-0.61 ± 0.08	-0.32 - 0.00	0.05
Monounsaturated fat (g/MJ)	0.04 ± 0.14	-0.24 - 0.33	0.76
Cholesterol (g/MJ)	5.66 ± 2.83	0.02 - 11.30	0.05
Carbohydrate(g/MJ)	-1.29 ± 0.74	-2.75 - 0.18	0.08
Fibre (g/MJ)	-0.4 0 ± 0.14	-0.69 - -0.11	0.01
Thiamine (mg/MJ)	0.00 ± 0.02	-0.03 - 0.04	0.77
Riboflavin (mg/MJ)	0.00 ± 0.02	-0.04 - 0.04	0.90
Niacin (mg/MJ)	-1.99 ± 0.13	-2.25 - -1.74	<.001
Vitamin C(mg/MJ)	-3.60 ± 1.50	-6.60 - -0.61	0.02
Folate (µg/MJ)	-0.65 ± 1.93	--4.5 - 3.21	0.74
Vitamin A (mg/MJ)	-50.66 ± 8.93	-68.47 - -32.85	<.001
Potassium (mg/MJ)	-30.32 ± 13.99	-58.18 - -2.46	0.03
Magnesium (mg/MJ)	-4.72 ± 1.27	-7.25 - -2.19	<0.001
Calcium (mg/MJ)	-79.1 ± 57.15	-193.24 - 35.04	0.17
Phosphorus (mg/MJ)	12.6 ± 10.72	-8.80 - 33.99	0.24
Iron (mg/MJ)	-0.21 ± 0.07	-0.36 - -0.07	<.001
Zinc (mg/MJ)	0.08 ± 0.07	-0.05 - 0.21	0.24

WAT= Walkabout Together Program
NNS = 1995 National Nutrition Survey data used to create matched sample
Mann-Whitney test on sample culled for implausible intake

The types of foods reported are summarized in Figure 3.4. In addition to take-away foods, frequently mentioned meals included stews, curries, roasts, and barbeque. Milk was most frequently consumed in tea or coffee or on breakfast cereal. 19% of the meats were processed (sausage, ham, bacon, and corned beef). Few traditional foods were mentioned (e.g. turtle, bush lemons, Burdekin plums), all of which were available in the Nuttab Bush Foods 2006 database. Lack of availability was often mentioned as a reason why bush foods were not eaten.

Figure 3.4: Summary of reported food intake from all Indigenous WAT participants

Food Group	Number of serves (mean ± sd)	Commonly reported foods (%of serves)
Grain	3.8 ± 1.4	White bread (49.5%), breakfast cereal (9.3%), white rice (11.9%), wholemeal bread 6.8%, crispbreads and savoury biscuits (6.5%), pasta 6.5%
Fruit	1.4 ± 1.5	Banana (19.7%), fruit juice (18.7%), orange (13%), apples (11.3%), dried fruit (5.3%, mostly in cereal)
Vegetable	2.2 ± 1.6	Potato (24.5, over one-third fried), tomato (12.8%), mixed vegetable (11.3), carrot (8.3%), lettuce (7.7%), pumpkin (4.4%)
Milk / Diary	1.2 ± 0.9	Cheese (47.5%), full cream milk (10.2%), low fat milk (9.2%), yoghurt (7.1%)
Meat / protein	1.9 ± 1.1	Beef (27.7%), chicken (24%), sausage (8.5%), fish (6.3%), egg (5.1%), pork (5.1%), bacon (5.1%)
Extras	3.9 ± 2.4	Butter (15.1%), added sugar (13.2%, mostly in tea and coffee), soft drink (11.0%), sweet biscuits/cookies (9.0%)

Based on 210 recalls from 100 participants,
Food groups and portions as defined by Australian Guide to Healthy Eating

The WAT satisfaction survey was completed by 33 / 100 participants. 84% reported that they felt comfortable answering questions about the foods they ate asked by the dietitian, nurse or doctor, while 9% acknowledged feeling embarrassed or shame when asked what they ate. 90% stated it was easy or very easy to remember what they ate the day before. Most (58%) did not have any specific suggestions to improve the interview process and "didn't have a problem with it", although 18% indicated suggesting foods or using a check-list of common foods might help.

3.3 Discussion

This study reported on diet amongst a group of Aboriginal and Torres Strait Islander people who participated in a medically-based lifestyle intervention program based in Townsville, Australia. Baseline dietary levels before intervention indicated poor nutrient density below the EAR, specifically in magnesium, vitamin A, thiamine, riboflavin, niacin, folate, vitamin C, calcium, phosphorus, iron and zinc. Our findings generally support the observation that Aboriginal and Torres Strait Islander people who live in urban communities eat a Western-style diet (2000) in that foods consumed were of Western origin. However, the average nutrient intake was found to be significantly different from the intake of non-Indigenous Australians. Importantly, 60% of the Indigenous people assessed had a dietary intake of magnesium below the estimated average requirement for half the population to have an adequate intake. Additionally, the average magnesium intake was found to be significantly lower than the intake of non-Indigenous Australians ($p<0.001$). The observed pattern suggests that diet, to include dietary magnesium intake, may be a potential contributor to the chronic disease burden experienced by the Indigenous population.

The difficulties of accurate dietary reporting have been well documented (Willett 1998). In the present study, under-reporting may be attributed to social desirability of lower reported intake and greater eating restraint. Other studies indicate obese individuals underestimate their energy intake by 30% to 47% (Amend *et al* 2007; Maurer *et al* 2006). The 35% rate of underreporting amongst the WAT participants is comparable to other studies (McCrory, McCrory et al. 2002), supporting the methodology chosen for this study. Recall strategies reported in the participation survey as being most helpful were similar to those used by other populations (Maurer, Taren et al. 2006) and were consistent with the

methods employed in the current study. This study therefore establishes the usefulness of standardized 24-hour recall procedures with urban Indigenous people.

Almost all of the 24-hour dietary interviews were completed on Tuesday through Friday, providing a snapshot of the week-day diet. However, adults consume more energy, fat, cholesterol and alcohol on the weekends, while vitamin and mineral intakes tend to be higher on week-days (McLennan and Podger 1995). That association may apply to this Indigenous population as well. One satisfaction survey respondent stated that "bush" foods were under-reported because we did not ask about weekend intake. Accordingly, the absence of recalls from the weekend was a potential flaw to this study. Another potential limitation of this study was the post-hoc study design. Some specificity on brand names and cuts of meat used in recipes was lost due to the time lapse between collection of data and entry into analysis. No attempt was made to assess the amount of added salt used, though heavy use of salt and salty foods has been described elsewhere (Guest and O'Dea 1993). The fact that the study cohort was actively seeking to improve their health and had received dietary advice suggests that the food and nutrient intakes reported by this study may also be different than that of the general Indigenous population.

Substantial barriers have been reported for the use of bush foods in urban areas. Whilst bush foods are still culturally important, the actual intake may be low due to lack of access, dislocation, living in larger settlements, decreased transfer of cultural knowledge to youth, and decreased time due to employment (Riley 2000) The frequency with which sweet biscuits (cookies), hot chips (french fries or fried potatoes), sausage, and full cream milk were reported was interpreted as an indication that the participants were sincere in their attempt to accurately report their intake. Although the foods reported were of Western

origin, the number of serves or the proportion of foods contributing to a food group differed from those reported in the National Nutrition Survey (McLennan and Podger 1995; McLennan and Podger 1995). There was a much higher use of white bread, red and processed meats, added butter and added sugar than what was reported in the National Nutrition Survey. A mean of 1.9 serves of meat, chicken and other protein foods is consistent with protein intake levels above the EAR cut-point. There was much less of use of fluid milk and more use of cheese amongst the WAT cohort. The higher use of cheese and full cream milk undoubtedly contributed to the saturated fat intake, although was insufficient as a source of calcium. While not assessed, the limited use of fluid milk might potentially be associated with lactose intolerance. The limited use of green vegetables combined with the low use of whole grains, legumes, and nuts is consistent with finding that magnesium intake is well below the EAR and significantly lower than the mean NNS magnesium level. The WAT cohort consumed on the average 1.4 serves of fruit and 2.2 serves of vegetable per weekday. Prior to the national 2005 "Go for 2 & 5" campaign to promote fruit and vegetable intake, the mean Australian daily intake was estimated at 2.0 serves of fruit and 2.6 serves of vegetables (2007). The current study may be useful in helping evaluate the population-based nutrition education campaigns being implemented through the National Aboriginal and Torres Strait Islander Nutrition Action Plan.

Historically there has been a perception that little if any benefit will occur through interventions with Aboriginal and Torres Strait Islander people (2000). This study demonstrated that measurable dietary intake change can be achieved through diet education and lifestyle modification. Decreases in food energy, total fat, saturated fat, and cholesterol were consistent with measured improvements in health parameters (Heath, Longstreet et al. 2006). The mean weight loss of 1.9kg, though significant, is less than the

2.7kg loss typically seen with ad lib low fat diets (2003). The nutrient patterns revealed in this study are still consistent with a potential association between diet and the chronic disease burden experienced by the Aboriginal and Torres Strait Islander population. The baseline high fat intake, especially high saturated fat, increases the risk of both cardiovascular disease and diabetes. Low levels of folate may contribute to homocystinuria, further increasing the risk of vascular disease. Lower intakes of magnesium, potassium, and calcium, combined with a previously observed high intake of sodium (Guest and O'Dea 1993), might foster hypertension. Lower fibre intake due to low use of whole grains, fruits and vegetables, further increases their risk for chronic disease. Low fibre has been associated with an increased risk of developing diabetes (Lopez-Ridaura, Willett et al. 2004), as has low dietary magnesium been identified as increasing the risk of developing type 2 diabetes in several large studies (Lopez-Ridaura, Willett et al. 2004; Song, Manson et al. 2004). There is clearly room for further qualitative improvements in dietary intake.

In conclusion, this study showed diet and lifestyle intervention can be successful with the urban Indigenous people. The study confirmed that this urban population typically consumed foods of Western origin, however the reported intake identified an average nutrient pattern that was significantly different from the typical Australian intake. It supports the premise that there is an association between diet and the chronic disease burden experienced by the Aboriginal and Torres Strait Islander population relative to the average Australian population. For this particular cohort, the study also confirms that dietary magnesium is low in the urban Indigenous diet, and significantly lower than in the general Australian population.

CHAPTER 4:

ENVIRONMENTAL CORRELATES

Diabetes in Aboriginal and Torres Strait Islander peoples occurs at a younger age and at almost four times the rate of non-Indigenous Australians (2001). In a cross-country comparison of four countries whose Indigenous populations were adversely affected by colonization by the British, Australia stands out as having the highest age standardized mortality rates for deaths due to diabetes amongst the Indigenous (Bramley, Hebert et al. 2004). While the cause for this disparity in diabetes incidence is multi-factorial, recent evidence suggests that nutrition, particularly magnesium intake, may play a role.

A significant relationship between magnesium and whole-body glucose homeostasis and insulin sensitivity has been previously demonstrated (Barbagallo *et al* 1997; Barbagallo *et al* 2003), with magnesium deficiency having been shown to induce insulin resistance even in normal subjects (Nadler, Buchanan et al. 1993). Dietary magnesium has also been found to be protective against the development of diabetes (Lopez-Ridaura, Willett et al. 2004; Song, Manson et al. 2004). Low serum magnesium has been identified in both Type 1 and Type 2 diabetes (Elamin and Tuvemo 1990; Saggese *et al* 1991; Walti *et al* 2003a), and the prevalence of low serum magnesium amongst diabetic outpatients has been reported to be 38% in Switzerland and 25% - 39% in USA (Nadler and Rude 1995; Walti, Zimmermann et al. 2003). Other populations with diabetes might, however, be more prone to low magnesium levels. For instance, Rodriguez-Moran and Guererro-Romero (2001) reported 73.1% of out-patient diabetics in Mexico had low serum magnesium, while 93.9% of diabetic subjects with foot ulcers had low serum magnesium. In a separate study, the same investigators found that up to 60% of diabetic subjects had low serum magnesium (Guerrero-Romero and Rodriguez-Moran 2000). Indeed, hypomagnesaemia is the most common electrolyte abnormality in diabetic outpatients and may be linked to the

development of both macrovascular and microvascular diabetic complications (Rodriguez-Moran and Guerrero-Romero 2001).

While the causes for low serum magnesium are unknown, a number of factors have been proposed including dietary intake, magnesium content in drinking water (Yang *et al* 1999; Zhao *et al* 2001), and magnesium loss through sweat (Franz and Bailey 2004). For Australia as well as the USA, the Recommended Dietary Intakes (RDI) for magnesium are 400 - 420 mg / day for adult men and 310 -320 mg / day for adult women (1997; NHMRC 2006). Population studies now use the Estimated Average Requirement (EAR), defined as the level needed to meet the daily requirements of half the healthy individuals in a particular life stage and gender group. The EAR for magnesium is set at 330-350 mg for adult men and 255-265 mg for adult women. The Australian National Nutrition Survey of 1995 (McLennan and Podger 1995) found that the mean daily intake for magnesium was 381mg for adult males and 283mg for adult females, both below the RDI but still meeting the needs of half the population. Similar dietary surveys in the USA also indicated magnesium intake was frequently below the RDI (1997). Moreover, the Australian 1995 survey showed that dietary intake of magnesium was lower for those at greater socio-economic disadvantage, and lowest amongst obese persons (McLennan and Podger 1995).

Magnesium content of water affects total body magnesium status (Rubenowitz *et al* 1998; Sabatier *et al* 2002; Verhas *et al* 2002). As magnesium salts in water may be highly bio-available (Sabatier *et al* 2003; Verhas *et al* 2002; Yokota *et al* 2004), they can make a significant contribution to dietary adequacy (Kiss *et al* 2004; Sabatier *et al* 2002; Verhas *et al* 2002). Azoulay et al (2001) reviewed the mineral content of tap water from 21 major USA cities. For half the tap water sources examined, 6% to 31% of the magnesium RDI was consumed from drinking two litres of water per day (Azoulay, Garzon et al. 2001).

Drinkers of magnesium-rich mineral water have significantly higher magnesium intake than those drinking low-mineralised or tap water (Galan, Arnaud et al. 2002). Evidence associating water hardness (from magnesium and calcium salts) with cardiovascular and other chronic diseases has gathered momentum in the last three decades (Ferrandiz *et al* 2004; Rubenowitz *et al* 1996; Rubenowitz *et al* 2000; Sauvant and Pepin 2002; Seelig 2002; Yang *et al* 1999). Not all studies have supported the association (Maheswaran *et al* 1999; Nerbrand *et al* 2003; Rosenlund *et al* 2005), and only a few ecological studies have specifically examined the association of magnesium in drinking water and diabetes (Mahaba 1998; Rosenlund *et al* 2005; Yang *et al* 1999; Zhao *et al* 2001). There may be a threshold effect involved (Durlach, Durlach et al. 1992). Overall the evidence seems to confirm the cardiovascular – water hardness link in areas with highest levels of water magnesium. The effect of water magnesium on diabetes morbidity and mortality has not been adequately investigated. Most urban water supplies remove magnesium as part of the water softening process.

Finally, blood magnesium levels are influenced by magnesium loss that can occur through sweat. Australia is called "the sunburnt country", with vast areas of blistering desert, and the northern third of Australia within the tropics. Accordingly, high temperatures and humidity are commonplace throughout much of the continent, with both of these factors expected to increase sweat loss. A review by Sawka and Montain (Sawka and Montain 2000) indicated that heat adaptation increases sweat loss. Residents of desert climates often experience sweat rates of 0.3 – 1.2 L/hour while performing occupational activities. Magnesium losses in sweat are approximately 0.8 mmol/L (range 0.2-1.5 mmol/LMg or 19-365 mg/day) (Sawka and Montain 2000). The only known studies on mineral sweat loss amongst Aboriginal people concluded differences in sweat concentrations was not

attributable to genetic differences but due to diet and lifestyle (2000), thus other studies on human sweat loss may be considered applicable to Aboriginal and Torres Strait Islander peoples. With consideration of the Australian climate, there may be potential for significant magnesium loss through sweat. Indeed, Franz and Bailey identified a climatic relationship to diabetes as well as heart disease, which they attributed to sweat loss of magnesium (Franz and Bailey 2004).

For the present investigation, we examined the relationship between magnesium and diabetes in Australia, especially in relation to Indigenous Australians. Specifically, we explore the associations between diabetes and the magnesium content of drinking water and diet, as well as climatic and socioeconomic factors that may impact on magnesium intake including temperature, rainfall, education, employment, and income.

4.1 Methods

157 local government areas (LGA) and Aboriginal Councils in Queensland were identified and contacted regarding magnesium content of municipal water supplies (delivered product). Contact by telephone, email and /or facsimile was repeated a minimum of three times in an effort to obtain data from all areas. Due to difficulties many LGA had in locating water test records, data collection was limited to only magnesium levels in the drinking water for 2002. All test results were derived from chemical analysis performed as part of routine water safety monitoring and met Queensland Health laboratory standards. Australian Drinking Water Guidelines specify both health-related and aesthetic guideline values. Although operational monitoring guidelines include a recommended assessment of water hardness as $CaCO_3$, it does not require specific testing for magnesium. Therefore, not all LGA were able to provide data on the magnesium content of drinking water.

Age-standardized death rates for diabetes mellitus (International Classification of Diseases ICD-10 E10-E14), inclusive of diabetes as either underlying or associated cause of death in 2002, were obtained from the Australian Bureau of Statistics (ABS). All mortality data was derived from the Queensland Registrar of Births, Deaths, and Marriages, where cause of death and usual residential address is recorded on death certificates. Due to small populations in some LGA, available data was limited to ABS statistical divisions. Because of concerns regarding confidentiality, data was not available by Indigenous status. For additional points of comparison, the age-adjusted mortality data for the largest city in each statistical division and the balance of statistical divisions were obtained.

The Office of Economic and Statistical Research provided data on the proportion of each local government area population with post-school education qualifications (diploma, certificate, advanced diploma, graduate diploma, post-graduate and bachelors), median weekly household income, percent unemployment for each LGA, and percent of population identified as Indigenous, with the original source being the ABS 2001 census. Annual rainfall and average maximum daily temperature were also obtained from Office of Economic and Statistical Research statistical division profiles, with original data from the Australian Bureau of Meteorology. A geographically dispersed selection of sample sites was obtained for each LGA to provide a representative sample of climate. The percentage of the population identified as Indigenous was also included in the analysis.

4.1.1 *Data analysis*

Available water data was evaluated at the local level with regard to mean, median, and range of magnesium content. Since the age-adjusted mortality data was only available for the largest city in each statistical division and the balance of statistical divisions, mean

water magnesium was aggregated to the same areas. All data was tested for normal distribution using the Kolmogorov-Smirnov test with Lilliefors correction. Where log-transformation successfully normalized distributions, Pearson's correlation was used. For non-normal distributions, nonparametric correlation of Spearman's rho was used to verify the bivariate unadjusted linear associations. An independent sample two tailed t-test was used to examine differences between the cities and townships and the balance of the statistical divisions. Additionally, replicating the statistical approach of Franz and Bailey (Franz and Bailey 2004), we determined z-scores for rainfall and mean maximum daily temperature, and summed the z-scores to create a climate variable. A sum of z-scores for post-school qualifications, percent of population employed (inverse of unemployment), and mean weekly household income were used as a variable for socioeconomic environment.

All statistical analysis was done using SPSS v13.0 (www.spss.com/). For spatial analysis, Map Info Professional v.7, a map software program used by the Australian Bureau of Statistics, was used to geo-code diabetes mortality and water magnesium levels (www.mapinfo.com/location/integration).

4.2 Results

Age standardized death rates for diabetes in 2002 for the statistical divisions of Queensland ranged between 52 and 191 per 100,000 population. Highest incidence occurred in the northern tropical statistical divisions while the lowest incidence occurred in the coastal divisions in the southern half of the state (Figure 4.1).

Figure 4.1.

Age-standardized diabetes death rates per 100,000 population by Statistical Dvision Queensland, Australia 2002
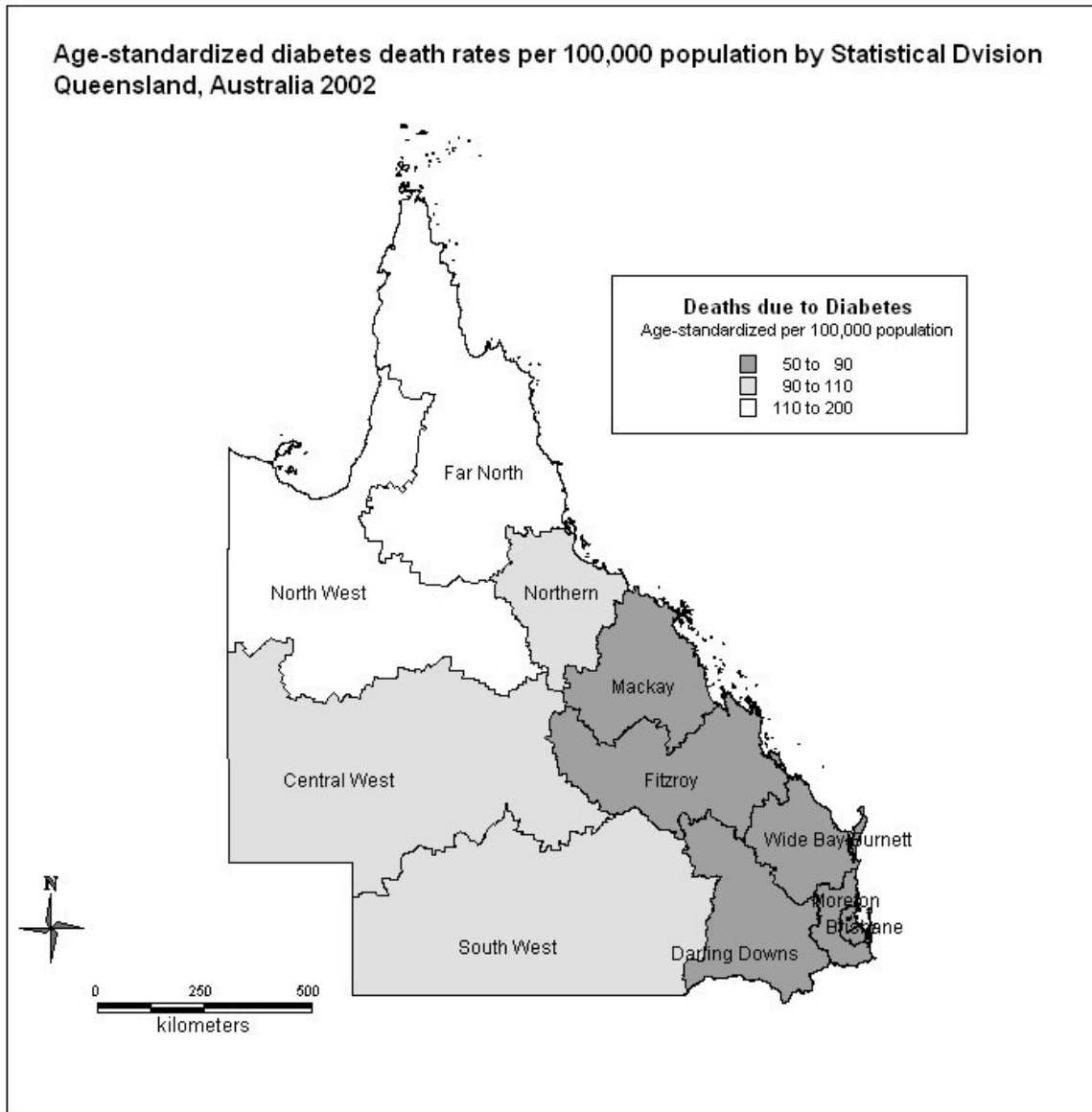
Magnesium data from 2002 was available from 88 of the 157 municipal councils and Aboriginal councils contacted. Councils who had data on water levels of magnesium covered 73% of the total population. 70.6% of the total Indigenous population was represented, though it was skewed toward coastal regions (Figure 4.2).

Figure 4.2. Missing magnesium data by coastal or inland region

Statistical Division (SD)	# LGA +AC	# no data	% SD with data	%Population represented	%Indigenous represented	Region
Brisbane	8	1	87.5%	77.4%	83.9%	Coastal
Moreton	9	1	88.9%	69.0%	74.9%	Coastal
Wide Bay Burnett	22	11	50.0%	47.5%	42.2%	Coastal
Fitzroy	13	3	76.9%	45.3%	47.3%	Coastal
Mackay	8	2	75.0%	91.5%	90.8%	Coastal
Northern	6	0	100.0%	100.0%	100.0%	Coastal
Far North	42	34	19.0%	80.2%	61.2%	Coastal
Total coastal	**108**	**52**	**51.9%**	**71.3%**	**72.7%**	
Darling Downs	19	5	73.7%	43.2%	39.5%	Inland
South West	10	4	60.0%	82.5%	92.0%	Inland
Central West	11	5	54.5%	81.8%	79.2%	Inland
North West	9	3	66.7%	78.5%	60.0%	Inland
Total Inland	**49**	**17**	**65.3%**	**53.7%**	**59.0%**	
Queensland	**157**	**69**	**56.1%**	**73.0%**	**70.6%**	

LGA + AC = Local Government Areas inclusive of Aboriginal Councils

Magnesium concentration in the drinking water ranged from a barely detectable 0.01 mg/L to as high as 150 mg/L. Outliers were validated by repeat inquiries to the original data sources and were retained in the analysis. The mean magnesium level was 13.0 mg/L and the median was 9.5 mg/ L. For the purposes of mapping (Figure 4.3), mean water magnesium data was sorted into three concentration levels indicated as low (0 to 6 mg/L), medium (6 to 11 mg/L), and high (11 to 30 mg/L).

Figure 4.3.
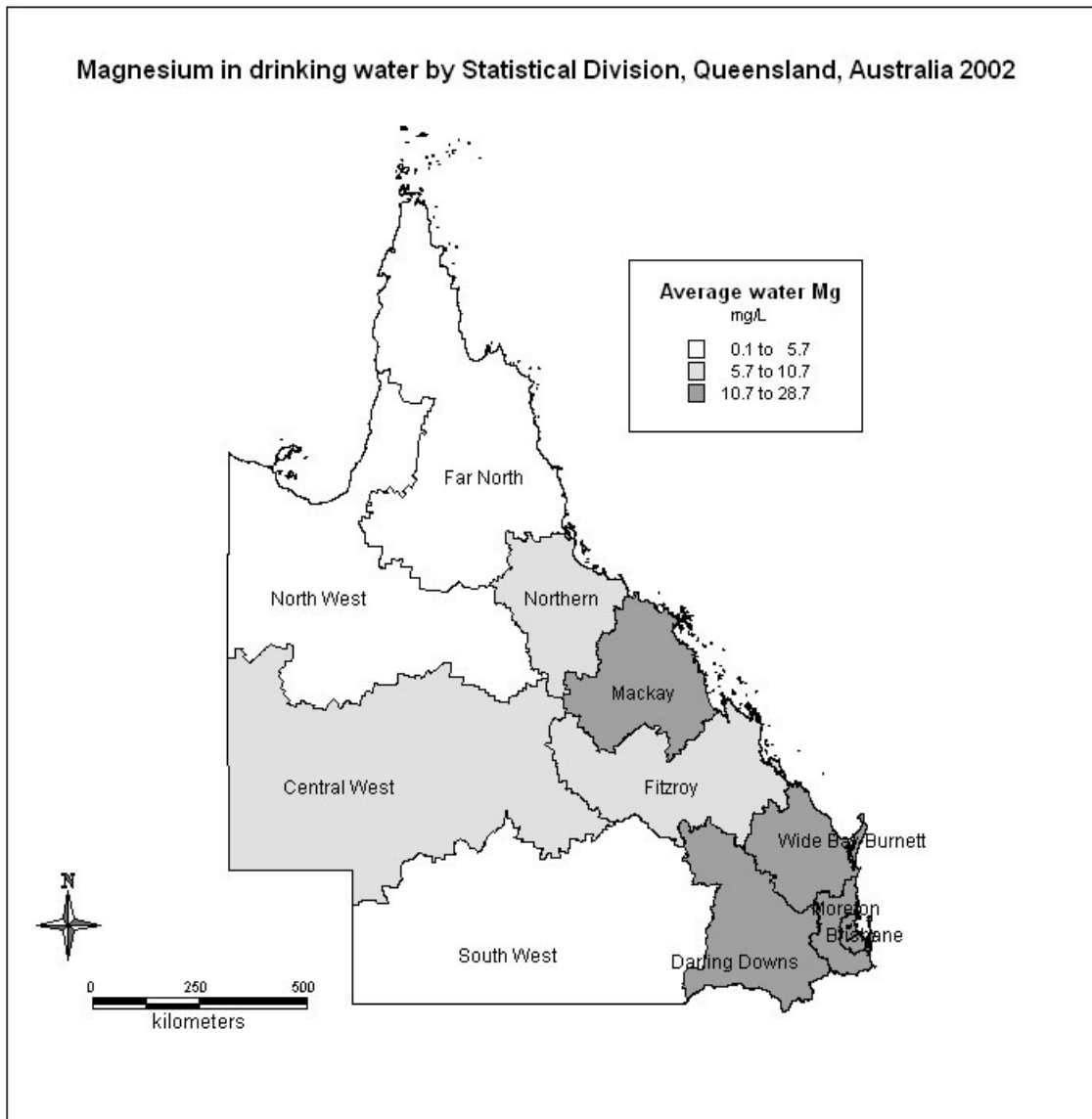
Interestingly, the area with the lowest reported levels of magnesium in the drinking water was the area with the highest reported Indigenous population (Figure 4.4).

Figure 4.4. Percent of the population identified as Indigenous in areas of low, medium, and high water magnesium.

Mg mg/L	% Indigenous
0 to 6	4.9%
6 to 10	1.7%
10 to 30	3.2%

Log transformation for water magnesium did not result in a normal distribution, so non-parametric methods were used for subsequent data analysis. Using SD and balance of SD data, there was a significant negative correlation between diabetes mortality rate and water magnesium levels ($r = -.422$, $p = 0.028$). No significant correlation was found between rainfall and the magnesium content of drinking water (Figure 4.5).

The diabetes mortality rate was positively correlated to the average maximum daily temperature ($r = 0.579$, $p = 0.002$), and to the percent of the population identified as Indigenous ($r = 0.673$, $p < 0.001$). The percent of the population identified as Indigenous was also positively correlated to the average maximum temperature ($r = 0.733$, $p < 0.001$), and negatively correlated to unemployment ($r = -.451$, $p = .027$). Higher proportions of the population with post-school qualifications correlated with higher incomes ($r = 424$, $p < .039$). Comparing cities and townships to rural areas revealed only one significant difference: post-school qualifications were more common in the urban areas ($t = 3.9$, 95% confidence interval 3.03-9.89, $p = .001$). Sum z-score variables for climate and socioeconomic factors did not reveal any additional correlations.

Figure 4.5. Summary of environmental data by area.

AREA	DM death rate (a)	Mean water Mg mg/L	% Indigenous Population	Average annual rainfall (mm)	Max Average Temp (C)	%Pop>15 yrs w/ post-school qualification (b)	% Unemployment (c)	Employment participation % (d)	Average Household weekly income
Brisbane City	61.00	10 ± 2.9	1.6	846	26.6	49.3	7.0	66.2	872
Balance Brisbane SD	76.00	9.5 ± 4.2	1.3	1103± 26	26± 1	40.0 ± 2.7	8.0 ± 2.0	65.7 ± 6.2	791 ± 138
Gold Coast City	46.30		1.1	1181	24.8	45.5	9.8	62.7	688
Sunshine Coast	50.30	5 ± 0.7	1.5	1597± 98	25± 1	45.1 ± 3.1	11.3 ± 0.3	57.6 ± 3.0	602 ± 23
Balance Moreton SD	69.10	36.3 ± 57.6	1.3	747± 62	25± 2	36.9 ± 1.9	9.7 ±2.4	57.5 ± 2.9	622 ± 36
Bundaberg	91.40	5.0	3.2	986	26.5	36.3	12.6	54.5	540
Hervey Bay City	99.50		2.3	967	26.1	39.5	15.2	45.7	482
Balance Wide Bay-Burnett SD	78.80	32.8 ± 29.7	3.1	883± 270	27± 1	35.9 ± 4.0	10.6 ± 4.6	55.6 ± 7.1	540 ± 73
Toowoomba	85.90		3	754	22.9	40.2	7.8	60.5	668
Balance Darling Downs SD	84.50	11.3 ± 9.7	2.5	658± 44	26± 2	34.7 ± 4.4	5.9 ± 2.2	64.6 ± 5.7	662 ± 116
Roma	93.50	0.01	7.3	600	27.7	35.9	5.3	72.3	778
Balance South West SD	108.70	0.4 ± .3	10.3	477± 115	28± 1	32.4 ± 2.5	4.3 ± 2.1	73.0 ± 6.8	659 ± 71
Gladstone	103.50	6.9	3.5	888	27.6	41.0	9.5	69	877
Balance Fitzroy SD	84.60	8.9 ± 9.7	4.4	666± 134	29± 1	38.0 ± 4.8	7.7 ± 5.7	64.5 ± 10.9	809 ± 203

AREA	DM death rate (a)	Mean water Mg mg/L	% Indigenous Population	Average annual rainfall (mm)	Max Average Temp (C)	%Pop>15 yrs w/ post-school qualification (b)	% Unemployment (c)	Employment participation % (d)	Average Household weekly income
Longreach	222.00	0.03	2	415	31.4	41.3	3.7	73.7	809
Balance Central West SD	51.20	6.9 ± 14.9	6.7	380± 114	30.9± 1	32.0 ± 3.0	4.1 ± 2.8	74.3 ± 5.9	750 ± 102
Mackay City Balance	107.00	8.0	3.7	1566	26.4	39.7	8.4	65.5	729
Mackay SD	65.70	24.4 ± 28.0	2.7	963± 298	29± 1	41.0 ± 6.2	6.0 ± 2.2	68.3 ± 7.2	900 ± 313
Townsville - Thuringowa	94.90	1.5 ± .7	4.8	1121	28.8	42.9 ± 4.0	8.4 ± 0.6	68.4 ± 3.0	850 ± 88
Balance Northern SD	107.30	13.1 ± 7.7	7.3	972±757	30±1	33.0 ± 2.1	6.2 ± 1.8	61 ± 5.9	668 ± 46
Cairns City Balance	97.70	1.1	8.3	2002	28.9	47.3	7.9	71.6	774
Far North SD	125.20	2.3 ± 2.4	16	1551±824	31±2	40.5 ± 7.9	7.5 ± 3.6	63.7 ± 6.7	633 ± 89
Mount Isa Balance	130.20	11.4	15.1	452	31.8	44.5	6.5	75.2	1112
North West SD	268.60	4.3 ± 5.6	30	624±286	33±1	35.5 ± 10.6	4.1 ± 1.4	71.1 ± 8.3	771 ± 112

(a) Age Standardized Death Rates using the indirect method and the 2001 Australian population; provided by Australian Bureau of Statistics. Expressed per 100,000 population, inclusive of diabetes as either underlying or associated case

(b) Post-school qualifications include postgraduate degree, bachelor degree, graduate diploma or certificate, and advanced diploma or certificate.

(c) Unemployment rate based on those identified as unemployed divided by the labour force (sum of employed and unemployed).

(d) Employment participation rate equals the labour force divided by the sum of those in the labour force plus those not in the labour force (age 15 and above).

4.3 Discussion

In the present study, we found the 2002 incidence of diabetes-related mortality in Queensland, Australia, was positively correlated to the percentage of the population that was of Aboriginal and Torres Strait Islander descent. These results are consistent with ABS reports that Indigenous deaths in Australia due to diabetes (2001-2002) accounted for 7.6% of the total deaths compared to 2.4% of the total non-Indigenous deaths (2001). We also report a correlation between the incidence of diabetes related mortality and the concentration of magnesium in drinking water. When considered in the context of lower magnesium intake that was observed in the regional Indigenous diet, total daily magnesium intake may be low in the Australian Indigenous population.

Extensive epidemiological evidence now exists suggesting that a high magnesium diet may protect against the development of diabetes. For example, the Women's Heart Study, with a cohort of 39,345 women and a 6-year follow-up, demonstrated a significant inverse relationship between dietary magnesium intake and the risk of developing diabetes (Song, Manson et al. 2004). An analysis that combined the Nurses Health Study and the Health Professionals' Follow-up Study, including 85,060 women (18 years follow-up) and 42,872 men (12 years follow-up), demonstrated that after adjusting for confounding variables, a magnesium-rich diet reduced the relative risk of developing diabetes by 34% in women and 33% in men (Lopez-Ridaura, Willett et al. 2004). In the Melbourne Collaborative Cohort Study, including 41,528 people between 1990 and 1994, total carbohydrates, sugars, and magnesium were inversely associated with diabetes incidence (Hodge, English et al. 2004). The Iowa Women's Health Study, evaluated by Meyer et al (2000) found a strong inverse association between the incidence of diabetes and intakes of total grains, whole grains, dietary fibre, cereal fibre, and dietary magnesium. This six year prospective

cohort study included 35,988 older women, and 1141 incident cases of diabetes. Fung et al (2003), in a study using a sub-set of the Nurses Health Study, found magnesium intake was inversely associated with fasting insulin concentration. Additional adjustment for glycaemic load and cereal fibre did not alter the association (Fung, Manson et al. 2003). One of the findings of the Atherosclerosis Risk in Communities study was a graded inverse relationship between serum magnesium levels and the risk of developing type 2 diabetes (Kao, Folsom et al. 1999). Humphries et al (1999) found a significant negative correlation of total dietary magnesium with the sum of insulin levels measured during an oral glucose tolerance test in a sample of young black Americans without diabetes. The association was interesting and may be indicative of magnesium being associated with insulin resistance prior to the development of diabetes in a black population (Humphries, Kushner et al. 1999). Clearly, most studies confirm that a high magnesium diet has a beneficial effect on reducing the risk of developing diabetes.

Both Australian and USA dietary surveys identified dietary intake was low in magnesium (McLennan and Podger 1995; 1997), and the study detailed in the previous chapter (Chapter 3) identified an Australian Indigenous cohort with magnesium intakes significantly lower than the average Australian diet. Magnesium deficiency has been shown to induce insulin resistance even in normal subjects (Nadler, Buchanan et al. 1993), and may be the underlying common mechanism by which insulin resistance links hypertension, lipid disorders, impaired glucose tolerance, type 2 diabetes, and metabolic syndrome (Barbagallo *et al* 2003; Guerrero-Romero and Rodriguez-Moran 2000), all of which are prevalent in the Australian Indigenous (2001).

The findings of the present study are consistent with prior studies that have correlated the level of magnesium in drinking water to diabetes (Mahaba 1998; Yang *et al* 1999; Zhao *et al* 2001). This does not imply causality, as ecological bias cannot be ruled out and the association observed at area level may be different than at the individual level. For example, the location of an individual's water consumption may not be reflected in the usual residence indicated on the death certificate. Furthermore, the water provided to one municipal area may be a blend from several sources with varying amounts of magnesium, and with seasonal variations (especially during times of drought). Finally, not all people living in an area receive their water from a municipal water system. While most Indigenous people live in cities and towns, and thus have access to municipal water, approximately one-quarter of the Indigenous population (108,085 of 458,520 in 2001) live in discrete Indigenous communities. Of these, 89,861 (20%) live in communities not connected to a municipal water supply (2004).

Despite these confounders, the fact that this study found a correlation between the incidence of diabetes-related mortality and the concentration of magnesium in drinking water suggests that further studies are warranted. When considered in the context of the lower magnesium dietary intake observed in the previous chapter, total daily magnesium intake may be low. This deficit might be exacerbated in an environment where magnesium sweat loss is increased. This supposition is supported by a highly significant correlation between average daily high temperature and incidence of diabetes related mortality observed in the present study. The correlations observed with lower post-school qualification amongst the indigenous, with subsequent association of lower socioeconomic status, may also contribute in that these individuals might be less likely to have access to

heat relief such as air-conditioning, either at home or in the workplace, and dietary choices may be limited by income.

The evidence presented here all contributes, in part, to a case suggesting that magnesium may be a potential contributor to diabetes in Australia, especially for Aboriginal and Torres Strait Islander peoples. It is plausible that a combination of lower magnesium intake in diet, increased loss of magnesium through sweat, and an inability to adequately replace lost magnesium through drinking water may create a unique situation predisposing an individual to hypomagnesaemia and diabetes. Indeed, Australia has the unenviable reputation of having the highest diabetes related mortality in the world in its Indigenous population (Bramley, Hebert et al. 2004).

Whether such hypomagnesaemia exists in this Indigenous population has not been determined. However, such an association between hypomagnesaemia and diabetes has been well described in other populations (Ma *et al* 1995; Rodriguez-Moran and Guerrero-Romero 2001; Walti *et al* 2002). Despite the growing body of evidence supporting the involvement of magnesium in diabetes, the need to consider magnesium status has not been integrated into primary health care for diabetes. This study adds additional support to the case for further investigation of potential relationship between magnesium and diabetes in the Australian Indigenous populations, especially since a deficiency might be readily addressed. The following two chapters examine the question of whether hypomagnesaemia is a factor amongst the Australian Indigenous population, and whether any such hypomagnesaemia may be associated with the presence of diabetes.

CHAPTER 5:

SERUM MAGNESIUM STUDY

The clinical correlation between low plasma magnesium and diabetes pathology has been investigated extensively over the past two decades (Fox *et al* 2001; Saris *et al* 2000; Walti *et al* 2003a) and both type 1 and type 2 diabetes have been associated with low serum magnesium concentration (Djurhuus *et al* 2001; Takaya *et al* 2003; Walti *et al* 2003a). Co-morbidities associated with both types of diabetes appear to be influenced by magnesium deficit (De Leeuw *et al* 2004; de Valk 1999; Rodriguez-Moran and Guerrero-Romero 2001) and accumulating evidence supports the concept that magnesium deficit may be an underlying common mechanism for the insulin resistance found in metabolic syndrome, hypertension, impaired glucose tolerance, and type 2 diabetes (Barbagallo *et al* 2003; Barbagallo and Dominguez 2007). As part of the Atherosclerosis Risk in Communities study, a 6-year follow-up of patients with initial low serum magnesium concentration (Mg_s) showed that there was an increased incidence of subsequent diabetes in these subjects (Kao, Folsom et al. 1999). Furthermore, epidemiological studies have identified that diets replete in magnesium protect against the subsequent development of diabetes (Lopez-Ridaura, Willett et al. 2004; Song, Manson et al. 2004). This evidence clearly suggests a role for magnesium in the metabolic pathology of diabetes.

The health disparity experienced by the Australian Indigenous population is profound. Diabetes comprises a significant part of the morbidity and mortality experienced by Aboriginal and Torres Strait Islander populations (Ring and Firman 1998; 2004). In a cross-country comparison, Australian Aboriginals and Torres Strait Islanders had the highest age-standardized diabetes mortality rates compared to the native populations in USA, Canada, and New Zealand (Bramley, Hebert et al. 2004). In 1999-2001, deaths due to endocrine and metabolic disorders (predominantly diabetes) were eight times higher for Indigenous males and 12 times higher for Indigenous females compared to their non-

Indigenous counterparts (2004). The cause of this health disparity is complex and potentially has its roots in the drastic changes Westernisation has wrought on the diet and lifestyle of the Aboriginal and Torres Strait Islander people. Despite an extensive history of research on Australian Aboriginal populations, there remains a significant research gap regarding urban Indigenous communities, especially considering the largest concentrations of Indigenous people are located in urban areas (2002).

No study has previously investigated the serum magnesium (Mg_s) status in an Aboriginal and Torres Strait Islander population and examined whether Mg_s might play a role in the health disparity experienced by this population. Accordingly, this study examined whether low Mg_s is associated with diabetes in urban Indigenous Australians, and whether low Mg_s is more prevalent in Indigenous versus non-Indigenous, diabetic and non-diabetic Australians.

5.1 Methods

5.1.1 *Subjects and setting*

Townsville Aboriginal and Islander Health Services (TAIHS) is a community-controlled Aboriginal Medical Service located in a suburban area of Townsville, Queensland, Australia that serves a community of over 16,000 Indigenous residents (Seebeck, Shepherd et al. 2006). About 20% of the TAIHS patient population are non-Indigenous, mostly pensioners from the immediate neighbourhood. Diabetes is the number one reason for TAIHS general practitioner (GP) consultations (11.3 times per 100 doctor consults) (Larkins *et al* 2006). All Aboriginal and Torres Strait Islander people recruited for this study were TAIHS patients who presented for health monitoring and subsequently required fasting blood tests as part of routine care between August 2004 and February 2006.

Additional non-Indigenous people were recruited from five GP practices in the Townsville area. Inclusion criteria included persons over the age of 15 (Tanner Stage 5) who had lived in the Townsville area for at least ten days. Exclusion criteria included chronic diarrhoea, alcoholism or binge drinking in the past two weeks, use of diuretics, consumption of magnesium supplements, reduced renal function (urinary albumin to creatinine ratio exceeding > 2.5 mg/mmol in men and > 3.5 mg/mmol in women), severe mental illness, pregnancy, or breastfeeding. Ethics approval was obtained from the Townsville Health Service District Ethics Committee and the TAIHS Board of Directors ethics sub-committee. Additional community consultation was obtained at community diabetes events and from small focus groups held at TAIHS. All subjects gave informed consent for participation in the study.

5.1.2 *Study design*

As part of ethics approval under Indigenous community consultation, this cross-sectional study was restricted to a convenience sample, integrated as part of on-going medical care, and included non-Indigenous subjects. The sample was divided into quarters comprising Indigenous people with type 2 diabetes, non-Indigenous with type 2 diabetes, Indigenous without diabetes, and non-Indigenous without diabetes. Power analysis determined that at least 92 subjects per group were required to achieve a power of 90% with a significance level of 5%, assuming a two-sided test.

A brief survey was administered to all subjects to verify the exclusion criteria. Noting that the previous environmental study had identified a significant positive correlation between diabetes mortality rate and the average maximum daily temperature ($r = 0.579$, $p = 0.002$), potentially mediated through magnesium sweat loss, the survey was expanded to include a

gross indicator of heat exposure / absence of heat relief from air conditioning. Additional questions pertaining to caffeine intake were included to assess any potential diuretic effect from excess caffeine intake. Medical records were reviewed for all TAIHS patients in the study. For subjects recruited from GPs, the most recent blood glucose level (BSL), glycosylated haemoglobin (HbA1c), blood pressure (BP), height, weight, and medications history were requested. The diagnostic standards were confirmed symptoms of diabetes and a random (non-fasting) blood glucose > 11 mmol/L, or fasting plasma glucose ≥ 7.0 mmol/L, or a 2-hour plasma glucose > 11 mmol/L during an oral glucose tolerance test (Harris, Joyner et al. 2003). Venous blood samples were collected in sterile blood separation tubes (Becton-Dickinson 5ml vacutainer), serum separated, refrigerated immediately, and analysed for Mg_s in less than 24 hours using a colorimetric method with chlorphosphonazo III (COBAS INTEGRA 400®, Diagnostics Australia Pty. Ltd., Castle Hill, NSW, Australia).

5.1.3 *Statistical analysis*

Data from three or more groups was analyzed for significance using one-way ANOVA followed by Newman-Keuls posthoc tests. When comparing only two group means (e.g., diabetic Mg_s versus non-diabetic Mg_s), a two tailed t-test was used. Odd-ratios were calculated using the reference range established by the international Society for Magnesium Research (Spatling, Classen et al. 2000). A multivariate analysis was performed to examine the effect of age, body mass index (BMI; kg/m^2), BSL, HbA1c, BP, use of BP medications, use of insulin, and exposure to air-conditioning / gross heat exposure. For BP analysis, in addition to diastolic and systolic BP, degree of hypertension based on the National Heart Lung and Blood Institute (NHLBI) BP categories was examined. Gross heat exposure was based on six levels of exposure to air-conditioning; no

air-conditioning at home or work, no air-conditioning at home but present at work, bedrooms only (night exposure) with none at work, night exposure and at work, most or all of the home air-conditioned but none at work, and full exposure to air-conditioning at both home and work. Gross estimates of exposure to air-conditioning were ranked based on the following assumptions; work exposure estimated at 40 hours per week, bedroom only exposure estimated at 56 hours per 24 hour day, combined night and work exposure estimated at 96 hours per week, full home air conditioning but none at work estimated at 128 hours per week, and full exposure to air conditioning estimated at 168 hours per week. A 95% confidence interval ($p=0.05$) was accepted as significant. Graph Pad Prism v. 5.01 and STATA v9.01 statistical software was used for all analysis (www.graphpad.com and www.stata.com).

5.2 Results

Of 425 subjects initially recruited, eight were removed from the study due to the presence of exclusion criteria in the medical record review (three diabetic subjects were in early renal failure, four were receiving diuretics and one non-diabetic subject was taking magnesium supplements). Overall, the sample distribution was more female (61.6%) than male (38.4%), and this was representative of the four individual groups (Figure 5.1).

There was no significant difference between the ages of males and females. The non-diabetic subjects were significant younger ($p<0.001$) and less obese ($p<0.001$) than the diabetic subjects. There was no significant difference in BMI between Indigenous and non-Indigenous subjects ($p = 0.739$), or male and female subjects ($p = 0.149$). Both Indigenous and non-Indigenous subjects with diabetes had significantly higher BMIs; the mean BMI

Figure 5.1: Serum magnesium study patient characteristics (n = 417)

Characteristic	Indigenous, Diabetics (n= 105)	Non-Indigenous, Diabetics (n= 104)	Indigenous, Non-Diabetics (n= 103)	Non-Indigenous, Non-Diabetics (n= 105)
Age (years)	51.9 ± 13.3	61.2 ± 12.2	44.2 ± 16.3	41.9 ± 18.4
Sex (percentage male)	37.1%	48.1%	36.9%	31.4%
BMI (kg/m^2)	31.4 ± 6.4	32.2 ± 6.1	29.0 ± 7.2	28.0 ± 7.3
Blood Glucose (mmol/L)	10.2 ± 5.2	8.7 ± 3.8	5.5 ± 1.0	5.2 ± 0.7
HbA1C (%)	8.4 ± 2.1	7.5 ± 1.9	6.2 ± 1.1	5.5 ± 0.5
Number years in Townsville	20.4 ± 16.1	24.9 ± 21.1	21.1 ± 16.5	17.8 ± 19.0
Hours heat exposure / week	3.84 ± 6.81	6.24 ± 11.51	6.84 ± 13.08	4.28 ± 5.68
Servings caffeine / day	2.97 ± 0.18	3.38 ± 0.26	3.17 ± 0.25	1.99 ± 0.19
Diabetic Medications			Not applicable	Not applicable
Oral Medications	69.5%	54.8%		
Insulin and tablets	10.5%	5.8%		
None	9.0%	17.3%		
Blood Pressure				
Normal BP	15.24%	4.81%	21.36%	25.71%
Prehypertension	41.90%	46.15%	41.75%	28.57%
Stage 1 Hypertension	28.57%	26.92%	19.42%	11.43%
Stage 2 Hypertension	12.38%	12.50%	14.56%	2.86%
Missing Data	1.90%	9.62%	2.91%	31.43%
Blood Pressure Medications	55.24%	53.85%	27.18%	13.33%
A2A	4.76%	8.65%	2.91%	3.81%
ACE Inhibitors	32.40%	25.00%	11.65%	6.67%
Beta blockers	7.62%	5.77%	6.80%	1.90%
CCB	1.90%	2.88%	1.94%	0.95%
Combination of 2+	8.57%	11.54%	3.88%	0.00%

BMI = Body Mass Index (kg/m^2); HbA1c = % glycosylated haemoglobin

BP= blood pressure; NHLBI blood pressure categories: Normal systolic <120mmHg and diastolic < 80mmHG, Prehypertension systolic 120 – 139 mmHG or diastolic 80-89 mmHG, Stage 1 hypertension systolic 140 -159 mmHG or diastolic 90-99 mmHG, and Stage 2 hypertension systolic >=160mmHG or diastolic >= 100 mmHG.

A2A = angiotensin 2 antagonist; ACE = angiotensin converting enzyme inhibitor; CCB = calcium channel blocker

of diabetics was 31.78±6.24 versus a mean BMI of 28.61±7.25 for non-diabetics (p = 0.001). Indigenous diabetics displayed negative correlations between blood glucose levels and Mg_s (r = -0.204, p = 0.004). Additionally, all diabetic subjects' Mg_s were negatively correlated with HbA1C levels (r = -0.188, p = 0.027). The Indigenous diabetic subjects had significantly poorer diabetes control than the non-Indigenous subjects as measured by blood glucose (p=0.018) as well as HbA1c (p=0.005). The non-Indigenous non-diabetics had a mean HbA1c of 6.2 ± 1.1, which might indicate undiagnosed glucose impairment, despite the fact all Indigenous medical records were completely reviewed for medical diagnosis. There was no significant difference in self-reported exposure to air-conditioning between Indigenous and non-Indigenous subjects, and no significant difference in the length of time each group had resided in the Townsville area.

**Figure 5.2: Serum magnesium (Mg_s) by group**

	Indigenous diabetic	**non-Indigenous diabetic**	**Indigenous non-diabetic**	**non-Indigenous non-diabetic**
Mean Mg_s ± sd	0.82 ± .08	0.83 ± .09	0.86 ± .07	0.88 ± .06
Low Mg_s	60	46	38	18
Normal	45	58	65	87
Total	105	104	103	105
% Low Mg_s	57.2%	44.3%	36.9%	17.2%

sd = standard deviation; Cut off for normal > 0.80 mmol/l per Society for Magnesium Research (Spatling, Classen et al. 2000)

Figure 5.2 reports the serum magnesium results. ANOVA of Mg_s revealed statistically significant differences in the mean Mg_s between the groups (Figure 5.3; p<0.001). As expected, Mg_s was significantly lower in the presence of diabetes (p<0.001) in both the Indigenous and non-Indigenous groups. 36.9% of non-Indigenous diabetic Australians had low Mg_s (mean Mg_s 0.83 ± 0.08), whereas 57.2% of the Indigenous had low Mg_s (mean of 0.82 ± 0.09). The mean Mg_s between the Indigenous diabetics and non-Indigenous

diabetics were not statistically different (p=0.233). However, in examining the prevalence of low Mg_s among diabetics, the Indigenous were 1.68 times more likely to have low Mg_s than non-Indigenous diabetics (confidence interval 0.97 – 2.9). More importantly, there was a significant difference in mean Mg_s between the Indigenous and non-Indigenous non-diabetic subjects (p =0.006). 36.9% of the Indigenous non-diabetics had low Mg_s in comparison to 17.2% of the non-Indigenous non-diabetics, representing an odds ratio of 2.8 times higher (confidence interval 1.48 – 5.39).

Figure 5.3. Serum magnesium concentration of both Indigenous and non-Indigenous subjects with or without diabetes (DM)
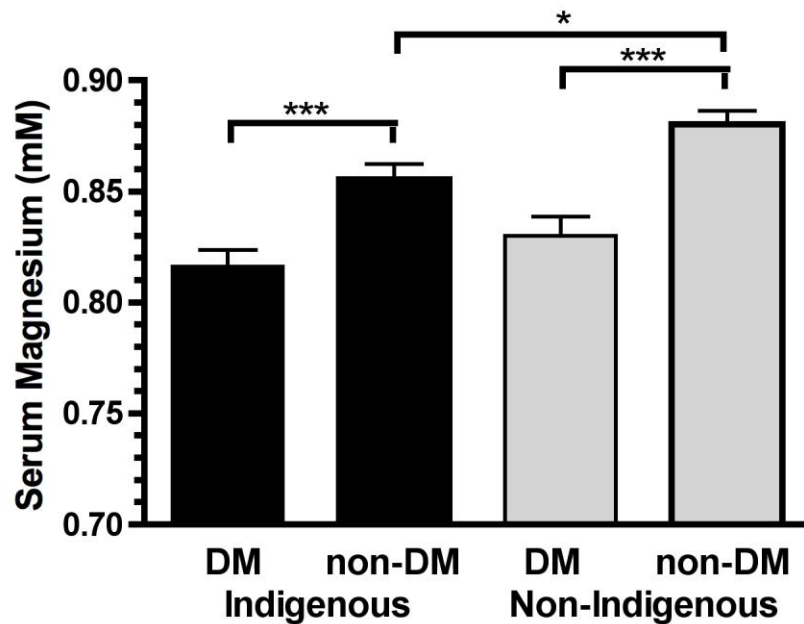
$*$ = p < 0.05; $***$ = p < 0.001.

Multivariate analysis demonstrated that there was no significant effect of Mg_s from age (p = 0.995), BMI (p = 0.437), systolic BP (p=.435), diastolic BP (p=.355), NHLBI hypertensive category (p =0.792), use of BP medications (p =0.485), insulin (p=0.142,

caffeine consumption (p=0.8392) or exposure to air-conditioning (p=0.1147). In regression analysis, the only variable that sustained a significant effect on Mg_s was BSL (p=.035).

5.3 Discussion

The current study is the first to demonstrate that diabetes in both Indigenous and non-Indigenous Australians is associated with reduced levels of Mg_s. This is consistent with a number other studies in different communities that have shown an association between reduced Mg_s and the presence of diabetes (Djurhuus *et al* 2001; Rodriguez-Moran and Guerrero-Romero 2001; Takaya *et al* 2003; Walti *et al* 2003a). A prevalence of about 14% hypomagnesaemia in the absence of diabetes has been previously identified (Schlingmann, Konrad et al. 2004) which is similar to levels found in non-Indigenous non-diabetics but much lower than the 36.9% low Mg_s found in the non-diabetic Indigenous Australian cohort. We suggest that this reduced Mg_s may place the Aboriginal and Torres Strait Islander community in Australia at greater risk for the subsequent development of diabetes.

Previous studies have shown that low Mg_s is associated with a higher incidence of subsequent diabetes onset over a six-year follow-up (Kao, Folsom et al. 1999). Those studies, however, indicated that this association was limited to middle-aged white populations, and did not apply to African-American participants. Our present results suggest that a similar association between hypomagnesaemia and incidence of diabetes exists in both the Australian Indigenous and non-Indigenous populations, and that ethnicity was not a factor. Nonetheless, the Mg_s in the non-diabetic groups suggested that there might be an ethnic influence in that the Indigenous population had a reduced Mg_s concentration. In a previous chapter (Chapter 3), we have shown a reduced dietary intake of magnesium in the Australian Indigenous population (Longstreet, Heath et al. 2008), and

assume this may be partly responsible for the reduced serum magnesium levels in this sub-group. Given the strong associations in large longitudinal studies that have been shown between reduced dietary magnesium intake and the incidence of diabetes (Kao *et al* 1999; Lopez-Ridaura *et al* 2004; Song *et al* 2004a), such reduced magnesium intake may therefore place the Australian Indigenous population at a higher risk of subsequent diabetes onset.

While magnesium has a protective role in the development of diabetes, the presence of diabetes also has been reported to have effects on magnesium metabolism (Fox *et al* 2001; Saris *et al* 2000). The present study found no significant difference between the Mg_s of Indigenous and non-Indigenous diabetics, implying that the effect of diabetes on Mg_s is identical for both populations. Low Mg_s has also been correlated with obesity (Randell, Mathews et al. 2008), most likely due to magnesium intake being lower amongst overweight and obese persons (McLennan and Podger 1995). However, in the current study the inverse correlation between Mg_s and BMI did not hold when controlling for diabetic status, suggesting that the association between low Mg_s and higher BMI in the present study was related more to the effect of diabetes rather than a nutrient deficient obesogenic diet.

The use of loop diuretics was an exclusion criterion for the current study since they are known to directly affect renal magnesium handling (Saris, Mervaala et al. 2000). Nonetheless, there was a correlation noted between the use of BP medications and reduced serum magnesium in the present study, although the type of medication (e.g. angiotensin-converting enzyme inhibitors, angiotensin 2 antagonists) did not seem to be a factor. However, the association between lower Mg_s and use of BP medication seemed related to

the higher use of these medications amongst diabetic subjects rather than the use of antihypertensives per se, and thus it is unlikely that BP medications directly affect Mg_s. Studies have identified mixed effects of caffeine on insulin secretion and sensitivity (Iso *et al* 2006; Pereira *et al* 2006; van Dam *et al* 2006). This study did not identify any significant effect from caffeine-containing beverages. The design of this study did not isolate coffee from tea consumption, therefore it is not comparable to studies associating high levels of coffee with lower diabetes risk (van Dam, Willett et al. 2006).

In 2004, Franz and Bailey (Franz and Bailey 2004) identified a significant correlation between climate and diabetes prevalence, with a hot climate and high precipitation being associated with a higher prevalence of diabetes. The authors attributed their findings to the potential for increased magnesium loss through sweating that might not be compensated by diet or water intake. Magnesium losses in sweat have been measured as 0.8 mmol/L, with a potential range of 0.2-1.5 mmol/L or 19-365 mg/day dependent on climate (Sawka and Montain 2000). The current study did not identify any apparent effect of heat relief on Mg_s. One of the limitations of this study was the survey tool used to assess heat relief was not validated and may have had limited sensitivity. A separate study using a validated assessment tool might generate different results.

Although total serum magnesium is measured in the current study, free magnesium is the biologically active form. The correlation between serum total and ionized magnesium has been previously shown to be 0.75 (Saha, Harmoinen et al. 1998), and while normal serum levels have been reported in the presence of reduced free levels, depleted serum total levels are widely accepted as reflecting reduced free levels (Heath and Vink 1998; Saha *et al*

1998). Therefore, the current results are likely to reflect a reduction in the biologically active pool, although further studies will be required to confirm this.

In conclusion, this study confirms that decreased serum magnesium was associated with the presence of diabetes in both Indigenous and non-Indigenous Australians. Significantly lower serum magnesium was found in non-diabetic, Indigenous Australians versus their non-Indigenous counterparts. This reduced Mg_s may place the Aboriginal and Torres Strait Islander community in Australia at greater risk for the subsequent development of diabetes. The following chapter will examine whether such changes in serum total magnesium concentration are also reflected in the free magnesium pool.

CHAPTER 6:

IONIC MAGNESIUM STUDY

The medicinal utility of magnesium dates back to ancient times, and to 1934 in terms of being recognised as an essential nutrient for human health (Vormann 2003). Optimal magnesium homeostasis has been associated with clinical well-being and numerous examples exist describing an association between altered magnesium status and disease states (Swaminathan 2003; Barbagallo, Dominguez et al. 2007). For example, chronic low magnesium levels have been associated with chronic diseases such as diabetes, hypertension, cardiovascular disorders, neurological disorders and osteoporosis whereas acute magnesium deficiency has been associated with hypocalcaemia and hypokalemia as well as asthma, stroke, cardiac arrhythmias and neurological dysfunction (Saris *et al* 2000; Swaminathan 2003).

Despite decades of research, many details of magnesium biochemistry still remain unknown and an effective clinical measure of magnesium status has not yet been well established. While a variety of methods are now available to determine magnesium status, the most commonly used methods in a clinical setting are colorimetric methods and ion selective electrodes for the determination of total or free serum concentration, respectively (Saris *et al* 2000). Total serum magnesium, the most frequently used measure, is a late and insensitive indicator of magnesium status (Kulpmann and Gerlach 1996; Sasaki *et al* 2000). Many deleterious changes in tissue magnesium concentrations have been found in the absence of lower total serum magnesium levels (Sasaki *et al* 2000; Takita *et al* 2004). In serum, roughly 40% of magnesium is bound to anions (Mikhail and Ehsanipoor 1999; Padgham *et al* 1993). In contrast, ionised or "free" magnesium is the largest fraction in serum. Due to its inherent bioavailability for immediate transport and use, a number of studies have accordingly suggested that only the ionic magnesium pool accurately reflects magnesium status and have recommended the routine use of ionic magnesium analysers for

magnesium assessments in serum samples (Barbagallo *et al* 2007; Corica *et al* 2006; Kulpmann and Gerlach 1996; Resnick *et al* 1993). As Sasaki et al (2000) have indicated, in terms of an early indicator for magnesium deficiency, serum ionised magnesium may be better than determination of serum total concentration. Few studies, however, have examined the interrelationship between total and free serum magnesium concentration in health and chronic disease, or across different ethnic groups, rendering the choice of appropriate methodology difficult.

A clinical correlation between low plasma magnesium and diabetes pathology has been investigated extensively over the past two decades (Saris *et al* 2000; Walti *et al* 2003a) and both type 1 and type 2 diabetes have been associated with low total serum magnesium (Djurhuus *et al* 2001; Takaya *et al* 2003; Walti *et al* 2003a). Theoretically ionic magnesium should be a more effective measure of magnesium deficit; however the results are not consistent. In 1993, in an early favourable evaluation of the use of magnesium specific ion electrodes, Resnick et al (1993) found that type 2 diabetes was uniformly associated with a significant suppression of ionic magnesium. A review of ion selective magnesium electrodes noted that the ionic magnesium concentration was significantly lower in diabetes compared to healthy subjects, even though total magnesium concentration was normal (Maj-Zurawska 1997). In contrast, one small cross-sectional study in chronic renal failure patients found that the prevalence of hypomagnesaemia was somewhat higher when ionic magnesium was considered (Dewitte, Dhondt et al. 2004). It should, however, be noted that evaluating hyper or hypomagnesaemia in chronic renal failure may be confounded by both catabolic magnesium release and alterations in urinary magnesium excretion. Higher levels of ionic magnesium have been reported amongst 32 diabetic patients compared to matched controls, although no details were given regarding

screening the control population to rule out undiagnosed diabetes (Mikhail and Ehsanipoor 1999). In all methods, care must be taken to address potential confounders as a result of choice of sampling tube and variations in lactate, citrate, and pH (Ritter *et al* 1996; Zoppi *et al* 1996). Moreover, an effect of circadian rhythm has been identified for both urinary magnesium excretion (Fox, Ramsoomair et al. 2001) and ionic serum magnesium concentration (Newhouse, Johnson et al. 2002). In healthy humans, this circadian pattern is not affected by glucose loading (Jacomella, Sauser et al. 1997), however it has been suggested that it is best to assess magnesium status with a morning sample, preferably after fasting (Ising *et al* 1995; Jacomella *et al* 1997).

The reference range for ionic magnesium has also been the subject of debate and several different reference ranges for ionic (free) magnesium have been suggested (Ising *et al* 1995; Mikhail and Ehsanipoor 1999; Newhouse *et al* 2002; Saris *et al* 2000; Swaminathan 2003). Normal range varies between 0.39 and 0.64 mmol/L depending on the blood collection method, storage method and ion selective analyser used (Greenway *et al* 1996; Hristova *et al* 1995; Huijgen *et al* 1999; Thode *et al* 1998), although most studies cite normal values of between 0.51 and 0.57 mmol/L. Issing et al (1995) recommended a cut-off for hypomagnesaemia of 0.46 mmol/L, although whether this is suitable to detect sub-clinical magnesium deficiency and its associated pathologies remains to be determined. The use of subject-based reference intervals has been suggested (Newhouse, Johnson et al. 2002). A consensus on a reference range for ionic magnesium has not yet been established, supporting a well-defined need for additional research into ionic magnesium.

The health disparity experienced by the Australian Indigenous population is profound. Diabetes comprises a significant part of the morbidity and mortality experienced by

Aboriginal and Torres Strait Islander populations (2004). The Australian Indigenous people have been described as having one of the highest prevalence rates of diabetes in the world, a rate that closely corresponds with the age-and sex-adjusted BMI-specific rates for the USA Pima Indians (Daniel, Rowley et al. 1999). The Pima Indians, who are well known for their high rates of type 2 diabetes, have been found to have low serum magnesium (Paolisso and Ravussin 1995) despite one study that assessed an adequate magnesium intake (Reid, Fullmer et al. 1971).

The current study has therefore measured serum total and free magnesium concentration, as well as ionic calcium/ionic magnesium ratios, in a group of 286 participants made up of Indigenous and non-Indigenous Australians, with and without a confirmed diagnosis of type 2 diabetes. Correlations between total and ionic magnesium were then described in each of the subgroups divided on the basis of ethnicity and diabetic status, and associations with other subject characteristics examined.

6.1 Methods

Townsville Aboriginal and Islander Health Services (TAIHS) is a community-controlled Aboriginal Medical Service located in a suburban area of Townsville, Queensland, Australia that serves a community of over 16,000 Indigenous residents. About 20% of the TAIHS patient population are non-Indigenous, mostly pensioners from the immediate neighbourhood. Diabetes is the number one reason for TAIHS general practitioner (GP) consultations (11.3 times per 100 doctor consults). All Aboriginal and Torres Strait Islander subjects recruited for this study were TAIHS patients who presented for care and subsequently required fasting blood tests as part of routine care between August 2004 and February 2006. Additional non-Indigenous people were recruited from five GP practices in

the Townsville area. Inclusion criteria included persons over the age of 15 (Tanner Stage 5) who had lived in the Townsville area for at least ten days. Exclusion criteria included chronic diarrhoea, alcoholism or binge drinking in the past two weeks, use of diuretics, consumption of magnesium supplements, reduced renal function (urinary albumin to creatinine ratio exceeding > 2.5 mg/mmol in men and > 3.5 mg/mmol in women), severe mental illness, pregnancy, or breastfeeding.

Ethics approval was obtained from the Townsville Health Service District Ethics Committee and the TAIHS Board of Directors ethics sub-committee. Additional community consultation was obtained at community diabetes events and from small focus groups held at TAIHS. All subjects gave informed consent for participation in the study. As part of ethics approval under Indigenous community consultation, this cross-sectional study was restricted to a convenience sample, integrated as part of on-going medical care, and included non-Indigenous subjects. The sample was divided into quarters comprising Indigenous people with type 2 diabetes, non-Indigenous with type 2 diabetes, Indigenous without diabetes, and non-Indigenous without diabetes.

A brief survey was administered to all subjects to verify the exclusion criteria. Noting that the previous environmental study had identified a significant positive correlation between diabetes mortality rate and the average maximum daily temperature ($r = 0.579$, $p = 0.002$), potentially mediated through magnesium sweat loss, the survey was expanded to include a gross indicator of heat exposure / absence of heat relief from air conditioning. Additional questions pertaining to caffeine intake were included to assess any potential diuretic effect from excess caffeine intake. Medical records were reviewed for all TAIHS patients in the study. For subjects recruited from GPs, the most recent blood glucose level (BSL),

glycosylated haemoglobin (HbA1c), blood pressure (BP), height, weight, and medications history were requested. The diagnostic standards were confirmed symptoms of diabetes and a random (non-fasting) blood glucose > 11 mmol/L, or fasting plasma glucose ≥ 7.0 mmol/L, or a 2-hour plasma glucose > 11 mmol/L during an oral glucose tolerance test. TAIHS clinic procedure included testing for HbA1c in the absence of diagnosed diabetes; not a standard for GP practice. Venous blood samples were collected in sterile blood separation tubes (Becton-Dickinson 5ml vacutainer), refrigerated immediately, serum separated in less than 24 hours and tested for total serum magnesium (Mg_s) and stored at -80C before being analysed for ionic magnesium (Mg_i) concentration and ionic calcium (Ca_i) using a NOVA-8 STAT analyser equipped with ion selective electrodes (Nova Biomedical Canada Ltd., Mississauga, Ontario). The Nova -8 STAT analyser automatically measures and controls for sample pH.

Data from three or more groups was analyzed for significance using one-way ANOVA followed by Newman-Keuls posthoc tests. When comparing only two group means (e.g., diabetic Mg_i versus non-diabetic Mg_i), a two tailed t-test was used. Linear regression was used to examine correlations between Mg_s, Mg_i, Ca_i, and the ratio of Ca_i / Mg_i. A multivariate analysis was performed to examine the effect of Indigenous status, diabetes, age, body mass index (BMI; kg/m^2), BSL, HbA1c, BP, use of BP medications, use of insulin, and exposure to air-conditioning / gross heat exposure. For BP analysis, in addition to diastolic and systolic BP, degree of hypertension based on the National Heart Lung and Blood Institute (NHLBI) BP categories was examined. Gross heat exposure was based on six levels of exposure to air-conditioning; no air-conditioning at home or work, no air-conditioning at home but present at work, bedrooms only (night exposure) with none at work, night exposure and at work, most or all of the home air-conditioned but none at

work, and full exposure to air-conditioning at both home and work. This was the first known attempt to evaluate heat exposure using this method. A 95% confidence interval (p=0.05) was accepted as significant. Graph Pad Prism v. 5.01 and STATA v9.01 statistical software was used for all analysis (www.graphpad.com and www.stata.com).

6.2 Results

Of 425 subjects initially recruited, eight were removed from the study due to the presence of exclusion criteria in the medical record review (three subjects were in early renal failure, four were receiving diuretics, and one subject was taking magnesium supplements) and 131 subjects were removed due to samples not being appropriately frozen. The excluded subjects were older (p=0.008), but otherwise there was no significant difference in gender (p=0.784), ethnicity (p= 0.058), diabetic status (p=0.596), BMI (p= 0.596), BP category (p=0.450), use of BP medications (p=0.916) or use of insulin (p=0.660). Overall, the sample distribution was more female (61.2%) than male (38.8%), and this was representative of the four individual groups (Figure 6.1). There was no significant difference between the ages of males and females (p=0.399). The subjects without diabetes were significantly younger (p<0.001) and less obese (p<0.001) than those with diabetes. There was no significant difference in BMI between Indigenous and non-Indigenous subjects (p = 0.954), or male and female subjects (p = 0.513). However, the mean BMI of people with diabetes was 32.00±0.59 versus a mean BMI of 27.65±0.60 for those without diabetes, which was statistically significant (p < 0.001). Both Indigenous and non-Indigenous people with diabetes displayed negative correlations between blood glucose levels and Mg_i (r = -0.216, p = 0.012). Additionally, in the presence of diabetes, ionic magnesium levels were negatively correlated with HbA1C levels (r = -0.188, p = 0.027). The Indigenous subjects with diabetes had significantly poorer diabetes control than the

Figure 6.1: Ionic magnesium study patient characteristics (n=286)

Characteristic	Indigenous with Diabetes (mean ± sd) n= 79	Non-Indigenous with Diabetes (mean ± sd) n= 60	Indigenous, No Diabetes (mean ± sd) n= 73	Non-Indigenous, No Diabetes (mean ± sd) n= 74
Age	50.25 ± 13.2	60.75 ± 13.7	43.16 ± 14.9	39.70 ± 19.3
Sex (% male)	36.70%	48.30%	39.70%	32.40%
Body mass index (kg/m^2)	31.50 ± 6.9	32.78 ± 5.8	28.15 ± 6.4	26.94 ± 6.5
Blood Glucose (mmol/L)	10.00 ± 4.9	8.23 ± 3.1	5.4 ± 1.0	5.2 ± 0.8
HbA1C (%)	8.5 ± 2.0	7.1 ± 1.6	6.2 ± 1.2	Missing
Serum magnesium (mmol/L)	0.818 ± .08	0.834 ± .09	0.857 ± .07	0.877 ± .06
Ionic magnesium (mmol/L)	0.533 ± .05	0.539 ± 0.5	0.559 ± .05	0.576 ± .04
Ionic calcium (mmol/L)	1.27 ± .06	1.29 ± .26	2.30 ± .24	1.26 ± .05
Low total serum magnesium *	60 (57.2%)	46 (44.3%)	38 (36.9%)	18 (17.2%)
Low ionic magnesium **	8 (10.1%)	4 (6.7%)	1 (1.4%)	0 (0%)
Number years in Townsville	19.7 ± 15.3	26.4 ± 22.5	18.5 ± 15.2	16.1 ± 20.5
Hours heat exposure / week	5.09 ± 12.8	13.05 ± 26.2	5.92 ± 9.9	5.93 ± 16.1
Servings caffeine / day	3.31 ± 2.2	3.38 ± 2.9	3.38 ± 3.3	2.17 ± 2.2
<u>Diabetic Medications</u> Tablets only Insulin and tablets None	 65.8% 11.4% 21.5%	 56.7% 6.7% 16.7%	*Not applicable*	*Not applicable*

Blood Pressure***				
Normal blood pressure	12.7%	6.7%	42.5%	23.0%
Prehypertension	45.6%	45.0%	15.1%	27.0%
Stage 1 Hypertension	26.6%	18.3%	16.4%	8.1%
Stage 2 Hypertension	12.7%	16.7%	24.7%	2.7%
Missing Data	2.5%	13.3%	1.4%	39.2%
Blood Pressure Medications				
No medications	45.6%	40.0%	78.1%	86.5%
Angiotensin 2 antagonist	5.1%	10.0%	2.7%	5.4%
ACE Inhibitors	30.4%	25.0%	8.2%	4.1%
Beta blockers	7.6%	6.7%	6.8%	2.7%
Calcium channel blocker	1.3%	5.0%	1.4%	1.4%
Combination of two or more	10.0%	13.3%	2.8%	0.0%

sd = standard deviation
HbA1c = glycosylated haemoglobin
*Cut off for normal > 0.80 mmol/l per Society for Magnesium Research(15)
** Cut off for normal > 0.46 mmol/L
*** NHLBI blood pressure categories:
Normal systolic <120mmHg and diastolic < 80mmHG
* Prehypertension systolic 120 – 139 mmHG or diastolic 80-89 mmHG*
* Stage 1 hypertension systolic 140 -159 mmHG or diastolic 90-99 mmHG*
* Stage 2 hypertension systolic >=160mmHG or diastolic >= 100 mmHG.*
ACE = angiotensin converting enzyme inhibitor

non-Indigenous subjects as measured by blood glucose ($p<0.001$) as well as HbA1c ($p=0.009$). There was no significant difference in self-reported exposure to air-conditioning between Indigenous and non-Indigenous subjects ($p= 0.933$), and no significant difference in the length of time each group had resided in the Townsville area ($p= 0.510$).

ANOVA revealed statistically significant differences in the mean Mg_i between the groups (Figure 6.1; $p<0.001$). As expected, Mg_i was significantly lower in the presence of diabetes in both the Indigenous (p= 0.002) and non-Indigenous groups ($p<0.001$). Similarly, diabetic subjects had 13.8 greater odds of having low Mg_i, compared to non-diabetic subjects (95% CI: 1.77 − 107.6). The mean Mg_i between the Indigenous people with diabetes (0.533 ± 0.055) and non-Indigenous people with diabetes (0.539 ± 0.007) were not statistically significantly different (p=0.527). More importantly, in the absence of diabetes there was a significant difference in mean Mg_i between the Indigenous and non-Indigenous subjects ($p =0.046$). 6.7% of non-Indigenous Australians with diabetes had low Mg_i, whereas 10.1% of the Indigenous had low Mg_i. In comparison, none of non-Indigenous non-diabetics had low Mg_i, while 1.4% of Indigenous non-diabetics had low Mg_i. Indigenous diabetics did not have greater odds of having low Mg_i than their non-Indigenous counterparts (95% CI of odds ratio: 0.45 − 5.51), and neither did Indigenous non-diabetics in comparison to non-Indigenous non-diabetics (95% CI of odds ratio: 0.12 - 76.98).

Multivariate analysis showed that there was no significant effect on Mg_i from age ($p = 0.586$), BMI ($p = 0.086$), systolic BP ($p=0.750$), diastolic BP ($p=0.249$), NHLBI hypertensive category ($p =0.585$), use of BP medications ($p =0.276$), insulin ($p=0.100$), caffeine consumption ($p=0.577$) or exposure to air-conditioning ($p=0.595$).

**Figure 6.2. Correlation between serum total magnesium and serum free magnesium across all participants**
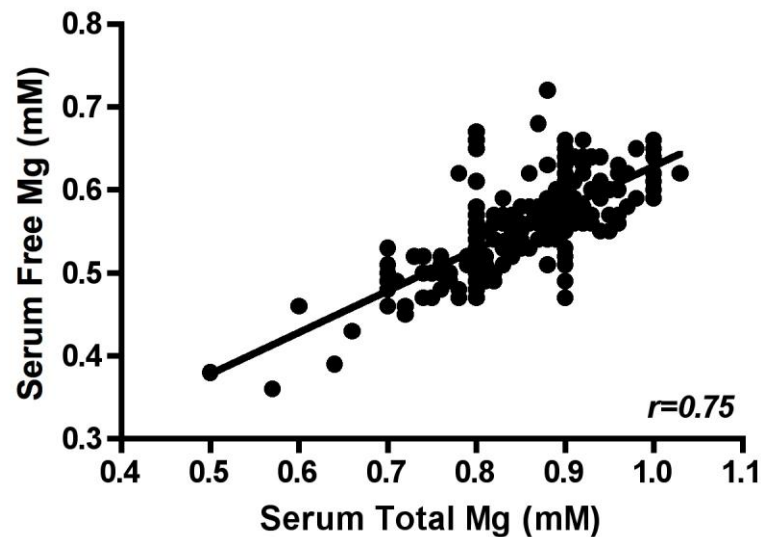
The correlation between Mg_i and Mg_s in all participants was highly significant (Figure 6.2; r = 0.75; p < 0.001). This relationship was independent of ethnicity (Figure 6.3), with a strong correlation being observed between Mg_i and Mg_s in non-Indigenous participants (Figure 6.3A; r = 0.81; p < 0.001) as well as in Indigenous participants (Figure 6.3B; r = 0.71; p < 0.001). There was no effect of diabetes on these correlations. The correlation between Mg_i and Mg_s in non-diabetic participants was highly significant (Figure 6.3C; r = 0.66; p < 0.001), with a similar strong correlation being observed between Mg_i and Mg_s in diabetic participants (Figure 6.3D; r = 0.81; p < 0.001).

Figure 6.3. Correlation between ionic and total magnesium among Indigenous and Non-Indigenous subjects with and without diabetes
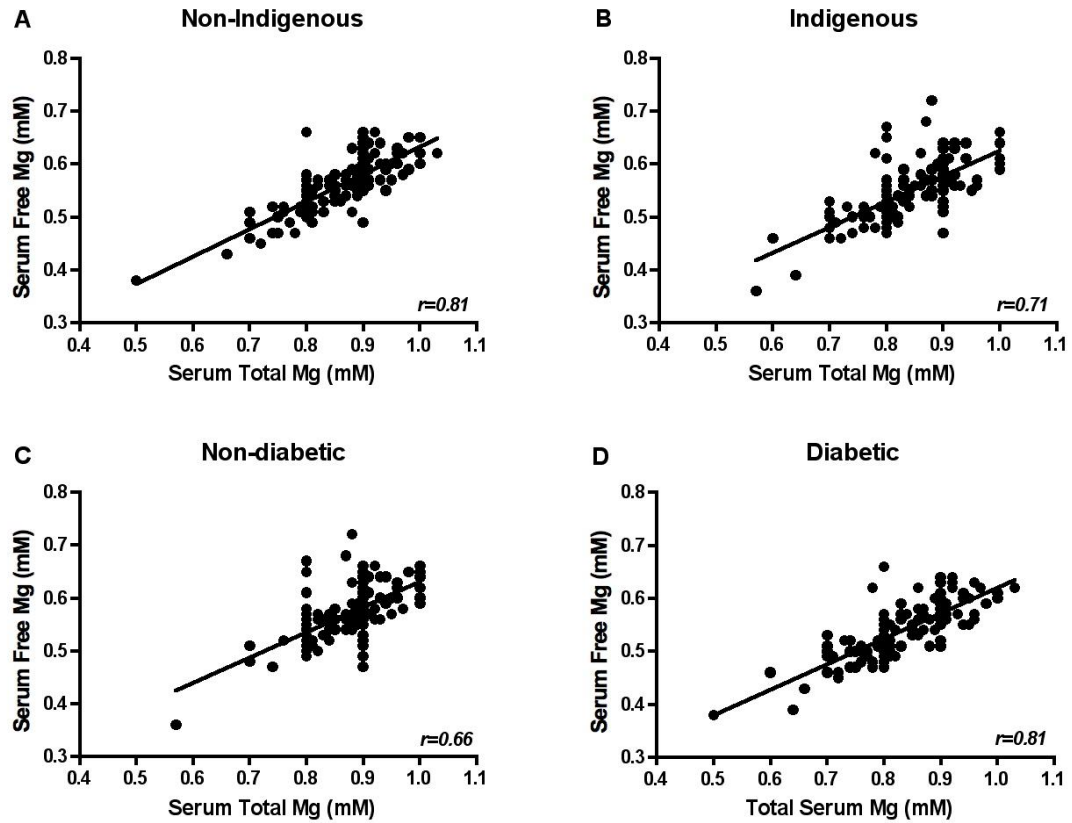
There was no correlation between Mg_s and Ca_i ($r=0.07$), and similarly none between Mg_i and Ca_i ($r = 0.26$). There was, however, a significant negative correlation between Ca_i/Mg_i ratio and Mg_s (Figure 6.4; $r = 0.80$; $p < 0.001$) across all participants. This significant relationship was independent of ethnicity ($r = 0.83$ for non-Indigenous and 0.77 for Indigenous participants) or diabetic status ($r = 0.71$ for non-diabetic and 0.83 for diabetic participants).

Figure 6.4. Correlation between serum total magnesium and serum free calcium/serum free magnesium ratio across all subjects
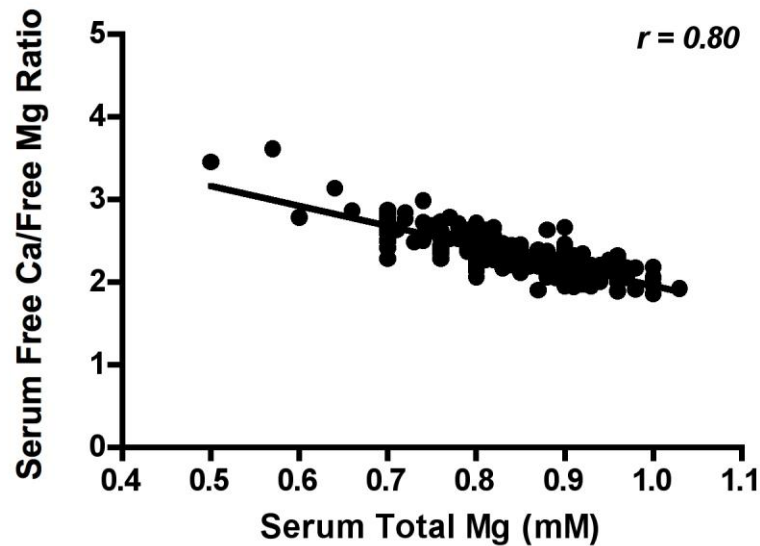
6.3 Discussion

The current study is the first to demonstrate that diabetes in both Indigenous and non-Indigenous Australians is associated with reduced levels of Mg_i. This is consistent with a number of other studies in different communities that have shown an association between reduced Mg_i and the presence of diabetes (Barbagallo *et al* 2007; Corica *et al* 2006; (Maj-Zurawska 1997)Resnick *et al* 1993). Also we have shown that Mg_i is strongly correlated to Mg_s concentration, with the relationship being apparent irrespective of either diabetic status or ethnicity. Similarly, the Ca_i/ Mg_i ratio was also correlated to Mg_s irrespective of diabetic status or ethnicity. Notably, there was no correlation between Mg_s and Ca_i suggesting that the change in Ca_i/ Mg_i ratio was dependent upon changes in Mg_s concentration, which were reflected in the Mg_i pool.

Although a number of reports have suggested that the Mg_s pool does not accurately reflect changes in the Mg_i pool ((Maj-Zurawska 1997), and vice versa, several studies have now reported correlations between the two parameters in various disease states. In particular, the group of Saha (Saha *et al* 1996; 1998) have published several reports showing strong correlations between Mg_s and Mg_i concentration in serum taken from heamodialysis patients, patients with intestinal disease, alcoholic liver disease, and chronic renal disease. In contrast, no significant correlations were noted in a study involving only critically ill patients(Johansson and Whiss 2007). Although the reasons for these differences are unclear, the chronic or acute nature of the patients' condition may be an important factor. In chronic conditions, a generalized magnesium deficiency would manifest as both a decline in the Mg_s and Mg_i pools, with the interrelationship between the two maintained. However, in acute conditions that more commonly require critical care, the rapid onset of a condition that alters the binding status of Mg_i (eg, hormonal changes, stress) may result in a dissociation of Mg_s and Mg_i. Thus, the chronic or acute nature of the condition should be considered when deciding whether to assess total of Mg_i in the assessment of magnesium status.

Diabetes is a chronic condition where declines in serum total magnesium have been well described (Djurhuus *et al* 2001; Takaya *et al* 2003; Walti *et al* 2003a). In the present study, we have shown a strong correlation between serum total and ionic magnesium in diabetic participants, as well as in non-diabetic controls. This strong correlation was also apparent irrespective of the participant ethnicity. Our findings are similar to a previous report by Mikhail and Ehsanipoor (Mikhail and Ehsanipoor 1999) who also report a correlation between the total and ionic magnesium, although their study reported that serum ionic but not serum total magnesium declined in diabetes. This is in contrast with the widely

reported phenomenon of decreased serum total magnesium in diabetes which has been reported by a number of different laboratories (Djurhuus *et al* 2001; Takaya *et al* 2003; Walti *et al* 2003a).

In conclusion, our results demonstrate that diabetes in both Indigenous and non-Indigenous Australians is associated with reduced levels of ionic mangensium. Moreover, the comparison of total and ionic serum magnesium levels in these individuals suggest that serum total Mg determination is perfectly adequate for the assessment of magnesium status in diabetic and non-diabetic patients, and that ethnicity does not play a significant factor. We propose that while serum ionic magnesium determination may be a better indicator of magnesium status in acute disease states, the correlation between serum ionic magnesium and total serum magnesium in more stable chronic states such as diabetes may not require assessment of ionic magnesium concentration as opposed to total magnesium concentration to ascertain the presence of hypomagnesaemia.

CHAPTER 7:

DISCUSSION

This thesis has provided a range of evidence supporting the argument that reduced magnesium intake may deleteriously affect the health of Australian Aboriginal and Torres Strait Islander people. By characterising dietary magnesium intake, magnesium concentration in drinking water, and then determining serum total and free magnesium concentration in a cohort of over 400 Indigenous and non-Indigenous Australians, we were able to demonstrate that Indigenous Australians had a lower dietary magnesium intake than their non-Indigenous counterparts, that the incidence of diabetes-related mortality throughout Queensland, Australia was significantly correlated to the degree of magnesium in drinking water, and that Australian Aboriginal and Torres Strait Islander people exhibited greater hypomagnesaemia than non-Indigenous Australians; this hypomagnesaemia was strongly correlated with the higher incidence of type 2 diabetes in this population.

Chapter 1 reviewed the existing literature exploring the clinical correlation between low plasma magnesium and diabetes (Fox *et al* 2001; Saris *et al* 2000) and specifically the evidence linking low serum magnesium to type 2 diabetes (Djurhuus *et al* 2000; Elamin and Tuvemo 1990; Saggese *et al* 1991; Sharma *et al* 2007; Walti *et al* 2003a). Clinically, a graded inverse relationship has been observed between serum magnesium levels and the development of type 2 diabetes (Kao, Folsom et al. 1999). Low serum magnesium was also observed to significantly increase the prediction of all-cause mortality in diabetic patients (Haglin, Tornkvist et al. 2007). Additionally, the co-morbidities associated with diabetes appear to be influenced by magnesium deficit (De Leeuw *et al* 2004; de Valk 1999; Rodriguez-Moran and Guerrero-Romero 2001). In many countries dietary magnesium has been identified as low, with an even more pronounced deficiency being noted in lower income and obese populations (McLennan and Podger 1995). Many large epidemiological

studies have identified that diets replete in magnesium protect against the development of type 2 diabetes, independent of being a marker for a healthy diet (Abbott *et al* 2003; Lopez-Ridaura *et al* 2004; Meyer *et al* 2000; Song *et al* 2004a) with several studies identifying magnesium supplementation as beneficial to the clinical management of diabetes (De Leeuw *et al* 2004; Rodriguez-Moran and Guerrero-Romero 2003; Sheth *et al* 2002; Yokota *et al* 2004). The mechanisms by which magnesium affected the pathological process associated with type 2 diabetes included upregulation of inflammation (Guerrero-Romero and Rodriguez-Moran 2006; King *et al* 2006; Mazur *et al* 2006; Xu and Whitmer 2006), alteration of insulin sensitivity via action on tyrosine kinases thus creating a post-receptor defect (Ferreira *et al* 2004; Takaya *et al* 2004), and altered glucose transport from either an inherited magnesium binding defect or induced by dietary magnesium deficit (Wells 2008). Additionally, in the presence of uncontrolled diabetes, high blood glucose increases magnesium diureses, further compounding the deficit and resulting in a progressive deterioration of both magnesium deficit and insulin sensitivity. At each stage in the course of disease, there is clear evidence that magnesium metabolism influences type 2 diabetes.

In Chapter 3 we evaluated the nutrient intake of a cohort of Aboriginal and Torres Strait Islander subjects and compared it to the average Australian dietary intake, making this study the first published nutrient assessment of urban Indigenous people (Longstreet, Heath et al. 2008). Baseline dietary levels had low levels of magnesium, vitamin A, thiamine, riboflavin, niacin, folate, vitamin C, calcium, phosphorus, iron and zinc. 60% of the Indigenous people that were assessed had a dietary magnesium intake below the EAR, the estimated average requirement for *half* the population to have an adequate intake. Additionally, the average magnesium intake was found to be significantly lower than the

intake of non-Indigenous Australians (p<0 .001). In other words, we found clear evidence that the Aboriginal and Torres Strait Islander people may be at a higher risk of inadequate magnesium intake.

In Chapter 4 we examined environmental factors, including the magnesium level in drinking water, and correlated them to diabetes-related mortality throughout the state of Queensland, Australia. A significant correlation was found between the incidence of diabetes related mortality and the concentration of magnesium in drinking water. This study was consistent with previous reports from other countries that correlated the level of magnesium in drinking water to diabetes (Mahaba 1998; Yang *et al* 1999; Zhao *et al* 2001). This does not imply causality, as ecological bias cannot be ruled out and the association observed at area levels may be different than at the individual level. However, when considered in the context of the lower magnesium intake that was observed in the regional Indigenous diet, low levels of magnesium in the drinking water supply may increase the risk of poor magnesium nutrition. Additionally, a deficit might be exacerbated in an environment where magnesium sweat loss is increased. This supposition was supported by the highly significant correlation between average daily high temperature and incidence of diabetes-related mortality. The additional correlations observed between diabetes-related mortality and lower post-school qualification amongst the Indigenous, with subsequent association of lower socioeconomic status, were also relevant given that these individuals might be less likely to have access to heat relief such as air-conditioning, either at home or in the workplace, and dietary choices may be limited by income.

In Chapter 5, a needs assessment study showed that diabetes in both Indigenous and non-Indigenous Australians is associated with reduced levels of total serum magnesium. More

importantly, in non-diabetics, the study found total serum magnesium is lower in Indigenous Australians compared to non-Indigenous Australians. The 17.2% prevalence of hypomagnesaemia in the absence of diabetes in non-Indigenous non-diabetic Australians was somewhat higher than the 14% prevalence rate previously reported (Schlingmann, Konrad et al. 2004). However, the much higher prevalence rate of 36.9% hypomagnesaemia found in the non-diabetic Indigenous Australian cohort is concerning. Low magnesium, probably derived from poor dietary intake, may be placing the Aboriginal and Torres Strait Islander community in Australia at greater risk for the subsequent development of diabetes.

Lastly, in Chapter 6, an ionic serum magnesium analysis confirmed the decreased serum magnesium levels and its association with diabetes that was reported in Chapter 5, and additionally demonstrated that serum free Mg is strongly correlated to serum total Mg concentration, with the relationship being apparent irrespective of either diabetic status or ethnicity. Our results suggest that serum total Mg determination is perfectly adequate for the assessment of Mg status in diabetic and non-diabetic patients, and that ethnicity does not play a significant factor. While serum free Mg determination may be a better indicator of Mg status in acute disease states, the correlation between serum free Mg and total Mg in more stable chronic states may not require such assessment.

The evidence presented here is not causal. However it does establish a strong case for magnesium being a potential contributor to diabetes in Australia, especially for Aboriginal and Torres Strait Islander peoples. This study clearly supports the need for further investigation of the potential relationship between magnesium and diabetes in the

Australian Indigenous populations, especially since a deficiency might be readily addressed.

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