School of Public Health

The Relationship Between Low Blood Thiamin Levels In Diabetes To Thiamin Intake And Diabetic Control

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This thesis is presented for the Degree of Master of Science of Curtin University of Technology

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: .............................................

Date: .................................
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RELATED PUBLICATIONS

ABSTRACT

Mild thiamin deficiency is prevalent in diabetes, and high dose thiamin ameliorates some diabetic complications, but there are no definitive studies addressing thiamin intake, diabetes control and thiamin status in diabetes.

Subjects were 113 people with diabetes (58 type 1, 55 type 2), 43 with and 70 without thiamin supplementation.

Dietary thiamin was estimated by 24-hour recall, diabetes control by HbA1c. Age, BMI, albumin excretion, activity level and smoking status did not correlate with red cell thiamin (RCT) in either group. RCT correlated with serum thiamin (ST) (p < 0.01).

In those unsupplemented, adequate dietary thiamin did not ensure normal RCT, with 15.7% of subjects below the reference range. Supplementation to intake > 4 mg/d, was significantly associated with normal RCT (p = 0.028), with 97.7% of supplemented subjects having normal RCT. Supplementation was also significantly associated with elevated serum thiamin 24 hours post supplementation, contrary to other reports. HbA1c was not significantly associated with RCT.

Conclusions: In diabetes, adequate dietary thiamin does not ensure normal red cell thiamin, but supplementation to > 4 mg/day does, raising questions about actual thiamin requirements in diabetes and supporting evidence that thiamin deficiency in diabetes is not primarily due to dietary deficiency. Diabetes control was not significantly related to thiamin status.
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ABBREVIATIONS

ADA – American Diabetes Association
ADS – Australian Diabetes Association
ADEA – Australian Diabetes Educators Association
AIHW- Australian Institute of Health and Welfare
AMA – American Medical Association
AusDiab Study - The Australian Diabetes, Obesity and Lifestyle Study
BMI – Body Mass Index
CHD – Coronary Heart Disease
CV - Coefficient of variation
CVD – Coronary Vascular Disease
DCCT - Diabetes Control and Complications Trial
ETKA - Erythrocyte transketolase activity
FSANZ – Food Standards Australia New Zealand
GABA - Gamma-aminobutyric acid
GAD - Glutamic Acid Decarboxylase
HDL – High Density Lipoprotein
HbA1c - HaemoglobinA1C measurement
HPLC - High Performance Liquid Chromatography
IDF – International Diabetes Federation
IDS - International Diabetes Society
IFG - Impaired Fasting Glucose
IGT - Impaired Glucose Tolerance
IV - Intravenous
JDRF - Juvenile Diabetes Research Foundation
NRM – Nutrient Reference Value
NHMRC – National Health and Medical Research Council
OGTT - Oral Glucose Tolerance Test
r - Pearson’s Product Moment Correlation Coefficient
RDI – Recommended Dietary Intake
SMBG - Self-monitoring of Blood Glucose
$r_s$ - Spearman’s Nonparametric Rank Correlation Coefficient
TPP - Thiamin Pyrophosphate
UKPDS - UK Prospective Diabetes Study
UL – Upper Level of Safety
WHO – World Health Organisation
CHAPTER 1  INTRODUCTION

1.1 Statement of the problem

Thiamin is present in a variety of foods, especially cereals. BMI, age, activity and smoking status can be modifiers of blood thiamin levels but in the absence of excessive alcohol, a low blood thiamin level is normally a marker of inadequate dietary intake.\(^1\) There are a number of reports citing a greater incidence of suboptimal blood thiamin levels in those with diabetes compared to those without,\(^2-4\) and also reports that conflict with this.\(^5\) A study on food intake in 50 people with type 2 diabetes showed that dietary intake of thiamin was well above the RDI in 98% of subjects\(^6\) and there is evidence that thiamin intake in type 1 diabetes is also adequate.\(^7\) However there is some evidence of increased excretion of thiamin in those with diabetes when compared to those without.\(^2\) Human, animal and in vitro studies have shown that high dose supplementation by thiamin and its derivatives ameliorate complications of diabetes.\(^8\) This suggests that low thiamin blood levels may be related to the diabetic state rather than to inadequate dietary intake. Although a recent researcher has stated that ‘decreased plasma thiamine concentration in clinical diabetes was probably not due to a deficiency of dietary input of thiamine’,\(^2\) this has not been demonstrated, and there are no definitive studies addressing dietary intake and thiamin status in diabetes. Unless this area is researched, it cannot be definitively stated how thiamin deficiency in diabetes is related to dietary intake, if indeed it is related at all. In addition, it has not been established that thiamin status in diabetes is not inappropriately diminished by other known modifiers, or by poor control of diabetes.

1.2 Benefits of the study

Thiamin status appears to be problematic in diabetes, and an understanding of thiamin’s role and metabolism has the potential to normalise some aspects of metabolism in diabetes. The role of thiamin in diabetes is currently being researched in a ‘jigsaw’ fashion.
It has been shown that:

- High dose thiamin has a potential role in ameliorating aspects of some complications of diabetes and currently several research centres are investigating the mechanism and implications of this finding in both animal and human studies.
- Mild thiamin deficiency is prevalent in diabetes, although various studies have shown conflicting results as to which markers of thiamin status are reduced, and the extent of this reduction.
- Excretion of thiamin in people with diabetes is probably increased in comparison to those without diabetes.

There is no definitive research addressing thiamin in diabetes ‘between the mouth and the red cell’ while considering diabetes control. This study addresses that area, and differs from other studies by including dietary intake data, and data on other possible modifiers of thiamin status. It contributes another piece to the jigsaw and adds to the emerging picture of the role of thiamin in diabetes. The completion of this picture has the potential to aid treatment, and hopefully to prevent some complications of diabetes.

1.3 Objectives of the study

The study will attempt to clarify the relationship between red cell thiamin levels and dietary intake and diabetic control using adequate numbers of subjects to provide sufficient power, and also to differentiate between the two types of diabetes.

The primary objectives of the study are, in type 1 and type 2 diabetes, to:

1. Determine the relationship between red cell thiamin levels and dietary thiamin.
2. Determine if red cell thiamin levels are related to diabetic control as measured by HbA1c.
The secondary objectives of the study are, in type 1 and type 2 diabetes, to:

1. Validate a method to accurately assess and describe the intake of thiamin, carbohydrate, energy intake,

2. Assess and describe the intake of thiamin, carbohydrate, energy intake, thiamin per unit of energy and percentage of carbohydrate.

3. Access and describe other known modifiers of thiamin status; age, BMI, duration of diabetes, activity, smoking status and urinary albumin excretion.

4. Access and describe indices of diabetic control and its possible modification by episodic hypoglycaemia.

5. Access and describe thiamin status as measured by serum and red cell thiamin.

6. Address the relationship of 2, 3 and 4 to 5.

1.4 Limitations of the study

- The thiamin assay used in this study has been well validated and is used for all medical thiamin assessments carried out in Western Australia. Non parametric reference ranges for both serum and red cell thiamin were determined on 505 healthy individuals aged 18 to 90 years. Mandatory enrichment of bread making flour in Australia since 1991 has increased the thiamin content of all breads with a concomitant increase in mean thiamin intake of 25% in men, and 20% in women in the Australian population. In those without diabetes and in the absence of excess alcohol, dietary intake of thiamin is the main determinant of thiamin status, therefore, it is reasonable to assume the reference levels used may now be too low in relation to current food supply. If this is so, it would not negate the study results, but would increase the numbers of subjects with low serum and red cell thiamin levels.

- Although the objectives of this study were primarily to examine the effects of parameters of dietary intake and diabetes control on thiamin status, it would have added to the completeness of the data to have also assessed urinary excretion of thiamin.
Nutrient assessment by 24-hour recall gave good correlation on repeat assessment for all nutrients assessed. Testing for agreement was less uniform; good agreement was obtained for thiamin intake on original and repeat assessments. Agreement for energy and carbohydrate intakes were less accurate.
CHAPTER 2 LITERATURE REVIEW

Literature review ceased 31/03/2008

2.1 Thiamin

Thiamin was one of the first organic compounds to be recognised as a vitamin; that is as one of thirteen organic compounds that are identified as being indispensable to the healthy functioning of the cells and tissues of the human body. The unique feature of the vitamins is that they generally cannot be synthesized by mammalian cells and, therefore, must be supplied in the diet. Vitamins occur in small quantities in food, and are thus part of the group termed ‘micronutrients’, distinguishing them from the ‘macronutrient’ group which includes the compounds present in larger amounts in foods. In 1926 thiamin was designated vitamin B1, and is part of the group of water soluble vitamins.12,13

2.1.1 History

Beri-beri was first described by the Chinese in about 2700 BC, but it was not until the 1880’s that Dr K. Takaki, the Director General of the Japanese Naval Medical Services, stumbled upon it again. Takaki tipped a relationship between the diet of sailors on naval ships, and the occurrence of beri-beri.14 In 1882 a navy boat returned from a 272 day journey with 61% of the crew sick with beri-beri. In 1884, Takaki loaded another boat with extra dry milk and meat, and after a 287 day journey, only 14 of the crew sickened with beri-beri.15 Because of this, Takaki initially thought beri-beri was connected with inadequate ‘nitrogenous substances’ in the diet of the sailors. To combat this he ordered the sailors’ diets routinely be diversified by decreasing the proportion of rice, and increasing meat, fish, barley and vegetables. On this diet, over the next six years, the incidence of beri-beri in the Japanese navy decreased from 40% to 0%.16

In 1886, Dr C. Eijkman, at a military hospital in Java, discovered that chickens fed white polished rice developed an animal form of beri-beri, while those fed unhusked
rice did not, and he and a colleague, Dr G. Grijns, concluded there was a substance in the outer coating of rice that was necessary for the chickens to maintain health.16

“There is present in rice polishings a substance different from protein and salts, Which is indispensible to health and the lack of which causes nutritional polyneuritis”.  C. Eijkman and G Grijns, 190616

Following this, Dr C. Funk, at the Lister Institute in London, crystallized an amine substance from rice bran, which he called ‘vitamine’, for ‘vital amine’. The crystallized substance turned out not to be thiamin, but the name stuck. Eventually, after more work by several other research groups, two Dutch chemists, Dr B. Jansen and Dr W. Donath, crystallized thiamin from rice bran in 1926. They named it antineuritic vitamin, however this name was not accepted by the American Medical Association (AMA). The correct formula of thiamin was determined by Dr R. Williams of the Bureau of Science, in Manila, who was asked by the AMA to come up with an alternative name. He chose the name thiamin.15, 16

2.1.2 Structure

The formula of thiamin is C₁₂H₁₇N₄OS. The structure of thiamin is a substituted pyrimidine ring (a 6 member ring containing two nitrogen atoms at position 1 and 3) and a thiazole ring (a 5 member ring with a sulphur and nitrogen atom at 1 and 3). The rings are connected with a one carbon link, or a methylene bridge. The nitrogen in the thiazole ring has a positive charge, which makes it serve as an important electron sink16, 17 (see section 2.1.14). Thiamin has a molecular weight of 266.4.18

![Structure of thiamin](image-url)
The main biologically active form of thiamin in the human body is thiamin pyrophosphate. Thiamin pyrophosphate is also known as thiamin diphosphate or cocarboxylase.

![The structure of thiamin pyrophosphate (TPP)](image)

Other forms of thiamin found in the body are thiamin triphosphate and thiamin monophosphate.

### 2.1.3 Food sources

The richest food source of thiamin is vegemite (a yeast extract) and brewers yeast, a dried, inactive yeast that is a byproduct of the brewing industry. Other foods that are naturally occurring good sources of thiamin are pork and bacon, wholemeal bread and legumes. Other, less rich, but still significant sources are milk, nuts, oats, oranges, brown rice, beef, and some seeds such as sunflower seeds. Refined sugar contains no thiamin.

### 2.1.4 Form

Thiamin can be found in food as the free form, thiamin monophosphate, thiamin diphosphate (thiamin pyrophosphate) and as a protein phosphate complex. There is limited data available on specific forms in specific foods, however, in general, thiamin occurs mainly as the free form in plants and phosphorylated in animal tissues but the phosphorylated form can also be found in plants. Thiamin protein phosphate complexes occur in some seeds. In milk, the vitamin is present both as free thiamin and as a vitamin-protein complex.
2.1.5 Availability from food

Properties
Thiamin is water soluble, so thiamin is lost into water during water-based cookery. Considerable losses of thiamin occur during heat processing of food, especially prolonged cooking.\textsuperscript{15} Estimated average loss in cooking in a mixed diet is 25%.\textsuperscript{13} Pasteurization of milk can also result in losses of thiamin of up to about 20%. Thiamin is destroyed by x-rays and by ultraviolet irradiation of food stuffs.\textsuperscript{14} It is relatively acid-stable\textsuperscript{23} and there is little loss from freezing.\textsuperscript{14} Thiamin has a high rate of loss in the extrusion process to make snack foods.\textsuperscript{24}

Thiaminases
These are enzymes found in fish and some other organisms which biologically deactivate thiamin by splitting the molecule. Thiaminase 1 is the most common and is found in some raw fish, shellfish and some types of ferns. Thiaminase 11 is much less common and is found in some other organisms, eg types of fungi.\textsuperscript{14} The process by which thiamin is split differs between the two types of thiaminases. Thiaminases are destroyed by heat.\textsuperscript{14} It is almost certain that the explorers Burke and Wills, who died in the outback in 1861, died as a result of eating large quantities of nardoo, an Australian fern with a high content of thiaminase 1. Burke and Wills prepared nardoo by grinding and cooking it, but as the fern is adapted to the high temperatures of central Australia, the thiaminase was not destroyed. The aboriginal people of the area also used nardoo, but ground and diluted it with water thus decreasing thiaminase activity.\textsuperscript{25}

2.1.6 Fortification of food

Thiamin content of foods can vary with processing. Wholegrain cereal and rice products are rich sources of thiamin\textsuperscript{14, 19, 26} whereas most of the natural thiamin content is lost in the production of white flour and polished rice.\textsuperscript{18} A need was seen to ensure adequate thiamin intake in some sectors of the Australian population because of the prevalence of Wernicke-Korsakoff Syndrome. Wernicke-Korsakoff Syndrome is a degenerative brain disease associated with a high alcohol intake and an inadequate thiamin intake\textsuperscript{27} (see section 2.1.18). Australia has a high incidence of Wernicke-Korsakoff syndrome compared to other countries.\textsuperscript{28} In response to this,
the National Health and Medical Council (NHMRC) recommended addition of thiamin to beer and flagon wine. This was supported by economic analysis but opposed by a variety of manufacturing groups, nutritionists and those opposed to alcohol.²⁹

The 1983 National Dietary Survey of Adults indicated that bread and cereals were the major sources of thiamin intake in the Australian diet so it was decided to fortify flour for bread-making rather than alcoholic beverages. An amendment to the Australian Food Standards Code was passed in 1991 making it illegal for bread-making flour to contain less than 6.4 mg/kg of thiamin and thus necessitating the addition of approximately 4 mg thiamin/kg to white bread-making flour and 2 mg/kg to wholemeal bread-making flour.³⁰ Thiamin that is added to bread-making flour is in the form of thiamin mononitrate. The amounts added are approximately 0.5 mg/100 g for white bread and 0.2 mg/100 g for wholemeal bread. It is premixed with a small amount of flour which is then remixed into the larger amount. Thiamin is fairly stable at the pH of most breads, which is about 5.³¹ Loss of thiamin during baking varies from 5% to 40%, depending on heat of baking and surface area. The thiamin content of both white and wholemeal bread is now 0.45 mg/100 g.³²

There has been a significant reduction in the prevalence of Wernicke-Korsakoff syndrome in Australia since the introduction of thiamin enrichment of bread flour, although the prevalence is still higher than in most other Western countries.²⁸ There is no evidence of adverse effects of Australian fortification of bread-making flour with thiamin.³³

2.1.7 Supplements

Thiamin is available in nutritional supplements. Over the counter thiamin supplements are typically found in the form of multivitamin, multivitamin and mineral or B-complex preparations. Single ingredient thiamin supplements are also available. The form of thiamin in supplements is usually thiamin hydrochloride or thiamin mononitrate.²³
Thiamin hydrochloride is more soluble than thiamin nitrate, but the latter is more stable at temperatures less than 95°C. The amount of thiamin in supplements varies from approximately 1 mg to 50 mg per tablet in some super multi B formulations. Lipid-soluble thiamin derivatives called allithiamins are also available. The most commonly used is benfotiamin. Benfotiamin is CS-benzoylthiamine-ο-monophosphateoral and it would appear that administration of benfotiamin is highly suitable for therapeutical purposes (see section 2.1.9).

Vitamins and/or mineral supplements must conform to the standards set down in the specific rules on vitamins and minerals laid down in the Guidelines set out by Food Standards Australia New Zealand (FSANZ). In Australia, vitamin and mineral supplements are regulated as complementary medicines under the Therapeutic Goods Act 1989 which regulates specific complementary medicines.

Oral toxicity in humans of thiamin is considered low, although it has been reported that oral doses of thiamin hydrochloride of greater than 700 mg have caused headache, nausea, rapid pulse and weakness which resolved following reduction in thiamin intake. There have been several single case studies of reactions at lower
doses than this. No mechanism of toxicity has been identified. Some hypersensitivity reactions have occurred after parenteral doses of thiamin. These reactions have ranged in severity from very mild to, very rarely, fatal anaphylactic shock. The NHMRC has determined that there is not enough evidence to set a safe Upper Limit (UL) for thiamin intake at this time.

2.1.8 Bioavailability

Thiamin in food appears to have a high bioavailability. A 1997 review on the bioavailability of thiamin suggests a high, but not total availability of naturally occurring and supplemental vitamin sources. There is limited data on the bioavailability of thiamin from specific foods, however the few studies do support a good availability. An animal study using pigs on the bioavailability of thiamin in eggs, bananas, white cabbage, corn, milk, fish, barley, soybeans, rice, wheat bran, brewer's yeast, rye and soybean meal showed a good bioavailability (73% to 94%) from all tested foodstuffs. An Indian study showed good bioavailability of thiamin from three types of curry leaves.

2.1.9 Digestion and absorption

Dephosphorylation

It would appear that phosphorylated forms of thiamin in food are readily dephosphorylated before intestinal absorption. Prior to absorption, phosphorylated forms of thiamin undergo complete hydrolysis involving a number of different intestinal phosphatases. Consequently, thiamin found in the intestinal lumen following a meal is in the free form.

Site of absorption

Thiamin is absorbed in the small intestine. Thiamin is variously said to be absorbed 'mainly in the jejunum', mainly in 'the jejunum and ileum', and 'maximally in the duodenum, with its rate decreasing caudally along the small intestine'.

Transport through the intestinal mucosa and rephosphorylation

When the concentration of thiamin in the gut is low, it is absorbed by active transport. The thiamin transporter is THTR1 and is Na+ dependent. Intracellular phosphorylation appears to facilitate transport by metabolic trapping.
Thiamin is phosphorylated (or rephosphorylated) in the intestinal mucosa during absorption.\cite{47} The upper level of concentration of thiamin in the gut for active transport is in the physiological range, and variously said to be less than 2 µmol/L\cite{14} and less than 1.5 µmol/L.\cite{43} At concentrations greater than this, the absorption is by diffusion.\cite{14} Thiamin is absorbed by passive diffusion in its unionised form.\cite{8} Intestinal transport of thiamin is rate limiting in humans,\cite{14} that is, at physiological concentrations of thiamin, absorption in the small intestine is by carrier-mediated transport which is saturable. At greater thiamin concentrations uptake is by slower passive diffusion.\cite{23}

**Water soluble thiamin supplements**

Water soluble supplements are almost always thiamin hydrochloride and thiamin nitrate. Literature reports on transport of thiamin supplements into the gut is a little confusing. Thiamin supplements almost ubiquitously contain amounts of thiamin well above physiological levels and are therefore largely subject to absorption by passive diffusion. It is variously asserted that “absorption of thiamin hydrochloride and other water-soluble forms of thiamin is dose-dependent”\cite{23}; that humans have a rate-limited capacity to absorb water soluble thiamin supplements such as thiamin hydrochloride\cite{48}; that the proportion absorbed of water soluble supplements is very low\cite{18} and that at a supplementation level of 2.5 – 5 mg of thiamin, the majority is largely unabsorbed.\cite{18} Daily absorption is usually limited to a maximum of 8-15 mg, although this may be increased by administration of divided doses with food.\cite{18} Animal studies indicate little difference between absorption of different water soluble supplemental thiamin salts, indicating that they have similar potency.\cite{49}

**Lipid soluble supplements**

Early studies on lipid soluble forms of thiamin were carried out on a disulphide of thiamin and allicin that was derived from garlic. This was called allithiamin, and appeared to be a highly available form of thiamin.\cite{50} Other lipid soluble forms of thiamin, such as CS-benzoylthiamine-o-monophosphate (benfotiamin) are better absorbed at higher intakes than are water soluble thiamin supplements.\cite{38} According to some investigators the allithiamins owe their good absorption to their fat-soluble property and their ease of transport across the intestinal wall without alteration\cite{48} although it has been postulated that some other process may be responsible.\cite{1} In one study benfotiamin had a significantly greater availability than water soluble supplements when being administered at 40% of the quantity of the water soluble
substances.\textsuperscript{51} Thornalley states that benfotiamin is a delivery vehicle for thiamin monophosphate and is probably absorbed by the RFC-1 transporter.\textsuperscript{8}

2.1.10 Transport around the body and thiamin transporters

Thiamin is transported around the body in both plasma and red blood cells however about 80\% is in the erythrocytes.\textsuperscript{52} An \textit{in vitro} study has shown that approximately 30\% \textsuperscript{53} of serum thiamin is bound to serum proteins under normal concentration of thiamin, and that as serum concentration of thiamin increases, percentage binding decreases.\textsuperscript{53} Thiamin is a cation, and at physiological concentrations is transported across cell membranes by high affinity cation transporters.\textsuperscript{8} Genes for the thiamin transporters are members of the SLC19A group of solute carriers. Three genes expressing thiamin carriers have been identified: they are SLC19A2 and SLC19A3 which encode transporters THTR1 and THTR2, respectively, and SLC19A1 which encodes RFC-1 which is a folic acid transporter, but also transports thiamin monophosphate into cells. At high expression, it may also transport thiamin pyrophosphate out of cells. It is widely expressed in the body, but especially in mitochondria. THTR1 is expressed on the basolateral surface of the gut, and in skeletal muscle, heart, liver, kidney and placenta and appears to be the primary transporter. THTR2 is expressed on the luminal surface of gut epithelial cells, and kidney, placenta and liver.\textsuperscript{8}

2.1.11 Uptake by erythrocytes

\textit{In vitro} studies of uptake of thiamin by the erythrocytes in humans show it is probably transported by facilitated diffusion, that is by a low capacity carrier mechanism that is Na\textsuperscript{+} independent. Uptake has two different mechanisms, saturable and nonsaturable. The saturable mechanism dominates at physiological concentrations. Only about 20\% of the thiamin taken up by the erythrocytes appears to be bound to membrane or intracellular protein.\textsuperscript{54} A study in humans indicated that thiamin and thiamin monophosphate are transported from plasma into the interior of the erythrocyte by the two carrier proteins THTR-1 and RFC-1.\textsuperscript{2}
2.1.12 Form in the body

Thiamin is present throughout the body as free thiamin, monophosphorylated thiamin, diphosphorylated thiamin (thiamin pyrophosphate) and triphosphorylated thiamin. Plasma and cerebrospinal fluid contain only the free and monophosphorylated forms, while erythrocytes contain the free and all phosphorylated forms. A thiamin binding protein has been purified from rat erythrocytes. It is also suggested that in the plasma, thiamin is bound to albumin via its phosphate moiety, so that it must be phosphorylated to be able to be bound to protein. In vitro studies have also shown that free phosphorylated thiamin in plasma is rapidly dephosphorylated. Thiamin is rapidly converted to the phosphorylated form in the tissues. Relatively high concentrations are found in skeletal muscle, heart, liver, kidney, and brain. Within the tissues, therefore, approximately 80% of thiamin is thiamin pyrophosphate, which is the main active form. Of the rest, 5 – 10% is thiamin triphosphate, and the rest is divided between free thiamin and thiamin monophosphate. The liver is the main site for storage. The total metabolic pool of thiamin in the human is approximately 30 mg. The half-life of thiamin in the body is approximately 9.5 – 18.5 days.

2.1.13 Excretion

Free phosphorylated thiamin in plasma is rapidly dephosphorylated. It, therefore appears likely that protein binding and urinary excretion of thiamin are linked to a dynamic process of phosphorylation and dephosphorylation, and that when the vitamin is present in increased concentrations it cannot be bound, and there will be consequent increased excretion. When intake of thiamin is low, virtually none is excreted in the urine. However, if intake is high, even though a proportionately small amount of the thiamin is absorbed by the gut, serum values are elevated and result in active urinary excretion. Over 200 urinary metabolites of thiamin have been identified.

The proportion of thiamin excreted appears to be dependent, at least partially, on whether it is taken orally or administered intravenously (IV). With a 50 mg dose of
thiamin hydrochloride, 53% of an IV dose is recovered in the urine within 24 hours. With a 50 mg oral dose only 2.5% of the dose was recovered in the urine.\textsuperscript{57} It is postulated this may be due to incomplete intestinal absorption of the thiamin, or perhaps retention by body tissues.\textsuperscript{1}

\subsection*{2.1.14 Function in the body}

As stated in section 2.1.2, the main biologically active form of thiamin in the human body is thiamin pyrophosphate. Ingested thiamin is rapidly converted to thiamin pyrophosphate in the brain and liver by a specific enzyme, thiamin diphosphotransferase which catalyses the replacement of the hydroxyl group attached to the thiazole group with a diphosphate ester group. Thiamin pyrophosphokinase would appear to be an adaptive enzyme whose activity depends on the thiamin content of the cells. Its activity is reduced in liver and heart muscle of thiamin deficient rats, but no decrease in enzyme activity has been found in the brain.\textsuperscript{58}

Thiamin pyrophosphate functions in the body as a coenzyme. Coenzymes are organic non protein molecules that bind with the protein enzyme (apoenzyme) to form the active enzyme.\textsuperscript{59} The 2 carbon of the thiazole ring is the reaction site of thiamin pyrophosphate. The hydrogen atom attached to this carbon bond readily dissociates, leaving a carbanion which is available for nucleophilic (literally ‘nucleus loving’ or positive seeking) addition to an unsaturated carbon bond, such as that in a carbonyl group.\textsuperscript{60}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{nucleophilic-addition.png}
\caption{Nucleophilic addition to an unsaturated carbon bond to a carbonyl group\textsuperscript{16}}
\end{figure}
Thiamin pyrophosphate is the coenzyme for two types of enzymes, alpha-ketoacid dehydrogenases and transketolases, both of which break a C-C bond next to a carbonyl group releasing carbon dioxide or an aldehyde.\textsuperscript{16}

Thiamin, in the form of thiamin pyrophosphate (TPP), is the coenzyme for three enzyme complexes that catalyse oxidative decarboxylation reactions; pyruvate dehydrogenase in carbohydrate metabolism, alpha-ketoglutarate dehydrogenase in the citric acid cycle, and a branched chain keto-acid dehydrogenase that catalyses metabolism of leucine, isoleucine and valine; TPP is also the coenzyme for transketolase in the pentose phosphate pathway. In all cases, apart from transketolase, the carbanion adds to a carbonyl group of the reactant eg pyruvate, which then decarboxylates eliminating CO\textsubscript{2}.\textsuperscript{61}

**Thiamin in carbohydrate metabolism and the citric acid cycle**

The citric acid cycle is an endpoint of the catabolism of all major products of digestion with all the pathways leading to the production of acetyl-CoA, which enters the citric acid cycle for complete oxidation to CO\textsubscript{2} and H\textsubscript{2}O.\textsuperscript{61}

![Diagram of the citric acid cycle](modified from 61)

Figure 2.6 The citric acid cycle is an endpoint (modified from\textsuperscript{61})
Thiamin pyrophosphate is the coenzyme for enzyme complexes that catalyse the oxidative decarboxylation reaction in the citric acid cycle.

Figure 2.7 Carbohydrate metabolism and the citric acid cycle showing the place of thiamin pyrophosphate\textsuperscript{16}
Figure 2.8  Decarboxylation of pyruvate in carbohydrate metabolism with thiamin pyrophosphate as a coenzyme (modified from\textsuperscript{16})

\begin{center}
\begin{tikzpicture}
\node[draw,align=center] (a) at (0,0) {\textbf{Pyruvate} \quad \textbf{TPP} \quad (electron transfer - TPP to pyruvate) \quad (nitrogen of TPP acting as an electron sink) \quad \text{resonance stabilized} \quad \text{transfer of decarboxylation product to coenzyme A} \quad [\text{several steps}] \quad \text{Acetyl CoA}};
\draw[->] (a) -- (0,-1);
\end{tikzpicture}
\end{center}

Figure 2.9  Decarboxylation of alpha-ketoglutarate in the citric acid cycle with thiamin pyrophosphate as a coenzyme (modified from\textsuperscript{16})

\begin{center}
\begin{tikzpicture}
\node[draw,align=center] (a) at (0,0) {\textbf{Alpha-ketoglutarate} \quad \textbf{TPP} \quad \text{electron transfer - TPP to alpha-ketoglutarate} \quad \text{nitrogen of TPP acting as an electron sink} \quad \text{resonance stabilized} \quad \text{transfer of decarboxylation product to coenzyme A} \quad [\text{several steps}] \quad \text{SuccinylCoA}};
\draw[->] (a) -- (0,-1);
\end{tikzpicture}
\end{center}
The citric acid cycle takes place in the mitochondria, and is aerobic, that is it requires mitochondrial enzymes and oxygen. This is in contrast to glycolysis (prior to the citric acid cycle) which takes place in the cytosol of all cells, including erythrocytes (which lack mitochondria) and can function aerobically or anaerobically.

**Thiamin pyrophosphate in the pentose phosphate pathway**

The pentose phosphate pathway is an alternative to part of the pathway of glycolysis in the metabolism of carbohydrate.

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**Figure 2.10  Metabolism of carbohydrate showing glycolysis and the place of the pentose phosphate pathway**

The enzymes for the pentose phosphate pathway, are, like the enzymes for glycolysis, found in the cytosol. It is firstly an anabolic pathway that uses the 6
carbons of glucose to generate 5 carbon sugars. However this pathway does oxidise glucose.16,61

Its main functions are:
1. To generate NADPH for biosynthesis reactions within cells.
2. To provide cells with ribose-5-phosphate for the formation of nucleotides and nucleic acids.
3. And less importantly it can operate to metabolize pentose sugars derived from the digestion of nucleic acids and also rearrange the carbon skeletons of dietary carbohydrates into glycolytic/gluconeogenic intermediates.

The pentose phosphate pathway accounts for 30% of the oxidation of glucose in the liver. Erythrocytes also use the pentose phosphate pathway to generate large amounts of NADPH used in the reduction of glutathione.17 There are two parts to the pathway. The first is an oxidative phase that is nonreversible, and the second is nonoxidative and reversible. The oxidation steps occur at the beginning of the pathway and are the reactions that generate NADPH. The non-oxidative reactions of the pentose phosphate pathway are primarily designed to generate ribose-5-phosphate for the formation of nucleotides. Equally important reactions of the pentose phosphate pathway are to convert dietary 5 carbon sugars into both 6 (fructose-6-phosphate) and 3 (glyceraldehyde-3-phosphate) carbon sugars which can then enter the pathways of glycolysis.17
2.1.15 Nutrient Reference Values

There are a variety of Nutrient Reference Values (NRV). They are determined from varied sources of evidence such as observations of populations, dietary intakes from national surveys, extrapolations from other populations, and in the case of thiamin, most importantly experimentation.\textsuperscript{62}
Estimated Average Requirement (EAR) is the daily intake level of the nutrient estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group.\textsuperscript{62}

The Australian Recommended Dietary Intake (RDI) is the level of intake of nutrient considered by the NHMRC, on the basis of available scientific knowledge, to be adequate to meet the known nutritional needs of practically all healthy people. The RDI includes a generous amount factored in to accommodate variations in absorption and metabolism of thiamin. Therefore the RDI exceeds the actual nutrient requirements of practically all healthy persons.\textsuperscript{62}

The NHMRC-endorsed review of NRV’s accepted several ways of estimating requirements of thiamin and inadequate thiamin status. These are low urinary excretion; low erythrocyte transketolase activity; low erythrocyte thiamin or elevated thiamin pyrophosphate effect.\textsuperscript{40} Urinary thiamin is the most widely used indicator, but erythrocyte transketolase activity is often regarded as the best functional test of thiamin status. However, erythrocyte transketolase activity has some limitations as it can be affected by factors other than diet. Erythrocyte thiamin is more stable in frozen erythrocytes, easier to standardise and less susceptible to other factors influencing enzyme activity.\textsuperscript{40} There is little information about the bioavailability of thiamin.\textsuperscript{40} Determination of the Australian RDI was published by the NHMRC in 1991, and a further revision was published in 2005, as there was a general consensus of opinion that there was sufficient new information to warrant another review. Thiamin, as a nutrient, was considered a low priority for revision.\textsuperscript{63} As the main function of thiamin is in the citric acid cycle, the pentose phosphate shunt and carbohydrate metabolism, the 1991 RDI for thiamin was expressed in terms of energy intake, as 0.1 mg/1000 kJ for all ages. This translated to an average intake of 1.1 mg for adults, which was revised in 2005, as follows:

The EAR for thiamin for adults were set on the basis of a number of metabolic studies using various endpoints.\textsuperscript{40} Consideration of these studies indicated a requirement of at least 0.8 mg/day of thiamin with intakes of 1.0 mg/day being marginally adequate for normal transketolase activity and generally adequate for urinary thiamin excretion. The EAR for thiamin was thus set at 1.0 mg/day for men
and 0.9 mg/day for women based on body size and energy needs. The RDI was set assuming a coefficient of variation for the EAR of 10%.

The upper level of safety (UL) for thiamin intake has not been estimated. There are no reports of adverse effects from consumption of excess thiamin from food but there were reports from the 1940s of sensitivity to continuous high doses of oral thiamin in fortified foods or supplements. There have also been reports of anaphylaxis and death after inappropriate parenteral thiamin.40

2.1.16 Determinants of thiamin status

Thiamin deficiency is most often the direct result of poor diet, which is complicated by the effects of alcohol.1 Thiamin deficiency may result from inadequate dietary intake of the vitamin as well as from decreased absorption, defective transport, increased requirements, and enhanced losses.14

Diet

The individual on a thiamin-free diet typically depletes in fewer than two weeks. Dietary thiamin intake is the major determinant of thiamin status, shown in a study on 206 subjects aged 25-64 years from the population of Seychelles,64 a study on 1108 French citizens of mixed ages,65 and a study on 200 Spanish schoolchildren.66 All studies related dietary intake directly to thiamin status.

Alcohol

Alcohol affects thiamin uptake and other aspects of thiamin utilization.67 High alcohol intake tends to be associated with poor nutritional intake and as thiamin is one of the shortest lived vitamins in the body, it is quickly affected. It has been shown using thiamin hydrochloride that high alcohol intake and the consequent malnutrition causes inhibition of oral thiamin absorption and that there are direct effects of ethanol on intestinal transport of thiamin.27 Also, in vivo studies on rats given high levels of ethanol showed a general trend toward a slower thiamin pyrophosphorylation and a faster thiamin phosphate dephosphorylation in the small intestine, kidney, heart and liver in the presence of adequate thiamin intake.68 It has also been postulated that thiamin deficiency in laboratory animals is not only associated with brain damage but also with a tendency to consume more ethanol.69 Low levels of alcohol intake appear to have no appreciable effect on thiamin status.70
Body Mass Index

The association between thiamin status and BMI is difficult to assess as there are very few studies that examine thiamin status with respect to body weight independent of dietary thiamin intake. There is some suggestion that obese women may have altered storage mechanism for thiamin. An Italian study of obese versus normal weight women suggested obese women maintain higher levels of thiamin compared to normal weight subjects by storing greater amounts of thiamin in cells but had relatively lower levels of extracellular thiamin. In contrast a study entitled ‘Blood thiamin status and determinants in the population of Seychelles’ studied 206 subjects aged 25-64 years with a wide range of BMI. The study showed diet and alcohol as the defining determinants of thiamin status, and BMI as having no significant influence. The situation with respect to the influence on thiamin status of excess body weight per se would appear to be far from clear cut.

Age

Elderly people would seem at increased risk for thiamin deficiency. Reduced food intake, decreased dietary variety, use of medications, and decreased absorption have been suggested. In spite of this, studies in Western populations have generally shown that most elderly people meet the current recommended intakes for thiamin, but that biochemical evidence of thiamin deficiency has been reported in both free living and institutionalized elderly people. Studies with rats have suggested that age is an important factor in the process of thiamin absorption in the small intestine, and that thiamin transport rate through the intestinal wall is significantly lower in ageing rats, compared to younger rats. Conversely other studies have indicated that age has no appreciable impact on thiamin status.

Exercise

Exercise stresses pathways that depend on thiamin, thus the requirements may be increased. There are a number of studies on the relationship between thiamin needs and exercise, but none are unequivocal.

Smoking

The evidence that smoking affects thiamin status is mixed. Information from the 1983 Australian Risk Prevalence Survey comparing 1809 smokers and 4395 non-smokers showed smokers had a significantly lower intake of thiamin than non-smokers and a similar result was obtained from a British study on 3430 teenagers, and a study in the US on 1338 adults in Michigan. However a study of 3390
Mediterranean subjects showed no impact of smoking on thiamin status as did a study of 243 young British adults, and of 206 adults in the Seychelles. Whether smoking *per se*, independent of dietary intake, affects thiamin status does not appear to have been determined.

### 2.1.17 Thiamin intake in Australia

The majority of dietary surveys in Australia have been carried out post 1983, including four national surveys of food consumed.

Table 2.1 Characteristics of major dietary surveys (modified from)

<table>
<thead>
<tr>
<th>Year</th>
<th>Type of survey</th>
<th>Assessment by:</th>
<th>Population surveyed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>National Dietary Survey of Adults</td>
<td>24-h recall</td>
<td>6255 adults (25-64yrs), all capital cities</td>
</tr>
<tr>
<td>1988</td>
<td>Australian Health and Nutrition Survey</td>
<td>Food frequency</td>
<td>2196 adults (18+ yrs), all states</td>
</tr>
<tr>
<td>1993</td>
<td>Australian Food Survey</td>
<td>Food frequency</td>
<td>2039 adults</td>
</tr>
</tbody>
</table>

It is difficult to compare these surveys due to sampling and demographic differences, collection methods, changes in the nutrient composition database and other essential survey differences. The ‘Bridging Study’ provides guidelines for comparison and interpretation of results from different national nutrition surveys with suitable adjustment to factor in the differences.
Table 2.2 A comparison of national thiamin intake for adults (25–64 y) from the 1983 National Dietary Survey of Adults and the 1995 National Nutrition Survey (modified from\textsuperscript{81})

<table>
<thead>
<tr>
<th></th>
<th>Sample size</th>
<th>Mean thiamin intake (mg)</th>
<th>95% confidence interval</th>
<th>Median intake thiamin (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>lower</td>
<td>upper</td>
</tr>
<tr>
<td>Males</td>
<td>1983</td>
<td>3021</td>
<td>1.47 ± 0.85</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td>1995</td>
<td>1114</td>
<td>1.94 ± 1.20</td>
<td>1.87</td>
</tr>
<tr>
<td>Females</td>
<td>1983</td>
<td>3233</td>
<td>1.10 ± 0.64</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>1995</td>
<td>1253</td>
<td>1.35 ± 0.76</td>
<td>1.31</td>
</tr>
</tbody>
</table>

This shows that the mean intake of thiamin at each survey exceeded the RDI of the time for both males and females. The intake at the 1995 survey was greater than the 1983 survey, as one would expect, with the institution of mandatory fortification of bread making flour in 1991, between the two surveys. The 1995 National Nutrition Survey showed that greater than 90% of Australian adults exceeded the RDI for thiamin.\textsuperscript{82}

2.1.18 Deficiency states and extent of deficiency in adults in Australia

There is very little thiamin stored in the body, so depletion can occur rapidly, even as quickly as 14 to 18 days.\textsuperscript{12, 67, 83}

Types of thiamin deficiency

Initial symptoms of thiamin deficiency are gastrointestinal, such as loss of appetite, nausea and vomiting.\textsuperscript{13} Marginal deficiency results in non-specific symptoms such as malaise, weight loss, irritability and confusion.\textsuperscript{52}

Beri-beri is the cover-all term for frank thiamin deficiency.\textsuperscript{13} ‘Wet’ beri-beri affects the cardiovascular system. ‘Dry’ beri-beri affects the nervous system.\textsuperscript{12} ‘Wet’ beri-beri is a cardiac failure, unlike that caused by other types of heart disease. It is due to intense peripheral vasodilatation caused by accumulation of lactate. The
lactate accumulates because the lack of TPP precludes conversion of pyruvate to acetyl CoA. There is oedema, warm extremities, and usually a normal ECG. Rapid response to thiamin treatment confirms the diagnosis. ‘Dry’ beri-beri affects the nerves in the legs and feet. There is loss of sensation in the feet and weakness. Unlike wet beri-beri, response to thiamin is slow.

"A certain very troublesome affliction, which attacks men, is called by the inhabitants Beri-beri (which means sheep). I believe those, whom this same disease attacks, with their knees shaking and legs raised up, walk like sheep. It is kind of paralysis, or rather tremor: for it penetrates the motion and sensation of the hands and feet indeed sometimes the whole body…”

Jacobus Bonitus, Java, 1630

Wernicke's encephalopathy involves damage to multiple nerves in both the central nervous system, brain and spinal cord and nerves to the rest of the body. It is a degenerative brain disease largely associated with high alcohol intake. The mechanism of how alcohol affects thiamin status has been discussed previously (section 2.1.16). It is possible to suffer from Wernicke's encephalopathy without drinking alcohol. Korsakoff psychosis tends to develop as Wernicke's symptoms fade and is part of the same complex as Wernicke's encephalopathy. It involves damage to areas of the brain involved with memory. Sufferers may try to hide their poor memory by confabulations.

**Extent of thiamin deficiency in Australia**

Beri-beri was a notifiable disease until 1991. The last figures listed were 302 cases (38 Northern Territory and 259 in WA). Current figures are not available. Wernicke-Korsakoff syndrome has a different pattern. In the 1980s, Australia had a higher incidence of Wernicke-Korsakoff syndrome than other comparable countries. Australia’s national incidence of acute Wernicke-Korsakoff syndrome was estimated at about 22 cases/100,000 adults per year. Thiamin fortification of bread making flour was made mandatory in 1991. Numbers of acute cases of Wernicke-Korsakoff syndrome were lower in 1992 and 1993 than for any of the years preceding fortification. Studies of cadavers however, give a different picture, with Wernicke-Korsakoff syndrome being more prevalent than is indicated by audits of acute cases.
A study was carried out in Sydney on the prevalence of Wernicke-Korsakoff syndrome in Australia post thiamin fortification of bread-making flour. Human brains were studied in 2212 sequential autopsies carried out in the New South Wales Institute of Forensic Medicine between 1 January 1996 and 31 December 1997. Wernicke-Korsakoff syndrome was present in 25 people, giving a 1.1% prevalence.28

2.1.19 Dietary assessment of thiamin intake

Overview of available methods
There are numerous methods of dietary assessment. They are generally divided into attempts to obtain either an average intake, such as in assessments by dietary history and food frequency questionnaire, or a chronological observation, such as in food records or 24-hour recall.86

Food frequency questionnaire
This is designed to assess usual eating habits over the longer term. This method is expensive but does not require an interview.87

Dietary history
This method also attempts to estimate usual food intake over a relatively long period of time, is less expensive, but does require an interview.87, 88 Histories are however, subject to problems of memory.89

Estimated food records
This method records recent dietary intake. If food is weighed, this type of assessment is more accurate but requires a minimum level of literacy and a high level of motivation in the participants. Subjects completing food records may change their eating pattern as a function of being observed.87, 88

The 24-hour recall
This method is often used in cross-sectional studies. It has the advantage of imposing less burden on the participants, so compliance is normally high.86 It is also more objective, being a retrospective recent record, which precludes subjects altering normal eating pattern due to observation.89 It requires a face-to-face interview, and a reasonable number of subjects.87 It minimizes the problem of memory, it being almost a given that most people remember what they ate yesterday better than they remember what they usually eat.89 It is a useful method of nutritional assessment when some participants have low levels of literacy, as shown by a WHO study on
nutritional intake in 82 subjects in Kenya with varying literacy,\textsuperscript{90} and another on 2134 subjects in San Antonio Texas, also with varying literacy.\textsuperscript{91} It is less useful in assessing ‘usual intake’ than dietary history,\textsuperscript{86, 87, 92} but its usefulness in this area is increased by repeat 24-hour recalls.\textsuperscript{87 93}

**Reproducibility of the 24-hour recall**

Reproducibility refers to the ability of a method to give similar results when repeated. Reducing error increases reproducibility.\textsuperscript{87}

Systematic error in 24-hour recall can be reduced by using a standard interviewing technique, interviewer training and/or having the same interviewer, using a standard method for food data entry and appropriate database for nutrient analysis.\textsuperscript{94}

Random error increases the variance of estimates, and the main source of random error in 24-hour recalls is day to day variation in food intake. It is also less in estimating nutrients which are present in many foods, and therefore less likely to vary from day to day.\textsuperscript{94} A test–retest method is normally used to check repeatability, with the same assessment method being used on the same subjects with a similar time gap.\textsuperscript{87} The gap should be short enough to minimize time-related change in food patterns, for example, seasonal change, but long enough that the memory of the first interview has dwindled, with 4 to 8 weeks being one recommendation.\textsuperscript{95} The use of a single 24-hour recall to assess the usual intake of an individual can be used, but the repeatability of the method should be assessed.\textsuperscript{87} The use of 24-hour recalls generally shows greater repeatability in adults than children.\textsuperscript{96}

**Statistical methods for assessing repeatability of 24-hour recall**

Correlation describes the linear relationship between two variables,\textsuperscript{97} in this case the first and second dietary assessment. The correlation coefficient $r$ is a measure of the closeness of the relationship between the two sets of assessments. The most commonly used are Pearson’s product moment correlation coefficient for data with a normal distribution and Spearman’s nonparametric rank correlation coefficient for data with other than a normal distribution.\textsuperscript{98} Demonstrating a high level of correlation between two sets of dietary assessments (by calculating significance) shows an association but not necessarily agreement between the two sets of results,\textsuperscript{86} that is, $r$ measures the strength of a relation between two variables, not the agreement between them.
A Bland Altman Plot is a method described by Bland and Altman in a paper in 1986.98 Broadly speaking they recommended that an initial plot of the data be made and the line of equality drawn on which all points would lie if the two assessments were exactly the same every time. They then recommended calculating the correlation coefficient (r) to see if the two sets of assessments were linearly related. There is perfect agreement only if the points on the plot lie along the line of equality, but there will be perfect correlation if the points lie along any straight line. Bland and Altman suggested that the difference between the assessments be plotted against their means.98 This would allow an investigation of any possible relationship between the measurement error and the closest approximation to the true value, which is the mean. The lack of agreement would be made clearer by calculating the bias, which is estimated by drawing a line parallel to horizontal representing the mean of the differences (d). It is assumed 95% of differences will lie between the limits d ± 1.96 SD (95% confidence intervals or ‘limits of agreement’). The standard error of d is then calculated as $\sqrt{s^2 / n}$, where n is the sample size, and the standard error of d ± 2s as about $\sqrt{3s^2 / n}$.98 Dietmar pointed out the importance of investigating whether the upper or lower 95% confidence limit of 1.96 SD of the differences between the methods is equal to or smaller than a predefined limit for total error.99

**Thiamin and the 24-hour recall**

Where 24-hour recalls are carefully planned and carried out, and sufficient numbers of subjects are interviewed they can give accurate results for many nutrients including thiamin.87 This was shown by Kigutha in a study on the nutritional intake of 82 subjects in Kenya, where results for thiamin intake obtained from both 24-hour recalls and weighed 3-day food records showed little variation.90 Other examples include Munger et al, in two urban areas in the USA, who showed a good correlation between 24-hour recalls and food frequency questionnaires for thiamin (r = .33 for micronutrients, including thiamin),100 although correlation with food frequency is a less convincing demonstration of accuracy. More convincingly, Sharma et al showed good agreement (10% variation) between a single 24-hour recall and weighed food records in haemodialysis patients.101 Knapp showed that the use of food models, household measures and containers to identify and quantify foods can increase the accuracy of 24-hour recalls and give a reasonable assessment of thiamin intake. The day of the week is normally a source of variation in 24-hour recalls however this appears to have little effect in the assessment of thiamin,102 which may be
attributable to the fact that food sources providing thiamin are largely cereal-based, which tend to be eaten on a daily basis. Also within-subject errors in nutritional assessment are less in the assessment of nutrients present in many foods\textsuperscript{87} such as thiamin. The converse of this is a study by Basiotis showing 24-hour recall can be a poor estimate of usual long term intake of thiamin with a minimum of 41 to 46 days food records necessary to obtain ‘a true average intake for an individual’\textsuperscript{103}

Overall there is evidence supporting 24-hour recall as a useful tool to assess thiamin intake and in view of the fact that thiamin has a half-life in the body of approximately 9.5 – 18.5 days, estimation of recent dietary intake rather than average dietary intake is considered relevant to current thiamin status\textsuperscript{18}

### 2.1.20 Biochemical assessment of thiamin status

In humans, there is not, at present, a universally agreed upon direct physiological functional indicator of thiamin status\textsuperscript{87} In animal studies whole blood thiamin levels relate to body thiamin stores although in the early stages of depletion they tend to be more depressed than total body stores and transketolase values underreact during early thiamin deprivation. Assessment of body stores in humans has been more problematic\textsuperscript{104} The common biochemical tests for assessment of thiamin status are: Erythrocyte transketolase activity (ETKA), urinary thiamin excretion, and whole blood and red cell thiamin levels\textsuperscript{18}

**ETKA**

This is probably the most widely used, and is an indirect assessment. Red blood cells only have one option for generating NADPH, namely the pentose phosphate pathway. Transketolase which catalyzes reactions in the pentose phosphate pathway requires thiamin pyrophosphate. So the level of transketolase activity in the red blood cell is a reliable diagnostic indicator of thiamin status. ETKA is determined by measuring the disappearance of pentose and appearance of hexose sugars in haemolysed red blood cells in the absence, and then presence of excess thiamin diphosphate\textsuperscript{14} The difference between stimulated activity, and basal activity is expressed as a percentage of the basal activity as an activity coefficient or percent stimulation\textsuperscript{87} Thiamin deficiency is then associated with a decreased ETKA and an increased stimulated ETKA\textsuperscript{18}
ETKA, however, has shortcomings. Some diseases cause abnormalities in ETKA. Low ETKA has been described in liver disease, uremic nephropathy, cancer, and diabetes.\textsuperscript{105} It has been postulated that low ETKA values in diabetes are due to a reduced apoenzyme level.\textsuperscript{106} Thornalley asserts that the conventional assessment of thiamin by the ratio of unstimulated to stimulated ETKA is masked in diabetes by increased thiamin transporter content of the erythrocytes.\textsuperscript{2} Kimura also noted reduced activities of glycolytic enzymes in erythrocytes from people with diabetes.\textsuperscript{107} Prolonged thiamin deficiency decreases the level of apotransketolase,\textsuperscript{108} and this will also confound the result. Age also can reduce transketolase activity, and there is a large within-subject variation in ETKA which makes interpreting transketolase activity coefficients difficult.\textsuperscript{87}

**Urinary excretion of thiamin**

In spite of the fact that the US study NHANES 1 used casual urine samples for thiamin assessment,\textsuperscript{109} in subjects with adequate thiamin, urinary excretion does not reflect body stores of thiamin,\textsuperscript{87} but rather is an index of recent dietary intake.\textsuperscript{14} In thiamin deficient subjects, it is not a good indicator of dietary intake or body stores.\textsuperscript{87} In addition, most urinary thiamin excretion is in the form of metabolites.\textsuperscript{87} The “thiamin loading” test involves the measurement of thiamin excreted in urine in the 4 hours following a 5 mg dose of thiamin. An excretion of < 20 µg is indicative of thiamin deficiency.\textsuperscript{14} This test will detect severe tissue depletion of thiamin.\textsuperscript{87} The most reliable urinary test for recent thiamin intake is the thiamin excretion in a 24-hour urine sample,\textsuperscript{33} however the within subject variation is high.\textsuperscript{87}

**Total thiamin or thiamin pyrophosphate in whole blood and erythrocytes**

Early studies were sceptical as to the usefulness of whole blood and erythrocyte thiamin as an indicator of thiamin status, however, improved methods have increased sensitivity.\textsuperscript{14} Methods vary, but many involve conversion of thiamin to thiochrome and analysis by High Performance Liquid Chromatography (HPLC).\textsuperscript{105, 110, 111} with efficiency improved with semi-automation.\textsuperscript{110} More recently direct assessment of thiamin pyrophosphate by HPLC has been developed and has correlated well with ETKA.\textsuperscript{108} Microbiological methods have also been developed, variously using *Ochromonas danica*\textsuperscript{112} and *Lactobacillus fermenti*\textsuperscript{9} as the test organisms. Good correlation between ETKA and erythrocyte thiamin levels has been noted,\textsuperscript{113, 114} although the converse has also been shown.\textsuperscript{115} Erythrocyte transketolase activity has some limitations when setting an EAR, as it can be affected by factors other than
diet. Erythrocyte thiamin is more stable in frozen erythrocytes, easier to standardise and less susceptible to other factors influencing enzyme activity. Work by Herve using a comparison of direct measurement of thiamin and its phosphate esters by HPLC and ETKA showed the former to be a more sensitive and specific index of thiamin status.

**Biochemical assessment of thiamin status used in the present study**

Assessment used in this study was an automated microbiological assay of thiamin in serum and red cells by the method of Icke and Nicol. This is a microbiological assay using *Lactobacillus fermenti*. This microbiological assay and an established thiochrome method were compared by assaying 10 red cell samples by both, and correlation was good (r = 0.99), although the microbiological assay gave results which were 21-28% higher than the thiochrome method. Comparisons with serum thiamin concentrations were not carried out as the thiochrome method used was not sensitive enough to measure serum, but the concentration of total thiamin found in the serum of healthy subjects by this method compared well with concentrations reported using sensitive HPLC methods. Non parametric reference ranges for both serum and red cell thiamin were determined on 505 healthy individuals aged 18 to 90 years. Ranges were: serum thiamin - 11.3-35-0 nmol/L and red cell thiamin - 190-400 nmol/L. Results were not related to age or gender and the method was sensitive to 2.0 nmol/L of thiamin.

### 2.1 Diabetes

Diabetes is a condition primarily defined as a level of hyperglycaemia giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life.

People with diabetes accumulate glucose in their blood, resulting in a high blood glucose level or hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. What is termed diabetes mellitus is actually several different metabolic
diseases characterized by hyperglycemia resulting from lack of insulin, or a defect in insulin action, or both.\textsuperscript{119}

\subsection*{2.2.1 Prevalence}

Diabetes mellitus is a significant health problem in Australia and throughout the world.\textsuperscript{118} The 2007 figures for the worldwide prevalence of diabetes stand at 246 million people. This indicates an average of approximately 5.1\% of people in the adult population have diabetes throughout the world. The projection is that by 2025 this will increase to 333 million people, bringing the percentage of the adult population having diabetes to 6.3\%. Diabetes is the fourth or fifth leading cause of death in most developed countries and there is strong evidence that there is an epidemic in many developing countries. These estimations are drawn from data available for 212 countries and territories.\textsuperscript{120}

The prevalence of diabetes in Australia has been investigated over the last eight years by The Australian Diabetes, Obesity and Lifestyle Study (AusDiab Study). The AusDiab study was made possible by a $2.6 million grant from the National Health & Medical Research Council, with support from state governments, pharmaceutical companies and individual donors.\textsuperscript{121} The AusDiab study is ongoing, with stage 1 being carried out in 1999/2000. This stage involved more than 11,000 people. From this study, the prevalence of diabetes in Australia in those aged 25 years and over was estimated to be almost one million, many of whom did not know they had the disease. The AusDiab 2005 study estimated that the incidence of diabetes for Australian adults is approximately 275 new cases every day.\textsuperscript{121} Studies also indicate that the rate of diabetes in some Aboriginal and Torres Strait Islander communities is as high as 30\%, compared to 7\% for the non-indigenous population of Australia.\textsuperscript{122}

It has been estimated that there are more than 80,000 people with diabetes in Western Australia\textsuperscript{118}. Diabetes as the sole principal diagnosis accounted for more than $5 million costs in Western Australian hospital services in 1997-98. This represents only a fraction of other morbidities associated with or caused by diabetes, and does not begin to address other diabetes-related issues.\textsuperscript{118}
2.2.2 Symptoms and signs of diabetes

Polyuria (frequent urination)
Polydipsia (increased thirst)
Unexplained weight loss
Polyphagia (constant hunger)
Fatigue
Slow healing
Infections
Blurred vision

2.2.3 Diagnosis of diabetes

In 1965 the World Health Organization (WHO) published the first internationally agreed guidelines for the diagnosis of diabetes. They were reviewed in 1998. In November 2005 a joint WHO and International Diabetes Federation (IDF) Technical Advisory Group met in Geneva to review and update the current WHO guidelines. In the absence of a more specific biological marker to define diabetes, plasma glucose estimation is used as the basis for the diagnosis of diabetes. The criteria are based on a level of blood glucose which defines a group of people with a significantly increased risk of premature mortality and microvascular and cardiovascular complications.

The WHO group stresses that the 2 hour post glucose load test, known as the oral glucose tolerance test (OGTT), be used preferentially for diagnosis, except where circumstances prohibit this, and then suggests using fasting plasma glucose level. For clinical diagnosis it is recommended venous blood be used rather than using a blood glucose meter, which tests capillary blood, however conversion factors for venous to capillary values are given in the WHO criteria.

The current WHO diagnostic criteria for diabetes are:
- Fasting plasma glucose $\geq 7.0$ mmol/L (126 mg/dL)
- 2 hour plasma glucose (after a 75 g oral glucose load) $\geq 11.1$ mmol/L (200 mg/dL).

There is a group of subjects whose glucose levels, although not meeting criteria for diabetes, are nevertheless too high to be considered normal. The term intermediate
hyperglycaemia is sometimes used to define those with blood glucose levels less than those diagnostic of diabetes but high enough to be associated with an increased risk of diabetes and cardiovascular disease. This has also been termed pre-diabetes. “Pre-diabetes” indicates the relatively high risk for development of diabetes in these people. People with pre-diabetes are defined as having impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT).

The current WHO diagnostic criterion for IFG is:

fasting plasma glucose $\geq 6.1$ mmol/L.

The current WHO diagnostic criterion for IGT is:

OGTT $\geq 7.8$ and $< 11.1$ mmol/L.

(OGTT necessary for individuals with fasting plasma glucose $\geq 6.1 – 6.9$ mmol/L).

In the AusDiab study, a cross-sectional survey of adults aged 25 years and older, IGT affected 10.6% of subjects, being more common in women (11.9% v 9.2% in men), and IFG was present in 5.8%, being more prevalent in men (8.1% v 3.4% in women). This represents an overall prediabetes prevalence of 16.4% in Australian adults aged 25 years and over. People with prediabetes have an increased chance of diabetes. The reported rates of conversion of those with impaired glucose metabolism to diabetes vary. However they have been quoted as 64.5% of patients with IGT developed diabetes over a follow-up of 5.8–6.5 years, and 32% within 3.5 years for IFG.

2.2.4 History of diabetes

The first mention of diabetes was in about 1500 BC when Ancient Hindu writings spoke of a deadly disease that caused intense thirst, large urine output and wasting away of the body. It was attributed to an over indulgence in food and drink, and it was recorded that insects and flies were attracted to the urine as it tasted sweet. Over the years many remedies were tried, however for thousands of years young people with diabetes died quickly, often within days of onset, and older people struggled with devastating complications.
Table 2.3  Significant events in diabetes (modified from \textsuperscript{127})

<table>
<thead>
<tr>
<th>Date</th>
<th>Significant events in diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1700</td>
<td>Two schools of thought: 1. Sugar lost in urine should be replaced in the diet. 2. Carbohydrate should be restricted to reduce the sugar in the urine</td>
</tr>
<tr>
<td>1800</td>
<td>First chemical tests to measure sugar in the urine</td>
</tr>
<tr>
<td>1869</td>
<td>Paul Langerhans discovered islet cells in the pancreas ‘islets of langerhans’</td>
</tr>
<tr>
<td>1889</td>
<td>Dr Joseph von Mehring and Dr Oskar Minkowski ascertain that removing the pancreas causes diabetes.</td>
</tr>
</tbody>
</table>
| 1897      | Average life expectancy at this time for someone diagnosed at:  
- 10 years old – 1.3 years  
- 30 years old – 4.1 years  
- 50 years old – 8 years  
Usual therapy was a diet $< 2000$ Kjs daily, prolonging life by a few months                                               |
| 1921      | Banting and Best discover insulin, a pancreatic extract that lowered blood glucose in dogs that had had their pancreas removed.                                                   |
| 1922      | Elizabeth Hughes, daughter of the Governor of New York, developed type 1 diabetes in 1918 and now weighs only 20 kgs. Banting treats her with insulin and her recovery is hailed as a miracle.  |
| 1922      | Eli Lilly manufacture insulin for use in Australia                                                                                                                            |
| 1923      | Banting and Macleod win Nobel Prize for Medicine for discovery of insulin                                                                                                     |
| 1940s     | The link between diabetes and complications was made.  
Life expectancy for a 10 year old diagnosed with diabetes is now 45 years.                                                                                         |
| 1950      | Recommended diabetic diet was 40\% fat, 40\% carbohydrate and 20\% protein                                                                                                    |
| 1955      | Two types of diabetes were recognized – type 1 and type 2 and oral drugs were introduced to help lower blood glucose levels.                                                        |
| 1959-1960 | Radioimmunoassay technology shows type 1 diabetes is associated with a lack of insulin and research into antibodies to insulin producing cells confirms type 1 diabetes is an autoimmune disease. |
| 1976      | Hb1Ac test developed to assess blood glucose in the preceding 3 months.                                                                                                    |
| 1980      | Recommended diet was only 30\% fat, to help reduce heart disease                                                                                                             |
| 2003      | The first islet cell transplantation in Australia is conducted at Westmead Hospital on a 37 year old man.                                                                   |
2.2.5 Types of diabetes

The vast majority of cases of diabetes fall into two broad categories; type 1 and type 2 diabetes, however there are many subsidiary types, the main being gestational diabetes. The situation is, however, far from clear, as it has happened that type 1 and type 2 diabetes can co-occur in the same families, and a possible common genetic susceptibility has been mooted. There is also evidence that some people have insulin resistance as well as anti-islet autoimmunity, perhaps indicating type 2 and type 1 diabetes might coexist. The level of hyperglycemia reflects the severity of the underlying metabolic process and its treatment more than the type of the process itself. Notwithstanding, the differences being generally marked, the two major types of diabetes will be treated separately.

Definition of type 1 diabetes
The defining feature of type 1 diabetes is ß-cell destruction, usually leading to absolute insulin deficiency.

Definition of type 2 diabetes
Type 2 diabetes results from a defect or defects in insulin secretion, usually with a major contribution from insulin resistance. People with type 2 diabetes show resistance to the action of insulin but in time there is also a progressive failure of the pancreatic beta cells and relative insulin deficiency as well. Hyperglycemia sufficient to cause pathologic and functional changes in various target tissues, but without clinical symptoms, may be present for a long period of time before type 2 diabetes is diagnosed. Although all causes of type 2 diabetes are not known, by definition autoimmune destruction of the pancreas does not occur. The majority of patients with this form of diabetes are obese, and obesity itself causes or aggravates insulin resistance. Many of those who are not obese by the criterion of BMI may have an increased percentage of body fat distributed predominantly in the abdominal region, although there is evidence to the converse indicating that BMI and measurements of central obesity may have similar associations with incident diabetes. Certainly, a reduction in weight per se improves insulin resistance, and a recent study by Haffner over 4 years targeting high-risk nondiabetic individuals (n = 3,234) with a mean BMI of 34.0 showed a group with an average weight loss of 5.6 kg reduced incidence of diabetes by 58%.
Definition of gestational diabetes (GDM)

GDM is IGT, IFG or frank diabetes with onset during pregnancy. The definition is independent of whether treatment is by diet or insulin and whether or not diabetes persists post pregnancy. A review of 28 studies on conversion of GDM to type 2 diabetes showed cumulative incidence of type 2 diabetes varied widely among studies, that progression to type 2 diabetes was highest in the first 5 years after delivery. The conversion rates to type 2 diabetes ranged from 2.6 to 70%, over a period from 6 weeks to 28 years.132

2.2.6 Demographics of different types of diabetes

Type 1 diabetes

People with type 1 diabetes constitute only about 5–10% of those with diabetes.119 Throughout the world there is a large variation in prevalence of type 1 diabetes. It is postulated this may be partly due to different distributions of genes increasing the risk of the disease as well as variation in possible environmental risk factors. It may also be due to methodological problems in determining prevalence in some geographical areas.120

In Australia the most recent national estimates of prevalence of type 1 diabetes comes from the 2004-05 National Health Survey, which collected self-reported information. From this survey it was estimated that 91,900 Australians (0.5% of the population) had type 1 diabetes.133 Data collected by the National Diabetes Supply Scheme indicates type 1 diabetes is on the increase in Australia.134

In Western Australia, the rate of type 1 diabetes in the 0-14 age group was measured in 1983 and showed a prevalence of 59 per 100,000 population and an annual incidence of 12.3 per 100,000 population, which is comparable to the rest of Australia. In 1998, a total of 740 children and young adults (0-21 years of age) were attending type 1 diabetic clinics around the State (Princess Margaret Hospital — personal correspondence 1998).118

More recently, the only reliable data on the incidence of type 1 diabetes is in those up to 14 years of age for Western Australia, and this also records a significant increase in the disease.135
Type 2 diabetes

Type 2 diabetes constitutes about 85% to 95% of all diabetes cases in developed countries and an even higher percentage in developing countries.\textsuperscript{119, 136} There are probably many different causes of this form of diabetes.\textsuperscript{119} Type 2 diabetes more often occurs in middle aged and elderly people but its incidence is increasing in younger people.\textsuperscript{118}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{diabetes_prevalence.png}
\caption{Self-reported prevalence of diagnosed diabetes by age in Western Australia.\textsuperscript{139}}
\end{figure}

In 2007, it was estimated that there were 246 million people with diabetes in the adult population in the seven regions of IDF. This means 7.3% of adults aged 20-79 in all IDF member countries have diabetes.\textsuperscript{136} The Western Pacific Region and the European Region have the highest number of people with diabetes, approximately 67 and 53 million, respectively. The highest rate of diabetes prevalence is to be found in the North American region (9.2%) followed by the European Region (8.4%).\textsuperscript{136}

There is a distinct impact of migration on the incidence of type 2 diabetes, which is paralleled by the prevalence of obesity in different ethnic groups. The risk escalation follows a gradient, as migrants become more affluent and urbanized, indicating an important role of environmental factors.\textsuperscript{137} The determinants appear to include
nutrition transition, physical inactivity, and some assert, stress. There appear to be
differences between ethnic groups in their susceptibility to diabetes under the same
environmental pressure. Generally speaking increasing diabetes prevalence is
projected to occur because of ageing populations, diet, increasing obesity and an
increasingly sedentary lifestyle.

![Ethnic origin](image)

**Figure 2.13** Differences in the prevalence of type 2 diabetes among two selected
ethnic groups, 2003

The prevalence of type 2 diabetes in Australia as determined by the AusDiab study
has been discussed in detail in section 2.2.1. In addition, the Australian Bureau of
Statistics National Health Survey 2004-05, reports prevalence of type 2 diabetes
based on self-reported information. According to this survey, 582,800 people
(approximately 3% of the population) reported having type 2 diabetes versus the
Ausdiab figures of over 7.2%.

The rate of diabetes in the indigenous population far exceeds that in the non-
indigenous Australians. The only national estimates of the prevalence of diabetes
among indigenous people come from population surveys conducted by the Australian
Bureau of Statistics. The most recent survey is the 2004-2005 National Aboriginal
and Torres Strait Islander Health Survey, where it was reported that the prevalence of
diabetes in participating aboriginal people was 6%, with the proportions being 9%
for those in remote areas and 5% in urban aboriginal people. This means, on age adjusted figures, diabetes is approximately 3.4 times more common in indigenous than in non-indigenous people. The ratio between indigenous and non-indigenous females of 4.1 was higher than that between indigenous and non-indigenous males, which was 2.9.

2.2.7 Differentiation of diabetes types

Once diabetes is diagnosed by blood glucose level it is necessary to determine the type of diabetes. The American Diabetes Association Expert Committee has proposed an etiologic classification of diabetes as below.

Type 1 diabetes
This is divided into type 1A diabetes representing immune-mediated diabetes and type 1B a non-autoimmune idiopathic form of type 1 diabetes.

Type 1A diabetes
In the majority of people with type 1 diabetes autoimmune destruction of the insulin producing β-cells of the pancreas occurs. Autoimmune destruction of β-cells is related to both genetic and environmental factors that are still poorly defined. The increasing incidence of type 1A diabetes is taken to suggest that environmental factors are of importance.

It has been stated that features of type 1 diabetes do not become evident until most of the beta cells are destroyed, with a ballpark figure given of about 80% destruction needed for obvious features of type 1 diabetes to be manifest. However other sources assert the exact β-cell mass remaining at diagnosis is poorly defined. People diagnosed with type 1 diabetes are rarely obese; however newly diagnosed type 1 diabetes can occur in the obese person. Genetic susceptibility to type 1A diabetes involves multiple genes.

Immune mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even in the 8th and 9th decades of life. It is estimated that, among patients with type 1 diabetes, 40% develop diabetes by 15 y of age, 30% between 15 and 34 y of age, and 30% thereafter. The rate of β-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and
slow in others (mainly adults).\textsuperscript{119} People with type 1A diabetes are at risk for development of thyroid autoimmunity, coeliac disease, Addison’s disease, pernicious anemia, and a series of other autoimmune disorders.\textsuperscript{129}

Various autoantibodies are markers of β-cell destruction. Markers used for clinical differentiation of type 1 diabetes are islet cell autoantibodies and autoantibodies to glutamic acid decarboxylase (65) (GAD).\textsuperscript{143, 144} GAD is the pancreatic islet beta-cell autoantigen which is a major target of autoantibodies associated with the development of type 1 diabetes. It is the biosynthesizing enzyme of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Pancreatic beta cells show high levels of this enzyme.\textsuperscript{145} One and usually more of these autoantibodies are present in 85–90\% of individuals at diagnosis of type 1 diabetes.\textsuperscript{119} The International Diabetes Society (IDS) and the Centres for Disease Control have initiated the Diabetes Autoantibody Standardization Program\textsuperscript{143}. Roughly 10\% of people diagnosed with type 1 diabetes on clinical parameters have no relevant autoantibodies.\textsuperscript{143}

It is asserted that insulin resistance plays a larger role in type 1 diabetes than was generally thought. As previously stated, the onset of type 1 diabetes is often preceded by an illness and/or the onset of puberty, both conditions associated with insulin resistance. In the face of beta-cell damage and thus reduced insulin secretion, this change is enough to cause hyperglycemia.\textsuperscript{146} What is undisputed is, residual functional beta cells may still exist but are insufficient in number to maintain glucose tolerance. The events that trigger the transition from glucose intolerance to frank diabetes are often associated with increased insulin requirements, as might occur during infections or puberty.\textsuperscript{141} After the initial clinical presentation of type 1A diabetes, a "honeymoon" phase may ensue during which time glycemic control is achieved with modest doses of insulin or, rarely the need for exogenous insulin temporarily disappears. As many as 40\% of all people diagnosed with type 1 diabetes enjoy a remission of some degree after starting insulin treatment\textsuperscript{147}. Remission is due to partial functional recovery of the surviving beta cells, whose capacity to secrete insulin is impaired by hyperglycaemia itself. It is generally stated that the honeymoon often lasts 3-6 months but may continue for 2 years.\textsuperscript{141} A study on 103 children less than 12 years of age showed that partial remission occurred in 71, and complete remission in 3. The length of time until remission was $28.6 \pm 12.3$ (mean ±
SD) days. The duration of remission was 7.2 ± 4.8 months. Remission rates were higher in those patients older than 5 y compared with those between 3 and 5 y of age.\textsuperscript{148} The honeymoon phase ends when the remaining beta cells are finally destroyed by the continuing autoimmune onslaught.\textsuperscript{147} For patients with long-term type 1 diabetes, there is often evidence via C-peptide secretion of some \( \beta \)-cell function remaining, although \( \beta \)-cell mass is usually decreased to less than 1\% of normal.\textsuperscript{129} The individual is, at this stage functionally insulin deficient, and will remain so.\textsuperscript{141}

![Figure 2.14: Development of type 1 diabetes\textsuperscript{141}](image)

**Type 1B diabetes**

A small minority of patients with type 1 diabetes have no evidence of autoimmunity. Most are of African or Asian ethnicity and the diabetes is strongly inherited. This type of diabetes has no known etiologies.\textsuperscript{119}
Type 2 diabetes

People with type 2 diabetes usually have at diagnosis insulin resistance and/or relative (versus absolute) insulin deficiency. The condition develops insidiously and the early symptoms may not be recognised. Subsequent to diagnosis, hyperglycemia sufficiently severe to cause damage and alteration in physiological behaviour to various target tissues in the body may be present. This may be without obvious clinical symptoms, and may precede the diagnosis of diabetes by a long period of time, although an abnormality in carbohydrate metabolism can be detected by measurement of plasma glucose in the fasting state or after a challenge with an oral glucose load.

Unless picked up incidentally, initial presentation of type 2 diabetes is often with the symptoms of hyperglycemia, but may even be with complications of diabetes. In type 2 diabetes, concordance between identical twins is very high, quoted as 60 – 100% (compared to 36% for people with type 1 diabetes), suggesting a much stronger genetic component in type 2 than in type 1 diabetes. However, the genetics of type 2 diabetes are complex and not well defined.

The majority of people, but not all, with this form of diabetes are obese. If genetically predisposed, the risk of developing this form of diabetes increases with age, obesity, and lack of physical activity. It is associated with at least some degree of insulin resistance. In addition to obesity and family history of type 2 diabetes, other features which may help to distinguish type 2 diabetes are a combination of: absence of specific autoimmune markers, especially GAD-65 antibodies; an absence of urinary ketones and basal or stimulated C-peptide.

Because of the prevalence of type 2 diabetes, testing for undiagnosed type 2 diabetes is recommended for high risk individuals, including:

- People with impaired glucose tolerance or impaired fasting glucose;
- Aboriginal and Torres Strait Islanders aged 35 and over;
- Certain high risk non-English speaking background groups aged 35 and over (specifically Pacific Islander people, people from the Indian subcontinent or of Chinese origin);
- People aged 45 and over who have either obesity or hypertension;
• All people with clinical cardiovascular disease and
• Women with polycystic ovary syndrome who are obese.151

**Differentiation of gestational diabetes**
Gestational diabetes is differentiated as any degree of glucose intolerance which commences or is first recognised during pregnancy, regardless of whether insulin or only dietary change is used for treatment or whether it persists after pregnancy.119

### 2.2.8 Complications of diabetes

Diabetic complications are the main cause of morbidity and mortality in those with diabetes.152 Poorly controlled diabetes can result in damage to small and large blood vessels throughout the body, leading to long-term complications.118 Cardiovascular disease (CVD) is the major cause of morbidity and mortality for people with diabetes. The common conditions coexisting with type 2 diabetes such as hypertension and dyslipidemia are risk factors for CVD, and diabetes itself is an independent risk factor. Microalbuminuria is also a well-established marker of increased CVD risk.153 Hypertension is a common comorbidity of diabetes and is a major risk factor for both CVD and microvascular complications. In type 1 diabetes, hypertension is often the result of nephropathy, while in type 2 diabetes it is usually associated with metabolic syndrome.153,154

Where insulin resistance is present, it is associated with abdominal obesity, high blood pressure and high blood fats. This cluster of risk factors predisposes people with type 2 diabetes to accelerated microvascular and macrovascular disease.118 People with type 2 diabetes have an increased incidence of lipid abnormalities, mainly low high density lipid (HDL) cholesterol levels and elevated triglyceride levels, which contributes to their high risk of CVD.153

Diabetic nephropathy is a microvascular complication of all types of diabetes and is a major cause of early death in these people.149 It occurs in 20–40% of patients with diabetes and is the single leading cause of all end-stage renal disease.155 Microalbuminuria has been shown to be the earliest stage of diabetic nephropathy in type 1 diabetes and a marker for development of nephropathy in type 2 diabetes.153
Diabetic retinopathy is a microvascular complication of both type 1 and type 2 diabetes. Diabetic retinopathy is the most frequent cause of all blindness among adults aged 20–74 years. People with diabetes are also more prone to glaucoma and cataracts than those without diabetes.\textsuperscript{153} Diabetic neuropathy is another microvascular complication of diabetes where effects on nerves can be acute or chronic and where cranial, peripheral and autonomic nerves can be affected.\textsuperscript{153}

In 2004, 11,735 deaths in Australia were related to diabetes. Where diabetes was cited as an underlying cause of death, coronary heart disease (CHD) was listed as an associated cause in 52.3\% of cases, kidney failure in 25.3\%, heart failure in 18.5\% and stroke in 17.2\%. When diabetes was listed as an associated cause, CHD was listed as the underlying cause of death in 30.5\% of cases and stroke in 7.5\% of cases.\textsuperscript{139}

The causes of the complications of diabetes are the subject of ongoing research. Body glucose metabolism uses a variety of metabolic pathways, therefore chronic hyperglycemia can induce diverse cellular changes.\textsuperscript{152} Genes also play a role in processes leading to diabetic complications, as does duration of diabetes,\textsuperscript{156} and there is some suggestion that increased oxidative stress may have a role in the pathogenesis of diabetic complications.\textsuperscript{157}

The Diabetes Control and Complications Trial (DCCT) was a clinical study conducted from 1983 to 1993 by the National Institute of Diabetes and Digestive and Kidney Diseases. The DCCT involved 1,441 volunteers with type 1 diabetes and 29 medical centres in the United States and Canada. Volunteers had diabetes for at least 1 year but no longer than 15 years. It showed that that keeping blood glucose levels as close to normal as possible reduces risk of eye disease by 76\%, kidney disease by 50\%, nerve disease by 60\%.\textsuperscript{158}

The second study targeted people with type 2 diabetes. The UK Prospective Diabetes Study (UKPDS) was a 20-year trial which recruited 5,102 people with type 2 diabetes in 23 clinical centres in the UK. The UKPDS has shown that: better blood glucose control reduces the risk of major diabetic eye disease by 25\% and early kidney damage by 33\%. It also showed better blood pressure control reduces the risk
of death from long-term complications of diabetes by 33%. Hyperglycaemia and hypertension are independent risk factors for diabetic complications in people with type 2 diabetes. Epidemiological analyses of the DCCT and UKPDS demonstrate a curvilinear relationship between markers of diabetic control and microvascular complications, and suggest that, generally speaking, the greatest number of complications will be avoided by improving diabetic control from very poor to fair or good control.

2.2.9 Treatment of diabetes

There is no cure for diabetes, but effective treatment exists. This entails keeping blood glucose levels as close to the non diabetic range as possible with a combination of diet, activity and medication. Clinical recommendations are that people with diabetes should receive care from a physician-coordinated team. Care is aimed at minimising symptoms of diabetes and long-term complications.

In type 1 diabetes recommended treatment is use of multiple dose insulin injections incorporating basal to control glucose produced by the liver and boluses of short acting insulin to cover food, all given subcutaneously. Insulin pump therapy using short acting insulin only is also available. It is necessary to dovetail insulin, carbohydrate intake and activity. The development of analogue insulins has made it possible to overcome some limitations in the pattern of action of conventional insulins. Insulin therapy needs to be individualized. Diabetes education should be part of the management of type 1 diabetes.

‘Therapy for type 2 diabetes’, a consensus statement by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes on individuals with type 2 diabetes states that control of hyperglycemia, the visible metabolic abnormality associated with type 2 diabetes, is paramount in context with treatment of obesity, insulin resistance, dyslipidemia and hypertension. Recommendations are immediate intervention at diagnosis with lifestyle changes and metformin. People with diabetes should be advised to perform at least 150 minutes per week of moderate-intensity aerobic physical activity, if medically able. They should receive individualised medical nutrition therapy, preferably provided by a
dietitian familiar with diabetes.\textsuperscript{153} They should receive continuing timely review of therapy and addition of other agents as needed, including initiation of insulin therapy.\textsuperscript{163}

\subsection{Markers of diabetic control}

Monitoring glycemic status is essential in the management of diabetes.\textsuperscript{165} The main techniques used to assess glycemic control are patient self-monitoring of blood glucose (SMBG) and haemoglobinA1C measurement (HbA1c). In addition, in recent years technologies for continuous monitoring of interstitial glucose have entered the market.\textsuperscript{153} Blood glucose testing provides essential information for day-to-day management of diabetes.\textsuperscript{165} Measurement of HbA1c is a longer term measure complementing day-to-day SMBG.\textsuperscript{165} It is a series of stable haemoglobin components formed slowly and nonenzymatically from haemoglobin and glucose. The rate of formation is directly proportional to glucose concentration in the blood. Because erythrocytes are permeable to glucose, the level of HbA1c is an indicator of the glucose level over the previous 120 days, which is the average lifespan of an erythrocyte.\textsuperscript{165} Glycohaemoglobins were first used in routine clinical laboratories for diabetes monitoring around 1977. Over the last few years the International Federation of Clinical Chemistry Working Group on Standardisation of HbA1c has been establishing International Reference Methods for HbA1c.\textsuperscript{166}

There are 3 basic types of assay currently commercially available; immunoassay, such as DCA2000 which is used for point-of-care testing used in WA by Princess Margaret Hospital; cation exchange, used by Sir Charles Gairdner and Royal Perth Hospitals, and affinity chromatography, which is considered to have the least problems with interference and abnormal haemoglobin variants (expert opinion). “Results of methods using different assay principles show excellent correlation, and there are no convincing data to show that any one method is clinically superior to any other”.\textsuperscript{165} Mathematical modelling and \textit{in vivo} studies have shown that a large change in mean blood glucose is accompanied by a large change in HbA1c within a matter of 1–2 weeks, however large variations in blood glucose levels will show an unrepresentative HbA1c and therefore frequent hypoglycaemia skews HbA1c.\textsuperscript{165}
HbA1c is normal if it is 5% or less. Normal ranges may vary slightly depending on the laboratory used.\textsuperscript{12} The correlation between HbA1c levels and outcome risks was demonstrated in the DCCT and the UKPDS.\textsuperscript{159, 167} However recent work has shown that individual biological variation in HbA1c, distinct from that that can be attributed to blood glucose levels, was evident among type 1 diabetic patients in the DCCT and was a strong predictor of risk for diabetes complications.\textsuperscript{168}

In diabetes, lowering HbA1c to 7% or less has been shown to reduce microvascular and neuropathic complications of diabetes and, possibly, macrovascular disease, but epidemiologic studies have suggested a benefit to a HbA1c of less than 6%.\textsuperscript{153}

2.3 Thiamin and diabetes

People with diabetes accumulate glucose in their blood, resulting in a high blood glucose level or hyperglycemia.\textsuperscript{118} Thiamin plays a major role in glucose metabolism and appears problematic in diabetes in a number of aspects.

2.3.1 Blood levels of thiamin in diabetes

There are mixed reports in the literature on the status of blood, plasma, and red cell thiamin levels in those with diabetes. The concentration of thiamin in red blood cells is approximately 12 times that in plasma. Numerous reports have cited a greater incidence of suboptimal blood thiamin levels in those with diabetes compared to non diabetics.\textsuperscript{3, 4, 170-172} Thornalley et al report in a recent study, plasma thiamin concentration decreased 76\% in type 1 diabetes and 75\% in type 2 diabetes compared with people without diabetes, and these decreased levels correlated with increased urinary clearance of thiamin\textsuperscript{2}. Conversely there are reports of normal blood thiamin levels in diabetes in humans\textsuperscript{5, 173} and in animal studies.\textsuperscript{174} Haugen showed low blood levels of thiamin in type 1 diabetes and normal levels in type 2 diabetes.\textsuperscript{175} Hobara, in a study on animals and humans with diabetes, documented that blood levels of thiamin increased as glucose levels increased.\textsuperscript{169}

There is little apparent reason for the variance in study results. Studies showing reduced blood thiamin levels in diabetes were carried out variously on animals,
adults and children, including those with type 1 and type 2 diabetes (with some studies not specifying diabetes type). Subjects in studies showing normal levels of thiamin varied similarly. Methods of assessment of blood thiamin varied, being mainly thiochrome method or microbiological in older studies, and high performance liquid chromatography in more recent studies, but with no particular trend in results. The two most recent papers supported reduced blood thiamin levels in diabetes.

### 2.3.2 Thiamin transport across membranes in diabetes

The transport of thiamin across membranes in people without diabetes has been reviewed in section 2.1.10. There appear to be some abnormalities in diabetes. It has been shown that a mutation of the gene SLC19A2 causes malfunction of the thiamin transporter THTR1, resulting in thiamin deficiency and thiamin responsive megaloblastic anemia, and it is thought this may be similar in diabetes. Experimental evidence suggests thiamin and thiamin monophosphate transport may be abnormal in diabetes and there is therefore decreased uptake from the gut of these. It is also suggested that mild thiamin deficiency in diabetes may induce increased expression of the thiamin transporters THTR1 and THTR2, to increase tissue scavenging of thiamin and a decrease in the expression of transporter RFC-1, to retain tissue thiamin pyrophosphate.

### 2.3.3 Thiamin excretion in diabetes

Thornalley found evidence of increased renal clearance of thiamin, with a 24 fold increase in type 1 and a 16 fold increase in type 2 diabetes. Plasma thiamin levels were negatively correlated with renal clearance. It is thought that the development of thiamin deficiency with increased renal clearance and albuminuria in diabetic rats may indicate abnormal renal handling of thiamin, and may be an early sign of declining renal function in diabetes.

### 2.3.4 Other aberrations in metabolism in diabetes relating to thiamin

As well as reports of suboptimal thiamin blood levels, there is also evidence of suboptimal activity of the thiamin dependent enzyme erythrocyte transketolase in
It has been postulated that these low levels are due to a reduced apoenzyme level. Transketolase has a short half-life (about 25 minutes) in the absence of thiamin pyrophosphate, so decreasing thiamin pyrophosphate decreases the activity of transketolase. Thornalley’s work has indicated that ETKA is not significantly reduced in diabetes and showed a thiamin stimulation effect of less than 15% in people with diabetes, indicating they were technically not clinically thiamin deficient. His work showed a masking of clinical thiamin deficiency in erythrocytes by increased thiamin content of erythrocyte membrane transporters THTR-1 and RFC-1 in both type 1 and type 2 diabetes. Kimura noted reduced activities of glycolytic enzymes in erythrocytes of people with diabetes.

As well as abnormalities in blood levels and enzymes, animal studies in diabetic animals have shown low levels of thiamin in liver and heart compared to non diabetic animals and Ariaey-Nejad has shown increased glucose turnover causes increased utilization of thiamin. Much research is being carried out in the area of thiamin and diabetes. Present results are confusing, but certainly highlight the presence of abnormal handling of thiamin in diabetes.

### 2.3.5 Dietary intake and blood levels

BMI and age can be minor modifiers of blood thiamin levels but in non diabetics, in the absence of excessive alcohol, a suboptimal blood thiamin level is normally a marker of inadequate dietary intake and one would presume that the same situation would prevail in those with diabetes. With the documented frequency of low blood thiamin levels in diabetes, it could therefore reasonably be presumed that there is a high incidence of low dietary thiamin intake in those with diabetes. However, it has been shown in a study of 50 people with type 2 diabetes that dietary intake of thiamin was greater than the RDI in 98% of cases, and similar results have been shown in a study on the nutritional content of subject-selected diets in people with type 1 diabetes. Saito, in a small study, reported no correlation between normal dietary intake of thiamin and blood and tissue thiamin levels in people with diabetes.
2.3.6 Results of thiamin supplementation in diabetes

Thiamin supplementation in diabetes appears to have mixed outcomes. A study of women with treated GDM found low blood thiamin levels in 19 of the 77 pregnancies after thiamin supplementation. Babies born to those mothers with low blood thiamin levels also had low blood thiamin levels. Valerio et al. showed that the plasma content of thiamin and thiamin monophosphate in patients with type 1 diabetes was significantly lower when compared with these measures in age-matched normal subjects after 3 months thiamin supplementation. Conversely, Kodentsova showed an improvement in blood thiamin level with ‘vitamin enriched drinks’ in children with type 1 diabetes and a separate study has shown that ETKA can be increased by pharmacological doses of thiamin.

2.3.7 Thiamin and diabetic complications

It is well accepted that complications of diabetes are related to sustained increased blood glucose levels. One theory is an increase in cytosol glucose results in accumulation of triose-phosphates, which are a potential trigger for biochemical dysfunction associated with diabetic complications. The triose-phosphates would normally be disposed of by the pentose phosphate pathway, however, in diabetes, this pathway is impaired by mild thiamin deficiency which decreases the activity of transketolase. This is consistent with the fact that a significant number of studies have shown that some complications of diabetes benefit from thiamin supplementation and that the benefit is not via an improvement in blood glucose levels. This is further supported in a study by Valerio et al which showed thiamin supplementation had no effect on HbA1c.

Increased bioavailability and dose of thiamin supplementation may enhance the effect on complications. Some studies have used lipophilic preparations of thiamin, such as benfotiamin, to increase the bioavailability. Karachalias et al showed in animal studies that high dose benfotiamin therapy countered incipient nephropathy. Conversely they also showed high dose thiamin, and not benfotiamin countered diabetic dyslipidemia. In another animal study Stracke showed benfotiamin reduced functional damage and the formation of advanced glycation end products.
products (AGE) associated with nerve damage in nerves of diabetic rats.\textsuperscript{188} Winkler et al showed a differential in therapeutic benefit on painful diabetic neuropathy of benfotiamin according to dose. Higher dose benfotiamin showed greater efficacy.\textsuperscript{186} However, conversely, studies in diabetic rats have indicated that benfotiamin is a better deliverer of thiamin in normal rats than in diabetic rats.\textsuperscript{8} Generally, it appears a larger proportion of research is being carried out using benfotiamin, rather than water soluble thiamin, but the benefits are certainly not clear cut.

Bender noted that ‘the metabolism of glucose is deranged in thiamin deficiency, but once any deficiency has been corrected there is no further effect of increased thiamin intake on the ability to metabolize glucose’.\textsuperscript{189} A study by Abbas and Swai showed blood thiamin levels were directly reduced proportional to the severity of symptoms in diabetic peripheral neuropathy.\textsuperscript{190} Conversely, as quoted previously, Hobara, in a study on animals and humans with diabetes documented that thiamin blood levels increased as glucose levels increased.\textsuperscript{169} As increased glucose levels are associated with diabetic complications, the last two studies appear contradictory. Thornalley has shown that plasma thiamin and thiamin excretion correlated negatively with vascular adhesion molecule, plasma sVCAM-1, which is increased in diabetes and is a marker of vascular disease.\textsuperscript{2} Again, there appears to be no particular effect of thiamin upon blood glucose levels. Overall, the relationship between thiamin and diabetic complications is a work in progress.

2.3.8 Possible mechanism by which thiamin relates to diabetic complications

A possible mechanism for the effect of thiamin on diabetic complications has been put forward by Hammes from a study using bovine aortic endothelial cells and is as follows: of the 4 metabolic pathways related to hyperglycemia-induced diabetic complications, 3 are activated by increased availability of the glycolytic metabolites: glyceraldehyde-3-phosphate and fructose-6-phosphate. The pathways are the hexosamine pathway, the AGE formation pathway and the diacylglycerol (DAG)-protein kinase C (PKC) pathway. Glyceraldehyde-3-phosphate and fructose-6-phosphate are also end products of the pentose phosphate pathway, and the activity of transketolase determines the rate in this pathway.\textsuperscript{181} Transketolase has been shown to have reduced activity in diabetes.\textsuperscript{3, 105} If thiamin pyrophosphate availability is
optimal, this increases the activity of transketolase which diverts metabolites into the pentose phosphate pathway.\textsuperscript{181} In another \textit{in vitro} study on human red blood cells in the presence of hyperglycemia, Thornalley et al demonstrated that adding thiamin increased the activity of transketolase, and decreased triose-phosphates and methyl glyoxal which are associated with development of diabetic complications.\textsuperscript{191}

Figure 2.15 Possible mechanism by which increased thiamin blocks pathways of hyperglycaemic damage (modified from\textsuperscript{181})

Hammes et al theorise that hyperglycemia may cause ‘functional thiamin deficiency’ in vascular endothelial cells, and while the mechanism is unknown, it may be that hyperglycemia renders these cells unable to downregulate glucose transport resulting in increased intracellular glucose and superoxide which oxidises thiamin to biologically inactive thiochromes.\textsuperscript{181} Ascher et al demonstrated in another \textit{in vitro} study that pharmacological amounts of thiamin reverse endothelial damage in the presence of high blood glucose levels,\textsuperscript{192} and similarly La Selve, showed that thiamin corrects cell replication and decreases AGE products in human umbilical vein endothelial cells cultured under high glucose.\textsuperscript{193} Thornalley, in a review paper, asserts that thiamin supplementation prevented multiple mechanisms of biochemical dysfunction: activation of protein kinase C, activation of the hexosamine pathway, increased glycation and oxidative stress, thus preventing incipient diabetic nephropathy, neuropathy and retinopathy.\textsuperscript{8}
CHAPTER 3  METHODS

3.1 Study population - The Royal Perth Hospital Diabetic Clinic Diabetic Survey

This project was carried out in conjunction with, and based on, participants in the Royal Perth Hospital Diabetic Clinic Diabetic Survey. The Diabetic Survey functions as a clinical review, designed to identify early indicators of morbidity allowing timely intervention, and also functions as a base for research. The Diabetic Survey was initiated by Dr John Calder in the late 1960’s. Computer records, and a database of enrolled patients commenced in 1973, allowing better organization and access to survey results and the recall of participants on a two yearly basis. Participants comprise selected patients with type 1 and type 2 diabetes attending the Royal Perth Hospital Diabetic Clinic. Selected private patients of diabetic clinic physicians are also enrolled. Patients are placed on the survey data base at the discretion of the treating physician. Exclusion criteria for selection are: diagnosed with diabetes for less than one year, significant other comorbidities, alcohol abuse, and aged over 70 at entry.

Diabetic Survey runs every Thursday morning with patients attending from 7 am until approximately midday. Patients attend fasting; are weighed, measured, vision tested and then have a barrage of blood tests. After breakfast, they attend a diabetic physician for a complete medical review, then non-fasting blood tests, and reviews in cardiology and ophthalmology. Results of blood tests are entered on the hospital database (‘i Soft’) and also on the Diabetic Survey database. All other parameters, including lifestyle factors, are entered only on the Diabetic Survey database. Apart from urgent problems, little medical intervention takes place at Diabetic Survey. When all results are available, patients are recalled for follow-up tests, dietary counselling where applicable, and a summary of results is sent to the patient’s nominated general practitioner.
3.2 Research design

This study was cross sectional, designed to look at factors affecting thiamin status in people with type 1 and type 2 diabetes. The major factors analysed were dietary, with data for other possible minor modifiers of thiamin status, including diabetes control, being accessed.

3.3 Subjects

3.3.1 Subject selection

Initial subjects were consenting patients with type 1 and type 2 diabetes attending the Diabetic Survey. Patients were selected consecutively on the day of attendance at Diabetic Survey and oral and written information was given on the study aims (see appendix 1), the information that would be required from participants, the results that would be accessed and the approval of the study by Royal Perth Hospital and Curtin University ethics committees. A consent form was then signed by subjects who agreed to participate (see appendix 2). Additional subjects were consecutive consenting patients with type 1 and type 2 diabetes on thiamin supplementation. Data obtained was as for initial patients, with the exception of a dietary assessment.

Exclusion criteria were:

- Daily alcohol intake of greater than 4 standard drinks for males and 2 standard drinks for females: Alcohol affects thiamin uptake, and may affect other aspects of thiamin utilization such as on oral thiamin transport, and intestinal pyrophosphorylation and dephosphorylation. Low levels of alcohol intake appear to have no appreciable affect on thiamin status. Alcohol and thiamin have been discussed in section 2.1.16. Alcohol intake was self reported.
- Significant other comorbidities: Excluded from this study as significant comorbidities may have an independent direct or indirect effect on thiamin status. Already excluded from Diabetic Survey Database.
- Pregnancy or lactation: This alters thiamin metabolism. Pregnancy and lactation were self reported.
• Currently on antibiotics: interferes with microbiological assay for thiamin. 
  Antibiotic status was self reported and cross checked on survey records.
• Unable to give a 24 hour food recall.

3.3.2 Sample size

The sample size for initial subjects was estimated based on the results of a previous small study by SV (prior to her Master's enrolment) and a colleague where dietary intake of thiamin was assessed and compared in 40 people with diabetes, 20 with normal serum thiamin levels and 20 with reduced serum thiamin levels. \(^{194}\) A power calculation showed a sample size of 43 subjects in each group (type 1 and type 2 diabetes) would have 80% statistical power to detect a correlation of at least 0.4 between dietary intake of thiamin and red cell thiamin levels based on a 5% level of significance for each group.

The sample size for the secondary subjects was estimated based on the results of the initial subjects which showed a trend with thiamin supplementation which did not reach significance with the numbers in the initial sample. A power calculation showing an extra 30 subjects on thiamin supplements was calculated to have 80% statistical power to detect an association between the presence or absence of thiamin supplementation on attainment of normal red cell thiamin levels based on a 5% level of significance.

Data collection for initial subjects took place from 23/2/2006 to 25/5/2006. Two potential subjects were excluded, one was on antibiotics and one was pregnant. A total of 89 subjects signed consent forms (44 type 1 diabetes and 45 type 2 diabetes). Consent was obtained from 98.9% of the potential sample, with 1.1% (1 subject) declining participation. Data collection for secondary subjects took place from 25/1/2007 to 22/11/2007. Data collection ceased with an additional 24 supplemented subjects (14 type 1 and 10 type 2), as a significant result had been achieved.
3.4. Methods

Data was collected and accessed primarily to assess the effect of dietary variables and diabetic control on serum and red cell thiamin levels in diabetes. Other variables that were potential minor modifiers of thiamin status were also included. Data collected or accessed were: demographic variables: weight, height, gender, age, smoking status, level of activity, diabetes type and duration, diabetes treatment; dietary assessment: intake of thiamin, carbohydrate, energy and alcohol. Blood test results: red cell thiamin, serum thiamin, HbA1c; and urinary albumin excretion. Self report: average number of hypoglycaemic episodes per week (hypos), currently taking thiamin supplements. Data was entered on a standard sheet (see appendix 3).

3.4.1 Demographic variables

All weights were measured to the closest 0.1 kg, on the same digital platform scales accurate to 200 kg (Wellsweigh Model JIS – 200, First In Scales, Perth, West Australia). Heights were measured to the closest 0.1 cm using an adult height measure (Pharmacia – Stadiometer, Perth, Western Australia), using standard procedure. Smoking status and activity level were self-reported. Diabetes type was assigned by the presence of anti-islet antibodies, anti-gad antibodies (indicating auto immune destruction of β-cells thus type 1 diabetes), level of C-peptide (high levels
indicating high levels of insulin production, therefore probably type 2 diabetes) and clinical judgement by examining physician. Duration of diabetes was as per recorded medical diagnosis, and thereby unreliable in type 2 diabetes where diagnosis may come years after the onset of symptoms. Diabetes treatment was as recorded by the examining physician at onset of interview.

3.4.2 Dietary Assessment

Rationale for use of 24-hour recall
General pros and cons regarding the use of 24-hour recall and thiamin assessment have been discussed in section 2.1.19. The 24-hour recall is relatively easy both in time and complexity, so has a high level of compliance, requires a face to face interview, and a reasonable number of subjects. From a practical point of view it was eminently suited to the Diabetic Survey, where participants have extended periods of time between other tests, interview rooms are available, and potential participant numbers are suitable. Royal Perth Hospital services a socially and ethnically mixed population with diverse literacy and linguistic levels and it has been shown that 24-hour recall is a useful method of nutritional assessment where some participants have low levels of literacy, with the interview situation allowing information to be transmitted orally, details can be clarified and visual aids such as food models are available. Kigutha, Munger and Knapp showed the 24-hour recall was suitable for assessment of thiamin intake and correlated with other methods of dietary assessment.

Procedure for the 24-hour recall
A standardised procedure was followed:

- Subjects were given no prior warning they would be interviewed regarding their food intake at the Diabetic Survey, thus eliminating alteration of food habits due to observation, and increasing objectivity.
- Interviews took place in a relatively quiet room in the diabetic clinic, with the door closed.
- Survey participants with poor English were assigned an interpreter for the morning of the Diabetic Survey, and these were available for the 24-hour recall interview.
• As participants came in fasting and were provided with a (probably atypical) breakfast by the hospital at the completion of fasting blood tests, the 24 hour recall commenced with the first food of the previous day after midnight and proceeded chronologically through until midnight of the night preceding Diabetic Survey. All interviews was conducted by the same dietitian (SV) and recorded on a standard form (see appendix 4).

• To help ascertain portion sizes and food constituents, food models, standard measuring cups and commercial containers of common foods (eg empty tins and packets) were used.

• Participants were questioned on food preparation and food processing methods.

• Where recipes were other than standard, recipe ingredients were ascertained. Where recipes could not be recalled, follow-up information was sought, with participants being requested to post, fax, email, or phone through recipe constituents. A reminder letter was sent where applicable.

• Where commercial foods were unknown, eg culturally specific foods, participants were asked to bring in, or send food containers pertinent to these foods.

• Fast food outlets were contacted or accessed on the World Wide Web, pages from Australia, to ascertain food ingredients where necessary.

• To cross check, an average weekly intake of several high thiamin foods was also assessed, viz: vegemite, nuts, sports or high protein supplementary drinks and vitamin supplements containing thiamin.

**Reproducibility of 24-hour recall**

Reproducibility was assessed by repeat 24-hour recalls on 31 subjects. This comprised all initial subjects who were willing to give a repeat recall (34.8% of initial subject pool). These were assessed for repeatability of thiamin, carbohydrate and energy intake.

**Dietary Analysis**

The 24-hour recalls were entered into the computer dietary analysis package ‘Foodworks’ (Xyris Software, 2006) by the same dietitian who obtained the 24-hour recalls. ‘Foodworks’ defaults to AUSNUT99 database (released by Australian New Zealand Food Authority in 1999) which is a compilation of seven data files
containing data on the food and nutrient composition of Australian foods. AUSNUT99 contains 4,500 foods and brand name pointers for most commercial foods and food analyses have been updated post mandatory fortification of breadmaking flour with thiamin (1991). It also has the facility for addition of food analyses, for foods not contained in the database, and thus ingredients of recipes, commercial foods etc were obtained and were hand converted by the dietitian into nutrients and added to the database.

3.4.3 Thiamin supplementation

Initial subjects
Initial subjects supplied a 24-hour recall and any vitamin supplementation was recorded in this interview. Participants were classified as unsupplemented if they had not taken a thiamin supplement within the 10 days preceding the 24-hour recall. A standard form and a self addressed envelope were then given to participants for detailed information on type of supplement (name and manufacturer), dose, and when last supplement was taken prior to Diabetic Survey. This was followed up by phone to clarify any details, and also if the form was not returned within 2 weeks. On receipt of the form, further information was obtained on the amount of thiamin in the supplement. Information was obtained from the manufacturer; often from the company website, or by email. Supplement composition was either thiamin hydrochloride or thiamin nitrate. The proportion of thiamin was calculated using molecular weights: thiamin 266.4; thiamin hydrochloride 337.3; thiamin nitrate 327.4; giving a proportion factor of 0.79 for thiamin in thiamin hydrochloride, and 0.81 in thiamin nitrate supplements.

Additional subjects
Additional subjects did not supply a 24-hour recall but were sent a short questionnaire on supplement use and requested to bring this when they came to Diabetic Survey (see appendix 5). Subjects were contacted by phone to clarify details on this questionnaire.
3.4.4 Diabetes control – HbA1c and hypoglycaemia

Haemoglobin A1c
This was assessed with cation exchange high pressure liquid chromatography, using BioRad Variant II, certified by the National Glycohaemoglobin Standardization Program (USA). With this method HbA1c elutes at a characteristic time and can be identified on a chromatogram (figure 3.1). Assessment of HbA1c has been discussed in 2.2.10.

Figure 3.2 Typical chromatogram A) normal, B) poorly controlled diabetes
(Courtesy laboratory Royal Perth Hospital)

Hypoglycemic episodes
Average number of hypoglycaemic episodes per week was a standard additional question in the 24-hour recall interview as hypoglycemia with subsequent rebound can be a possible modifier of HbA1c. The level at which a subject had symptomatic hypoglycaemia was used as a criterion, however there is obviously some variation in level of blood glucose where subjects perceive symptoms.
3.4.5 Urinary albumin excretion

Urinary albumin was collected from a fresh urine sample with no preservative required. Collection volume was 10 – 15 mL, analysed by Immunoturbidimetric on Roche Modular Autoanalyser. Normal range < 30 mg/L.

3.4.6 Serum and red cell thiamin

As discussed previously (2.1.20), there are several methods of biochemical assessment of thiamin status. The most common is an indirect method measuring change in erythrocyte transketolase activity with thiamin stimulation, but this is unsuitable for use in diabetes, as a documented increased content of red cell membrane thiamin transporters probably falsely increases red cell content of thiamin at the expense of serum thiamin content thus elevating unstimulated transketolase activity. A more direct method of measurement is therefore required.

The methodology used in this study was an automated microbiological assay of thiamin in serum and red cells by the method of Icke and Nicol. This is a microbiological assay using Lactobacillus fermenti. Chloramphenicol and cycloheximide were added to suppress bacterial and yeast contamination. Spectrophotometric analysis was automated using a robotic liquid handling system. One serum sample was assayed 24 times in one batch to determine intrabatch precision, giving a coefficient of variation (CV) of 3-4%. Three serum samples covering a wide range of values were assayed 12 times in different batches over nine months to determine interbatch precision giving a CV for red cells of 7.4% and for serum of 6.1 – 10%. At the time of development the microbiological and an established thiochrome method were compared by assaying 10 red cell samples by both, and correlation was good (r = 0.99), although the microbiological assay gave results which were 21-28% higher than the thiochrome method. Comparisons with serum thiamin concentrations were not carried out as the thiochrome method used was not sensitive enough to measure serum, but the concentration of total thiamin found in the serum of healthy subjects by this method compared well with concentrations reported using sensitive HPLC methods. Non parametric reference ranges for both serum and red cell thiamin were determined on 505 healthy
individuals aged 18 to 90 years. None of these individuals were known to be currently receiving any therapy. Ranges were: serum thiamin 11.3-35.0 nmol/L (3.4 – 10.5 µg/L) and red cell thiamin 190-400 nmol/L (57.2 – 120.3 µg/L). Results were not related to age or gender and the method was sensitive to 2.0 nmol/L of thiamin.³

3.5 Data Analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 13 for Windows.

3.5.1 Validation of 24 hour recall

Data was entered for the 31 subjects with repeat 24 hour recalls on a separate database. Variables entered were ID, first and repeat thiamin values, first and repeat carbohydrate values and first and repeat energy values (as calculated by ‘Foodworks’).

Statistical testing was initially testing for normality, t-test for equality of means (age, duration of diabetes, BMI) and calculation of parametric and non parametric correlation coefficients between first and repeat values for each nutrient. A line of equality was drawn, and outliers identified. Means and differences for the initial and repeat values for thiamin, carbohydrate and energy were then calculated and recoded as new variables. A Bland Altman plot (means on the x-axis and differences on the y-axis) was generated, a mean of the differences (d) was calculated, 95% confidence intervals (d ± 1.96SD) was also calculated and all three lines entered on the graph. Standard error of both were ascertained. These were the limits of agreement and were compared to acceptable criteria for difference in these two nutrients.

3.5.2 Initial subjects (89 total)

The database was split into two groups. type 1 and type 2 diabetes and baseline demographics examined for each group; weight, height, gender, age, duration of diabetes and diabetes treatment. Although groups were examined independently (not as comparison groups) t-tests were carried out for descriptive purposes.
Descriptive data including normality tests were then generated for the following continuous variables: dietary: dietary thiamin, supplementary thiamin, total thiamin intake, carbohydrate, mg thiamin per % carbohydrate, energy, mg thiamin per 1000 kJ; indicators of diabetic control: HbA1c and average number of hypos; urinary albumin excretion and thiamin status: red cell and serum thiamin.

Correlation coefficients (Pearson’s product moment correlation coefficient for data with a normal distribution and Spearman’s nonparametric rank correlation coefficient) were generated to assess correlation with red cell and serum thiamin of dietary variables, indicators of diabetic control, age, BMI and duration of diabetes (for type 1 group only, as diagnosed duration of diabetes is not accurate in type 2 diabetes).

To more accurately assess association, categorical variables were generated as follows:
Thiamin intake:
Adequate/Inadequate: Adequate thiamin intake was taken as ≥ 1.1 mg/d for females and 1.2 mg/d for males and inadequate < these levels. This was based on the current RDI values, which have a very generous margin for metabolic variation.40 The results of the National Nutrition Survey showed that 90% of Australians exceeded the RDI,82 and a study by Anderson196 showed that absolute daily thiamin requirement was more useful in predicting biochemical thiamin status than thiamin intake expressed as mg/1000 kJ.
Thiamin per kJ
Adequate thiamin per kJ/Inadequate thiamin per kJ: As there was literature supporting the requirement for thiamin being dependent on energy intake197 a categorical variable was generated based on this, as per previous RDI,40 with adequate ratio being ≥ 0.0001 mg/kJ and inadequate < 0.0001 mg/kJ.
Thiamin supplementation:
Thiamin supplementation/No thiamin supplementation
Gender
Male/female
Age:
Divided into tertiles, based on percentiles within the two groups:

Type 1 Group:
1. $< 40$ y
2. $40 – 50$ y
3. $51 – 65$ y
4. $> 65$ y

Type 2 Group
1. $< 56$ y
2. $56 – 61$ y
3. $62 – 68$ y
4. $\geq 69$ y

Duration (type 1 group only):
a) Based on the earliest onset of problems with complications of diabetes$^{167}$:
1. $< 5$ y
2. $\geq 5$ y

b) Based on tertiles:
1. $< 12$ y
2. $12 – 22$ y
3. $23 – 30$
4. $> 30$

Body Mass Index:
Based on values accepted internationally and for Australians$^{198}$:
1. $< 20$ (underweight)
2. $20 – 25$ (normal range)
3. $> 25 – 30$ (overweight range)
4. $> 30$ (obese)

Haemoglobin A1c:
Based on standardised values used for this test at this venue:
1. $< 6\%$ - normal
2. $6.1 – 7\%$ - good glycemic control
3. $7.1 – 8\%$ - acceptable glycemic control
4. $> 8.1\%$ - poor glycemic control
Hypoglycemic episodes (type 1 group only):
Based on greater and less than average number of hypoglycaemic episodes per week experienced by people with type 1 diabetes.\textsuperscript{199-201}

1. $< 1.6 \text{ /week}$
2. $\geq 1.6/\text{week}$

Urinary albumin
Based on greater or less than the normal range for albumin excretion.

1. $< 30 \text{ mg/L}$
2. $\geq 30 \text{ mg/L}$

Red cell thiamin:
Adequate/Inadequate based on standardised levels in microbiological assessment.\textsuperscript{9}

1. Adequate $\geq 190 \text{ nmol/L}$
2. Inadequate $< 190 \text{ nmol/L}$

Serum thiamin:
Adequate/Inadequate
Adequate/Inadequate based on standardised levels in microbiological assessment.\textsuperscript{9}

1. Adequate $\geq 11.3 \text{ nmol/L}$
2. Inadequate $< 11.3 \text{ nmol/L}$

Statistical analysis using the $\chi^2$ test and Pearson’s correlation was then carried out.

3.5.3 Additional plus initial subjects (113 total)

Grouping was by thiamin supplement, yes or no. Normality tests and t-test for equality of means were generated for continuous variables (age, BMI, HbA1c, urinary albumin excretion, duration of diabetes) and categorical variables were as above.

Additional categorical variables generated were:
Type of supplementation:
Thiamin nitrate/thiamin hydrochloride
When last supplement taken:
Morning of survey/night before survey/ morning of day preceding survey
Thiamin status:
Below normal range/within normal range/above normal range on standardised levels in microbiological assessment.9

Serum thiamin within or above the normal range (11.3 – 35 nmol/L):
1. < 11.3 nmol/L
2. 11.3 – 35 nmol/L
3. > 35 nmol/L

Statistical analysis using the $\chi^2$ test and Pearson’s correlation was carried out to determine relationship of supplementation to red cell and serum thiamin levels adding additional subjects to initial subjects.
CHAPTER 4       RESULTS

4.1 Initial subjects - demographics

There were 89 initial subjects, 44 with type 1 diabetes (27 male, 17 female) and 45 with type 2 diabetes (23 males, 22 females). The intention was to analyze groups separately, rather than compare them. Initial subject demographics are shown in Table 4.1. Age, BMI and duration of diabetes were all normally distributed in type 1 subjects (Shapiro-Wilk statistic 0.980, 0.950, 0.973; p = 0.635, 0.054, 0.372, respectively). In type 2 subjects age and BMI were normally distributed (Shapiro-Wilk statistic 0.986, 0.975; p= 0.860, 0.427) but duration of diabetes was not (Shapiro-Wilk statistic 0.940, p = 0.021). Data for duration of diabetes in type 2 is of limited value as diagnosis of onset of type 2 diabetes is notoriously unreliable, with many people remaining undiagnosed for prolonged periods. The Ausdiab study addressed prevalence of type 2 diabetes in Australia and showed one person undiagnosed for every person diagnosed.121

There was a large age range in both groups, with more variation in people with type 1, as expected from a disease that is diagnosed in all age groups. Mean age was significantly higher in people with type 2 diabetes, which is largely diagnosed in older adults. Similarly mean years since diagnosis was significantly longer and varied more in type 1 subjects reflecting varied age at diagnosis, but shorter with less variation in type 2 subjects, who were, in the main, diagnosed at older ages (see Table 4.1).

In 2007, 52% of people in the Australian population aged 15 years and over were classified as overweight or obese based on their calculated BMI.203 Compared to this, all subjects were more likely than the general population to be classified as overweight or obese, but type 1 subjects less so (type 1 59%, type 2 75.6% overweight or obese). There was similar variation in BMI in both groups but mean BMI in type 1 subjects was significantly lower than for type 2 subjects (see Table 4.1).
Table 4.1 Initial subjects - characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Type 1 diabetes</th>
<th>Type 2 diabetes</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>53.4 ± 14.8</td>
<td>62.0 ± 10.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Time since diagnosis (y)</td>
<td>22.6 ± 11.7</td>
<td>12.6 ± 7.1</td>
<td>0.000</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.7 ± 4.4</td>
<td>31.2 ± 6.4</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Medications for diabetes treatment are shown in figure 4.1 and 4.2. As would be expected, all subjects with type 1 diabetes were on insulin, with the majority (75%) on a basal bolus regime of subcutaneous insulin with 4 or more injections a day. An additional 9.1% were on insulin pumps. The remainder were on less physiologically-based insulin regimes. Subjects with type 2 diabetes were on a wide variety of medication combinations, with the largest proportion being on the often used and simpler regimes, biguanide (17.8%), and ‘double therapy’ (biguanide plus sulphonylurea). The small numbers on diet alone (8.9%) probably reflect exclusion criteria for the diabetic survey (< 1 year since diagnosis) and the population serviced by a specialist diabetic clinic. Increasing duration of type 2 diabetes, and referral for specialist attention signal increasingly more complicated diabetes with increased need for medication. Almost 20% of type 2 subjects had progressed to insulin regimes, with 6.6% on background insulin and 13.3% on multi insulin regimes. In all but 7.9%, insulin was accompanied by some sort of oral diabetes therapy.
Figure 4.1  Diabetes treatment – subjects with type 1 diabetes
Figure 4.2 Diabetes treatment – subjects with type 2 diabetes

4.2 Initial subjects - validation of 24-h recall

Reproducibility of the 24-h recall was assessed by repeat 24-h recalls on 31 subjects (34.8%) of the sample.

4.2.1 Characteristics of repeat subjects

Characteristics of repeat subjects versus subjects undergoing single 24-h recall and independent t-test on equality of means are shown in Table 4.2.
Table 4.2 Characteristics and t-test for equality of means for subjects undergoing single versus repeat 24-h recalls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Single recall</th>
<th>Repeat recall</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>57.2 ± 13.9</td>
<td>59.0 ± 12.6</td>
<td>0.55</td>
</tr>
<tr>
<td>Time since diagnosis (y)</td>
<td>18.6 ± 10.5</td>
<td>14.8 ± 11.5</td>
<td>0.12</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.2 ± 5.7</td>
<td>30.3 ± 6.1</td>
<td>0.12</td>
</tr>
</tbody>
</table>

T-test for equality of means was carried out to determine equivalence of groups with respect to these characteristics. As Levene’s test for equality of variance was not significant, equal variance was assumed. Mean age, duration of diabetes and BMI did not differ significantly between the two groups. Similarly, using cross tabulation and chi square for categorical variables there was no significant difference between the two groups in gender (p = 0.85), activity (p = 0.95) and smoking status (p = 0.125). The 31 subjects undergoing repeat recalls were assumed representative in these respects.

4.2.2 Correlation of repeat and original 24-h recalls

Reproducibility of thiamin, carbohydrate and energy intakes were assessed by repeat 24-h recalls. Normality testing was carried out and showed intake of carbohydrate was normally distributed in original and repeat recalls (Shapiro-Wilk statistic 0.949 original carbohydrate and 0.952 repeat carbohydrate; p = 0.168 and 0.211 respectively), but thiamin and energy intake were not (Shapiro-Wilk statistic 0.767 and 0.831 for original and repeat thiamin, respectively, and 0.805 and 0.908 for original and repeat energy, respectively; p < 0.01. Parametric and nonparametric testing were carried out for thiamin and carbohydrate as sample size > 30, however only nonparametric testing was carried out for energy (non-normal distribution, sample size < 30).

Thiamin

Mean original thiamin intake was 1.22 ± 0.55 mg, mean repeat 1.33 ± 0.69 mg. Correlation of original and repeat thiamin intake was good, with Pearson’s product moment correlation coefficient (r) of 0.861 and Spearman’s nonparametric rank correlation coefficient (r_s) of 0.770, both significant at the 0.01 level.
Carbohydrate
Mean original carbohydrate intake was 156.88 ± 65.34 g, mean repeat 162.85 ± 69.64 g. Correlation of original and repeat carbohydrate intake was good with r of 0.567 and rₚ of 0.629, both significant at the 0.01 level.

Energy
Mean original energy intake was 6387 ± 2558 kJ, and mean repeat 6497 ± 2460 kJ. Correlation of original and repeat energy intake was good with rₚ of 0.608, significant at the 0.01 level.

4.2.3 Repeatability as assessed by Bland Altman plot.

Thiamin
Initially, a scatterplot of original and repeat thiamin values was generated with the line of equality plotted (figure 4.3). There was clustering around the line of equality but also several outliers. A Bland Altman plot was then generated (figure 4.4). Mean of differences (0.112 ± 0.35) was calculated. This was the bias (standard error - 0.002) with the limits of agreement (95% confidence limits) being 0.8054 and -0.5812 (standard error - 0.0035). Variability was consistent across the graph, with no increasing or decreasing trend in variability with increasing mean. The interpretation of this was that we can be confident that 95% of the time the value of thiamin intake as assessed by repeat 24-hour recalls will be within 0.6 mg of the original estimate across the range of measurement. This was 46% of mean thiamin intake so was considered acceptable agreement.
Figure 4.3 Correlation of thiamin intake with line of equality

Figure 4.4 Bland Altman plot – thiamin
Carbohydrate

Initially, a scatterplot of original and repeat carbohydrate values was generated with the line of equality plotted (figure 4.5). There was reasonable clustering around the line of equality but more outliers than for the thiamin plot. A Bland Altman plot was then generated (figure 4.6). The bias was 5.97 (standard error – 11.30) with the limits of agreement being 129.30 and -117.3 (standard error – 19.57). Variability was consistent across the graph, with a slight trend towards increasing variability of difference with increasing mean. The interpretation was that we can be confident that 95% of the time the value of carbohydrate intake as assessed by repeat 24-hour recalls will be within 123.3 g of the original estimate with possibly larger variation at higher levels of carbohydrate intake. The agreement between original and repeat carbohydrate values was much less than for thiamin values, and variability increased with increasing carbohydrate intake. Correlation coefficient was good but a limit of agreement of 123.3 is substantial, and suggests that assessed carbohydrate values, although useful, should be interpreted with some caution.

Figure 4.5  Correlation of carbohydrate intake with line of equality
Energy

Initially, a scatterplot of original and repeat energy values was generated with the line of equality plotted (figure 4.7). Clustering around the line of equality was good, with only one obvious outlier. A Bland Altman plot was then generated (figure 4.8). The bias was 38.2014 (standard error – 433.04) with the limits of agreement being 4609 and -4533 (standard error – 750.05). With the exception of one outlier, variability was quite consistent across the graph. The bias was relatively small so there appeared little systematic error in energy estimation, and the consistent variability supported this, however the limits of agreement were relatively large. This suggests that assessed energy values, although still useful, should be interpreted with caution.
Figure 4.7 Correlation of energy intake with line of equality

Figure 4.8 Bland Altman plot – energy
4.3 Initial subjects - dietary intake profiles

4.3.1 Initial subjects - dietary thiamin

Dietary thiamin had the greatest reproducibility on repeat recall among the nutrients studied. It had a non-normal distribution in both groups (Shapiro-Wilk statistic 0.782 and 0.789, \( p = .000 \)). In initial subjects, people with type 1 diabetes had a median thiamin intake of 1.43 mg, and those with type 2 diabetes, a median intake 1.11 mg. As the RDI for thiamin is gender specific, thiamin intake of initial subjects according to both diabetes type and gender was considered (Table 4.3).

Table 4.3 Initial subjects - dietary thiamin intake (mg)

<table>
<thead>
<tr>
<th>Diabetes type</th>
<th>Type 1</th>
<th>Type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Male</td>
</tr>
<tr>
<td>Median thiamin intake (mg)</td>
<td>1.43</td>
<td>1.50</td>
</tr>
<tr>
<td>Mean thiamin intake (mg)</td>
<td>4.78 ± 8.04</td>
<td>4.43 ± 6.82</td>
</tr>
</tbody>
</table>

In both types of diabetes males had a higher median intake of thiamin than females, consistent with the trend noted in the 1995 National Nutrition Survey that males consumed larger quantities of vitamins and minerals than females\(^82\). With the exception of females with type 2 diabetes, median thiamin intake in all groups was \( \geq \) than the RDI (1.2 mg M, 1.1 mg F). In females with type 2 diabetes 87.6% (78 subjects) had a dietary thiamin intake above the RDI.

4.3.2 Initial subjects - energy intake

Energy intake was normally distributed in type 1 subjects (Shapiro-Wilk statistic 0.976, \( p = 0.495 \)) but not in type 2 (Shapiro-Wilk statistic 0.858, \( p = .000 \)), possibly reflecting a skew in energy intake in a group with a mean BMI in the overweight range. Subjects with type 1 diabetes had a mean energy intake of 6273 ± 1900 kJ. Mean energy intake was less in those with type 2 diabetes being 6020 ± 2702 kJ.
Looking at energy intake according to gender, as well as type of diabetes (see Table 4.4), all males had a significantly higher mean energy intakes than females (p = 0.000) (as expected).

4.3.3 Initial subjects - thiamin intake per 1000 kJ

Until the 2005 revision of NRV’s the RDI for thiamin was expressed per energy value\(^{204}\) (see section 2.1.15) and thiamin intake is shown expressed thus in Table 4.4. This shows a slightly different pattern of intake, with total mean mg thiamin per 1000 kJ being greater in type 1 than in type 2 subjects, but males with type 2 having a smaller mean ratio than type 2 females, possibly due to a higher mean energy intake without a concomitantly increased thiamin intake (Table 4.4). By assessing adequacy of intake in this form, taking 0.1 mg per 1000kJ as adequate, it can be easily seen that mean intake was highly adequate in all groups.

4.3.4 Initial subjects - carbohydrate intake

Carbohydrate intake similarly had a normal distribution in type 1 subjects (Shapiro-Wilk statistic 0.963, p = 0.169) and non normal in type 2 subjects ((Shapiro-Wilk statistic 0.900, p = 0.001). Subjects with type 1 diabetes had a higher mean carbohydrate intake (166.49 ± 63.82 g) compared to those with type 2 diabetes, (149.76 ± 61.24 g). Males in both groups had a significantly higher mean carbohydrate than females (p = 0.000) (Table 4.4). Carbohydrate as a percentage of energy intake was unremarkable, with mean percent intake varying little between groups (Table 4.4), and standard deviation being a little greater in type 2 than type 1 subjects.

4.3.5 Initial subjects - thiamin intake per % carbohydrate

Mean thiamin intake per percent of carbohydrate (Table 4.4) was the converse of the pattern of mean thiamin intake, with all subjects with type 2 diabetes having higher mean intakes per percent carbohydrate than those with type 1, and males having a lower intake than females in each group.
Table 4.4 Initial subjects – dietary thiamin proportional to energy and carbohydrate intake

<table>
<thead>
<tr>
<th>Diabetes</th>
<th>Type 1</th>
<th></th>
<th>Type 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
</tr>
<tr>
<td>Mean energy (kJ)</td>
<td>6273 ± 1901</td>
<td>7060 ± 1618</td>
<td>5024 ± 1659</td>
<td>6020 ± 2702</td>
</tr>
<tr>
<td>Thiamin/1000 kJ</td>
<td>1.70 ± 6.29</td>
<td>2.04 ± 7.86</td>
<td>1.16 ± 2.35</td>
<td>1.07 ± 2.42</td>
</tr>
<tr>
<td>Mean carbohydrate (g)</td>
<td>166.49 ± 63.82</td>
<td>193.09 ± 60.92</td>
<td>124.25 ± 42.74</td>
<td>149.76 ± 61.24</td>
</tr>
<tr>
<td>[ % of energy intake]</td>
<td>[45.93 ± 9.98 %]</td>
<td>[45.73 ± 9.66 %]</td>
<td>[42.32 ± 9.24 %]</td>
<td>[45.28 ± 12.12 %]</td>
</tr>
<tr>
<td>Thiamin/ % carbohydrate</td>
<td>0.10 ± 0.17</td>
<td>0.08 ± 0.13</td>
<td>0.13 ± 0.23</td>
<td>0.14 ± 0.30</td>
</tr>
</tbody>
</table>

4.3.6 Initial subjects - supplementary thiamin

Of the 89 initial subjects, 19 were taking thiamin-containing supplements. Subjects taking thiamin-containing supplements were evenly distributed between groups and gender, with 9 (4 female, 5 male) having type 1 diabetes and 10 (6 female, 4 male) having type 2 diabetes. Supplements consisted of either thiamin hydrochloride (10), or thiamin nitrate (9) and were taken on survey morning (1) or the morning of the day preceding survey (6) with 12 not able to supply this information. Mean thiamin content of supplements was 19.87 ± 15.25 mg. The greatest content was 59.25 mg, giving that subject a total thiamin intake of 60.28 mg. The least was 2.43 mg giving a total thiamin intake of 4.01 mg. Total thiamin intake of all supplemented subjects exceeded 4 mg, almost 4 times the RDI.

4.4 Initial subjects - other possible modifiers of thiamin status

Activity and smoking were reported as categorical variables. Indices of control and complications were continuous variables.
4.4.1 Initial subjects - level of activity

Level of activity was self reported, as ‘sedentary’, ‘moderate’ and ‘heavy’ and frequencies of each level are shown in Table 4.5.

Table 4.5 Initial subjects - level of activity

<table>
<thead>
<tr>
<th>Diabetes type</th>
<th>Activity level</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 (n = 44)</td>
<td>Sedentary</td>
<td>14</td>
<td>31.8</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>21</td>
<td>47.7</td>
</tr>
<tr>
<td></td>
<td>Heavy</td>
<td>9</td>
<td>20.5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>44</td>
<td>100.0</td>
</tr>
<tr>
<td>Type 2 (n = 45)</td>
<td>Sedentary</td>
<td>23</td>
<td>51.1</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>22</td>
<td>48.9</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>45</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Level of activity is, as might be expected, higher in the younger, leaner type 1 subjects, with 68.2% reporting higher than sedentary activity compared to the type 2 group’s 48.9%. No type 2 subject reported heavy activity, versus 20.5% in type 1 subjects.

4.4.2 Initial subjects - smoking status

Only present smoking status was assessed, with both groups being distinguished by a paucity of smokers. Only 13.6% (6) of type 1 subjects and 8.9% (4) type 2 subjects were smokers.

4.4.3 Initial subjects - diabetes control and complications

Haemoglobin A1c

Haemoglobin A1c levels were based on standardised values used for this test at this venue which are:

- < 6% - normal
- 6.1 – 7% - good glycemic control
- 7.1 – 8% - acceptable glycemic control
- > 8.1% - poor glycemic control
HbA1c had a normal distribution in both groups (Shapiro-Wilk statistic 0.980, 0.981, p = 0.621, 0.675). Mean HbA1c was 7.8% in both groups (type 1 - 7.87 ± 1.38%; type 2 - 7.84 ± 1.55%), which is in the acceptable only range. There was a large standard deviation, especially in the type 2 subjects, with maxima in both groups well into the poor glycemic control range (type 1 - 10.80%; type 2 - 11.40%).

**Frequency of symptomatic hypoglycaemia (hypos)**

Hypoglycemia is a feature of type 1 diabetes, but rarely a problem in type 2. As discussed previously (section 2.2.10), episodes of hypoglycaemia modify HbA1c, which is essentially an average, and this may result in the appearance of good control, as assessed by HbA1c, in the presence of fluctuating blood glucose levels. Symptomatic hypoglycaemia (hypos) which are usually recognised, and often recorded by people with type 1 diabetes are an indicator of the frequency of hypoglycaemia. Mean number of self reported hypos in type 1 subjects was 1.5 per week, with a large variation, with some subjects reporting no hypos and others almost one a day.

**Urinary albumin**

Normal range for urinary albumin at this venue was < 3.0 mg/L. Urinary albumin had a non normal distribution in initial subjects with Shapiro-Wilk statistic 0.431 and 0.383 with p = 0.000. Data on urinary albumin excretion was available for only 28 (total 44) type 1 subjects and 37 (total 45) type 2 subjects. Median value of urinary albumin was slightly above the normal range in type 1 subjects (3.0 mg/L) and more markedly above in type 2 subjects (5.3 mg/L). Minimum values (2 mg/L) were the same in both groups. Maximum values were 41.5 mg/L in type 1 and 208 mg/L in type 2 subjects.

**4.5 Initial subjects - markers of thiamin status**

**4.5.1 Initial subjects - serum thiamin**

Serum thiamin had a non-normal distribution in both groups (Shapiro-Wilk statistic 0.886, 0.849; p = 0.000). Median serum thiamin was within the normal range (11.3 – 35 nmol/L) for initial subjects, but lower among unsupplemented subjects (Table 4.6). Median values were lowest in unsupplemented type 2 subjects and highest in type 1 subjects when supplemented subjects were included; type 2 subjects had a
slightly higher rate of supplementation. Minimum values were below, or just reached the lower level of the normal range for serum thiamin.

Table 4.6  Initial subjects - serum thiamin

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Diabetes type</th>
<th>Median serum thiamin (nmol/L) [range]</th>
</tr>
</thead>
<tbody>
<tr>
<td>All initial subjects (89)</td>
<td>Type 1 (44)</td>
<td>19.1 [11.4 – 50.9]</td>
</tr>
<tr>
<td></td>
<td>Type 2 (45)</td>
<td>18.1 [9.8 – 52.0]</td>
</tr>
<tr>
<td>Unsupplemented initial subjects (70)</td>
<td>Type 1 (35)</td>
<td>18.4 [11.4 – 30.5]</td>
</tr>
<tr>
<td></td>
<td>Type 2 (35)</td>
<td>16.5 [9.8 – 43.5]</td>
</tr>
</tbody>
</table>

4.5.2  Initial subjects - red cell thiamin

Red cell thiamin had a normal distribution in both groups (Shapiro-Wilk statistic 0.979, 0.980; p = 0.594, 0.605). Mean red cell thiamin levels were lower in all unsupplemented subjects than the whole group (Table 4.7). Minimum values in all groups were below the normal range, with unsupplemented type 1 subjects having the lowest mean.

Table 4.7  Initial subjects - red cell thiamin

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Diabetes type</th>
<th>Mean red cell thiamin (nmol/L) [range]</th>
</tr>
</thead>
<tbody>
<tr>
<td>All initial subjects (89)</td>
<td>Type 1 (44)</td>
<td>251.5 ± 59.1 [142.0 – 398.0]</td>
</tr>
<tr>
<td></td>
<td>Type 2 (45)</td>
<td>276.8 ± 72.2 [139.0 – 450.0]</td>
</tr>
<tr>
<td>Unsupplemented initial subjects (70)</td>
<td>Type 1 (35)</td>
<td>244.9 ± 61.4 [142.0 – 398.0]</td>
</tr>
<tr>
<td></td>
<td>Type 2 (35)</td>
<td>263.1 ± 67.4 [139.0 – 384.0]</td>
</tr>
</tbody>
</table>
4.6 Initial subjects – preliminary correlations using continuous variables

Continuous variable correlations were carried out as a preliminary investigation.

4.6.1 Correlations with serum thiamin

Looking at possible minor modifiers of thiamin status, there was no correlation between serum thiamin and BMI, HbA1c, HbA1c controlled for hypoglycaemic episodes, or urinary albumin. Serum thiamin correlated with age in subjects with type 2 diabetes but not in type 1; it also correlated with duration in type 1 diabetes (all $p < 0.05$) (see Table 4.8). An attempt to assess correlation of serum thiamin with duration was not carried out in type 2 subjects due to the difficulty of assessing duration in type 2 diabetes. For dietary variables, there was no correlation between serum thiamin and carbohydrate, energy, % of energy as carbohydrate, thiamin per 1000 kJ or thiamin per % carbohydrate. There was also no correlation between serum thiamin and total thiamin intake in unsupplemented subjects (Table 4.8), but there was a correlation ($p < 0.05$) in type 2 subjects when supplemented subjects were included.

Table 4.8 Initial subjects – correlations, serum thiamin and possible modifiers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subjects</th>
<th>Type diabetes</th>
<th>Pearson’s correlation</th>
<th>Spearman’s correlation</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>Whole group</td>
<td>2</td>
<td>0.313</td>
<td>0.350</td>
<td>0.036 p &lt; 0.05</td>
</tr>
<tr>
<td>Duration diabetes (y)</td>
<td>Whole group</td>
<td>1</td>
<td>0.343</td>
<td>0.325</td>
<td>0.023 p &lt; 0.05</td>
</tr>
<tr>
<td>Total thiamin intake (mg)</td>
<td>Unsupplemented</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>n.s</td>
</tr>
<tr>
<td></td>
<td>Whole group</td>
<td>2</td>
<td>0.619</td>
<td>0.369</td>
<td>0.000 p &lt; 0.05</td>
</tr>
</tbody>
</table>
Figure 4.9  Initial subjects - scatterplot, serum thiamin versus total thiamin intake in type 2 subjects  a) unsupplemented,  b) whole group
Figure 4.10  Initial subjects - scatterplot, serum thiamin versus total thiamin intake in type 1 subjects  a) unsupplemented,  b) whole group
4.6.2 Correlations with red cell thiamin

Correlations with red cell thiamin were similar to those of serum thiamin (Table 4.9), with all minor modifiers failing to correlate except for a correlation with age (p < 0.05) in type 2 subjects only. There was a correlation (parametric only) between total thiamin intake and red cell thiamin in type 2 subjects (including supplemented), however total thiamin intake had a non normal distribution so this was discounted.

Table 4.9  Initial subjects - correlations, red cell thiamin and possible modifiers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subjects</th>
<th>Pearson's correlation</th>
<th>Spearman's nonparametric correlation</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>Age (y)</td>
<td>Whole group 2</td>
<td>0.387</td>
<td>0.009</td>
<td>0.411</td>
</tr>
<tr>
<td>Total thiamin intake (mg)</td>
<td>Without supplement 1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Whole group 2</td>
<td>0.315</td>
<td>0.035</td>
<td>-</td>
</tr>
</tbody>
</table>

Red cell thiamin
Figure 4.11  Initial subjects - scatterplot, red cell thiamin versus total thiamin intake in type 2 subjects  a) unsupplemented,  b) whole group
Figure 4.12   Initial subjects - scatterplot, red cell thiamin versus total thiamin intake in type 1 subjects  a) unsupplemented,  b) whole group
4.6.3 Correlation red cell versus serum thiamin

There was a consistent strong correlation between red cell and serum thiamin on both parametric and nonparametric testing. Non parametric testing was the strongest indicator in view of the non normal distribution of serum thiamin, and was valid at the level $p < 0.01$.

Table 4.10 Initial subjects – correlation - red cell thiamin and serum thiamin

<table>
<thead>
<tr>
<th>Serum thiamin</th>
<th>Type diabetes</th>
<th>Pearson’s correlation</th>
<th>Spearman’s nonparametric correlation</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
</tr>
<tr>
<td>1</td>
<td>diabetes</td>
<td>0.344</td>
<td>0.022</td>
<td>0.388</td>
</tr>
<tr>
<td>2</td>
<td>serum</td>
<td>0.551</td>
<td>0.000</td>
<td>0.647</td>
</tr>
</tbody>
</table>

Figure 4.13 Initial subjects - Scatterplot, red cell thiamin versus serum thiamin (Equation: $RCT = 3.47ST + 191.67$)
4.7 Initial subjects – nonparametric testing using the \( \chi^2 \) test

Categorical variables were generated as described in section 3.4.2. Dietary variables, and other possible modifiers were tested for association with attainment of red cell thiamin and serum thiamin within the normal range using cross tabs and Pearson’s chi square.

4.7.1 Initial subjects – association of serum and red cell thiamin

There was a consistent association between attainment of red cell thiamin within the normal range and attainment of serum thiamin within the normal range, with 33.3% (1) of subjects with serum thiamin below the normal range attaining red cell thiamin within the normal range, versus 89.5% (77) of subjects with serum thiamin within or above the normal range (p = 0.039).

4.7.2 Initial subjects – dietary variables versus thiamin status

Dietary variables were assessed for all initial subjects and then separately in those without thiamin supplementation. It was not possible to carry out Pearson’s chi square on the supplemented group as there were too few subjects. There was no association between attainment of red cell thiamin within the normal range and thiamin intake or thiamin per 1000kJ in either group (type 1 and type 2) in all initial subjects or unsupplemented subjects alone. Similarly there was no association between attainment of serum thiamin within the normal range and the same variables.

4.7.3 Initial subjects – non-dietary variables versus thiamin status

There was no association in either group (type 1 and type 2) between attainment of red cell thiamin within the normal range and gender, BMI, HbA1c (categorised by both probability of complications and also tertiles; see section 3.4.2), frequency of hypoglycaemia, urinary albumin excretion, smoking status, activity and duration of diabetes (type 1 only). Similarly there was no association between attainment of serum thiamin within the normal range and the same variables.
Figure 4.14 Initial subjects - Scatterplot, red cell thiamin versus HbA1c
a) unsupplemented, b) whole group
4.8 Initial subjects - trends in thiamin status in supplemented subjects

The number of supplemented subjects was insufficient for statistical analysis by Pearson’s chi square, therefore, frequency counts were carried out to see if there was a trend toward or away from attaining adequate thiamin status.

4.8.1 Initial subjects - serum thiamin

The frequency of subjects attaining serum thiamin within the normal range (11.3 – 35 nmol/L) was delineated comparing supplemented and unsupplemented subjects. In subjects with type 1 diabetes, 35 were unsupplemented and 9 supplemented. Data for serum thiamin showed 0% of supplemented and unsupplemented type 1 subjects failed to attain serum thiamin within the normal range. In subjects with type 2 diabetes 35 were unsupplemented and 10 supplemented. In type 2 subjects 8.6% (3) unsupplemented and 0% of supplemented subjects failed to attain serum thiamin within the normal range.

4.8.2 Initial subjects – red cell thiamin

Similarly numbers of supplemented subjects were insufficient for statistical analysis by Pearson’s chi square for attainment of red cell thiamin within the normal range (190 – 400 nmol/L). Frequency of subjects attaining red cell thiamin within the normal range was delineated comparing supplemented and unsupplemented subjects in type 1 and type 2 diabetes. In subjects with type 1 diabetes, 35 were unsupplemented and 9 supplemented. Of those unsupplemented 17.1% (6) failed to attain red cell thiamin within the normal range, whereas in the supplemented group 0% failed to attain red cell thiamin within the normal range. Similarly in subjects with type 2 diabetes 35 were unsupplemented and 10 supplemented. A total of 14.3% (5) of type 2 subjects failed to attain red cell thiamin within the normal range, whereas 0% of supplemented subjects failed to attain red cell thiamin within the normal range.

The small numbers of supplemented subjects precluded testing to significance, however, although in initial subjects a dietary intake above the RDI was not
associated with attainment of red cell thiamin within the normal range, there was a
trend that supplementation might be.

4.9 Summary

There were 89 initial subjects, 44 with type 1 diabetes (27 male, 17 female) and 45
with type 2 diabetes (23 males, 22 females) with 70 taking diet alone and 19 taking
thiamin-containing supplements (9 [4 female, 5 male] having type 1 diabetes and 10
[6 female, 4 male] having type 2 diabetes).

Using chi square and Pearson’s correlation:

- There was no association in either group (type 1 and type 2) between
  attainment of serum and red cell thiamin within the normal range and gender,
  age, BMI, HbA1c frequency of hypoglycaemia, urinary albumin excretion,
  smoking status, activity and duration of diabetes (type 1 only).
- There was a consistent significant correlation between red cell and serum
  thiamin on both parametric and nonparametric testing. Non parametric
  testing was the strongest indicator in view of the non normal distribution of
  serum thiamin, and was valid at the level $p < 0.01$.
- There was no association between attainment of serum and red cell thiamin
  within the normal range and thiamin intake or thiamin per 1000kJ in either
  group (type 1 and type 2) in all initial subjects or unsupplemented subjects
  alone.
- The number of supplemented subjects was insufficient for statistical analysis
  by Pearson’s chi square. However in the 19 subjects taking supplements, all
  attained serum and red cell thiamin levels within the normal range.

4.10 Extension of study – additional subjects

Additional subjects (24) on supplementation were recruited to ensure sufficient
numbers for statistical testing comparing supplemented and unsupplemented subjects
(see section 3.3.1). This section of the study aimed to investigate the effect of
thiamin supplementation *per se* on thiamin status, therefore a 24h-recall was
considered unnecessary. This gave a total of 113 subjects (58 type 1 and 55 type 2) with 43 supplemented and 70 unsupplemented.

In the initial subjects, there had been little significant difference in correlations between dietary and non-dietary variables and markers of thiamin status between the type 1 and type 2 diabetes groups. In addition, testing using the $\chi^2$ test and Pearson’s correlation showed no significant difference in distribution of subjects with type 1 and type 2 diabetes between the supplemented (53.5% [23] type 1 and 46.5% [20] type 2) and unsupplemented groups 50% [35] type 1 and 50% [35] type 2) (p = 0.847). Therefore both type of diabetes were analysed together in this section.

4.10.1 Descriptive data – supplemented and unsupplemented group – dietary

Distribution of total thiamin intake was non-normal in the unsupplemented group (Shapiro-Wilk statistic 0.704, p = 0.000). In the supplemented group total thiamin intake could only be determined for the 19 initial supplemented subjects (who had dietary intake assessed by 24-h recall), but not for the 24 additional subjects. Distribution of thiamin content of supplements was non-normal (Shapiro-Wilk statistic 0.704, p = 0.000). In the supplemented group, 25 subjects were supplemented with thiamin nitrate and 18 with thiamin hydrochloride. Of these, 7% (n = 3) took last supplement on survey morning, 4.7% (n = 2) the night before, 51.2% (n = 22) the morning of the day preceding survey and 37.2% (n = 16) did not remember. Median total thiamin intake was 1.1 mg in the unsupplemented group (equivalent to the RDI for women), with minimum intake 0.37 mg and maximum 4.44 mg. Variation in thiamin content of supplements was large, giving great variability in thiamin intake in the supplemented group. The minimum total intake in this group was 4.01 mg in an initial supplemented subject, which is almost four times the RDI. The maximum was 122.60 mg plus dietary intake in an additional subject. This is over 100 times the RDI.

4.10.2 Supplemented and unsupplemented groups – other thiamin modifiers

Basic demographic variables (age and BMI) and possible modifiers of thiamin status (HbA1c and urinary albumin excretion) was similar between the groups in both mean
values and variation. Duration was assessed in subjects with type 1 diabetes only and was similar between groups, in both mean values and variation. Independent samples t-test was carried out on these continuous variables to determine the equivalence of the supplemented and unsupplemented groups in these respects. Variances were assumed equal for BMI, HbA1c, duration in type 1’s and urinary albumin excretion as Levene’s test for equality of variances did not reach significance. Equal variance was not assumed for age (Levene’s test for equality of variances, p = .011). T-test for equality of means did not reach significance, and the groups were presumed equivalent with respect to basic demographics and possible thiamin status modifiers HbA1c and urinary albumin excretion.

Table 4.11  Supplemented and unsupplemented groups – comparison of demographics and minor modifiers - independent t-test

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>Unsupplemented</td>
<td>58.5 ± 13.0</td>
<td>0.390</td>
</tr>
<tr>
<td></td>
<td>Supplemented</td>
<td>56.0 ± 15.9</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/cm²)</td>
<td>Unsupplemented</td>
<td>28.7 ± 6.0</td>
<td>0.900</td>
</tr>
<tr>
<td></td>
<td>Supplemented</td>
<td>28.9 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>Unsupplemented</td>
<td>7.9 ± 1.5</td>
<td>0.720</td>
</tr>
<tr>
<td></td>
<td>Supplemented</td>
<td>7.8 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Urinary albumin (mg/l)</td>
<td>Unsupplemented</td>
<td>13.9 ± 35.9</td>
<td>0.680</td>
</tr>
<tr>
<td></td>
<td>Supplemented</td>
<td>11.0 ± 28.0</td>
<td></td>
</tr>
<tr>
<td>Duration diabetes (y)</td>
<td>Unsupplemented</td>
<td>23.1 ± 11.5</td>
<td>0.101</td>
</tr>
<tr>
<td>(Type 1 subjects only)</td>
<td>Supplemented</td>
<td>26.6 ± 15.0</td>
<td></td>
</tr>
</tbody>
</table>

Equivalence of self reported categorical variables, smoking status and activity level between the supplemented and unsupplemented groups was tested by statistical analysis using the $\chi^2$ test and Pearson’s correlation. Smoking status was expressed as smoker or nonsmoker, and there was no significant difference between the supplemented and the unsupplemented groups in smoking status with 88.6% (62) of supplemented subjects and 93% (40) of unsupplemented subjects being nonsmokers (p = 0.528).
Activity level was expressed as sedentary, moderate or heavy (see Table 4.12). Statistical testing using the $\chi^2$ test and Pearson’s correlation showed no significant difference between the supplemented and unsupplemented groups in activity levels ($p = 0.468$).

Table 4.12  Supplemented versus unsupplemented groups – activity level

<table>
<thead>
<tr>
<th>Group</th>
<th>Activity level</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sedentary</td>
<td>Moderate</td>
</tr>
<tr>
<td>Unsupplemented</td>
<td>41.4% (29)</td>
<td>47.1% (33)</td>
</tr>
<tr>
<td>Supplemented</td>
<td>44.2% (19)</td>
<td>51.2% (22)</td>
</tr>
</tbody>
</table>

In summary, there was no significant difference between the supplemented and unsupplemented groups in type of diabetes, age, BMI, HbA1c, urinary albumin excretion, duration of diabetes in type 1 subjects, smoking status and activity levels.

4.10.3 Supplemented (n = 43) and unsupplemented (n = 70) groups – markers of thiamin status

Serum thiamin

Median, minimum and maximum values of serum thiamin were markedly higher in the supplemented group than the unsupplemented. All values were above the normal range in the supplemented group, with the maximum value in the supplemented group being approximately three times the upper level of the normal range. The range of values was also greatly increased in the supplemented group (see Table 4.13).

Table 4.13  Supplemented and unsupplemented groups – serum thiamin – descriptives

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum thiamin (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
</tr>
<tr>
<td>Unsupplemented (70)</td>
<td>17.35</td>
</tr>
<tr>
<td>Supplemented (43)</td>
<td>33.10</td>
</tr>
</tbody>
</table>
**Red cell thiamin**

All values of red cell thiamin were markedly higher in the supplemented group than in the unsupplemented group, with over twice the standard deviation (see Table 4.14).

Table 4.14 – Supplemented and unsupplemented groups – red cell thiamin – descriptives.

<table>
<thead>
<tr>
<th>Group</th>
<th>Red cell thiamin (nmol/L)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>Unsupplemented (70)</td>
<td>253.97 ± 64.63</td>
<td>139.00</td>
<td>398.00</td>
</tr>
<tr>
<td>Supplemented (43)</td>
<td>320.91 ± 148.58</td>
<td>185.00</td>
<td>1170.00</td>
</tr>
</tbody>
</table>

**4.10.4 Supplemented and unsupplemented groups – dietary thiamin intake-correlations and attainment of adequate thiamin status.**

**Serum thiamin**

Preliminary correlation with continuous variables showed no correlation between total thiamin intake and serum thiamin in the unsupplemented group (r = 0.098, p = 0.4180) but a strong correlation between total thiamin intake and serum thiamin in the supplemented group (r = 0.591, p = 0.000).

Categorical variables were as described in section 3.4.2. Total thiamin intake was tested for association with attainment of serum thiamin within the normal range. Categorical variables used were < 11.3 nmol/L and ≥ 11.3 nmol/L (the lower limit of the normal range for serum thiamin). Using cross-tabulation, and testing with Pearson’s chi square, the likelihood of attaining an adequate level of serum thiamin was not significantly different between the supplemented and unsupplemented groups (p = 0.287).

Three categorical variable were then used; categorising serum thiamin as below (< 11.3 nmol/L), within (11.3 – 35 nmol/L) and above (> 35 nmol/L) the normal range. Distribution within these categories can be seen in table 4.15. In the unsupplemented group 4.3% of subjects were below the normal range, but the majority of subjects (91.4%) were within the normal range, with only 4.3% above the normal range,
whereas in the supplemented group, no subject was below the normal range and 44.2% of subjects were above the normal range. Type of supplement (thiamin hydrochloride or thiamin nitrate) was not significantly associated with level of serum thiamin (p = 0.60). Levels of serum thiamin in subjects who had last supplemented ≥ 24 hours previously were almost identically distributed (0% below normal range, 54.5% within normal range and 45.5% above normal range).

Table 4.15  Supplemented versus unsupplemented groups – distribution of serum thiamin

<table>
<thead>
<tr>
<th>Group</th>
<th>Below normal range (≤ 11.3 nmol/L)</th>
<th>Within normal range (11.3 – 35 nmol/L)</th>
<th>Above normal range (&gt; 35 nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsupplemented</td>
<td>4.3% (3)</td>
<td>91.4% (64)</td>
<td>4.3% (3)</td>
</tr>
<tr>
<td>(70)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplemented</td>
<td>0.0% (0)</td>
<td>55.8% (24)</td>
<td>44.2% (19)</td>
</tr>
<tr>
<td>(43)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Using cross-tabulations and Pearson’s chi square there was a significant association between thiamin supplementation and having a serum thiamin above the normal range in all unsupplemented subjects (p = 0.00) and a similar significant association between thiamin supplementation ≥ 24 hours previously and having a serum thiamin above the normal range (p = 0.00).

**Red cell thiamin**

Preliminary correlation with continuous variables showed no correlation between total thiamin intake and red cell thiamin in either the unsupplemented group (r = -0.025, p = 0.839) or the supplemented group (r = 0.066, p = 0.674). It can be seen however, that a much lower percentage of supplemented subjects than unsupplemented had red cell thiamin levels below the normal range (Table 4.16) (2.3% versus 15.7%).
Table 4.16  Supplemented versus unsupplemented groups – distribution of red cell thiamin.

<table>
<thead>
<tr>
<th>Group</th>
<th>Red cell thiamin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Below normal range (&lt; 190 nmol/L)</td>
</tr>
<tr>
<td>Unsupplemented (70)</td>
<td>15.7% (11)</td>
</tr>
<tr>
<td>Supplemented (43)</td>
<td>2.3% (1)</td>
</tr>
</tbody>
</table>

Using cross-tabulations and Pearson’s chi square there was a significant association between thiamin supplementation and having a red cell thiamin within the normal range (p = 0.028).

4.11 Summary

In the initial subject group (n = 89, 70 unsupplemented, 19 supplemented):

- There was a consistent strong association between serum and red cell thiamin on both continuous and categorical testing using Pearson’s chi square.
- There was an initial correlation between age, total thiamin intake and serum thiamin in type 2 subjects, and duration and serum thiamin in type 1 subjects but this was not supported on categorical testing using Pearson’s chi square.
  There was an initial correlation between age, total thiamin intake and red cell thiamin in type 2 subjects, similarly unsupported by categorical testing using Pearson’s chi square.
- There was a strong trend toward supplemented subjects attaining a red cell thiamin within the normal range but subject numbers precluded testing to significance.
- There was no significant association between indicators of diabetes control (HbA1c and episodes of hypoglycaemia) and serum or red cell thiamin.
In the total group (initial plus additional subjects n = 113, 70 unsupplemented, 43 supplemented):

- There was a consistent, highly significant association between serum and red cell thiamin on both continuous and categorical testing using Pearson’s chi square.
- Using continuous variables, there was a correlation between total thiamin intake and serum thiamin in the supplemented group but not in the unsupplemented group.
- Using categorical variables and Pearson’s chi square there was a strong association between being supplemented and having a serum thiamin above the normal range.
- Of subjects who had taken last supplement on the morning of the day preceding survey, 45% had elevated serum thiamin levels 24 hours later.
- Using categorical variables and Pearson’s chi square there was a strong association between being supplemented and having a red cell thiamin within the normal range.
CHAPTER 5: DISCUSSION

5.1 Reduced thiamin status

The study aim was to investigate the relationship between thiamin intake, diabetes control and thiamin status in people with diabetes in Perth, Australia. Subjects were 113 people with diabetes (58 type 1, 55 type 2). The study showed reduced thiamin status in subjects with type 1 and type 2 diabetes, with 15.7 % (n = 11) of unsupplemented subjects having a red cell thiamin below the normal range. Serum thiamin levels were less reduced, with 3.4% (n = 3) unsupplemented subjects having serum thiamin below the normal range. There is not an enormous number of studies on this topic in the literature but most record some sort of reduction in thiamin status in diabetes. Comparisons can be difficult as studies variously report results for whole blood, plasma, but rarely red cell thiamin levels. (The concentration of thiamin in red blood cells is approximately 12 times that in plasma so whole blood is the nearest approximation to red cell levels). This study is essentially concordant with studies by Hobara, Kodentsova and Saito, who documented reduced thiamin levels in whole blood.3, 169, 170, 172 Haugen also showed low levels of thiamin in whole blood in type 1 diabetes (but normal levels in type 2 diabetes).175 This study differs from studies by Tamai and Havivi who reported decreased plasma thiamin levels in diabetes,4, 171 and also Thornalley, who reported plasma thiamin concentrations decreased 76% in type 1 diabetes and 75% in type 2 diabetes compared with people without diabetes. Conversely there are reports of normal blood thiamin levels in diabetes in both humans,5, 173 and also in animal studies.174

Compounding the difficulties in making comparisons between this study and others is the differing methodology used in assessment of thiamin status in different laboratories. Hobara used a microbiological method of assessment using Lactobacillus viridescence.170 Kodentsova measured transketolase activity172 and Saito and Haugen used a fluorometric thiochrome method.3, 175 More recent studies, for example that by Thornalley have assessed thiamin levels in plasma and red blood cells using high performance liquid chromatography.2 The thiamin assessment used in the present study was an automated microbiological assay of serum and red cell thiamin using Lactobacillus fermenti by the method of Icke and Nicol.9 These
authors standardised their method against an established thiochrome method, with good correlation ($r = 0.99$), but the microbiological assay gave results which were 21-28% higher than the thiochrome method. Comparisons with serum thiamin concentrations were not carried out in this way as the thiochrome method used was not sensitive enough to measure serum thiamin, but the concentration of total thiamin found in the serum of healthy subjects by this method compared well with concentrations reported using sensitive HPLC methods. Differences in methodology, and specifically the elevated readings by the microbiological method used may partly account for the smaller numbers of subjects with reduced serum thiamin levels in this study, compared with the study by Thornalley. In addition, for the assay method used in this study, the non parametric reference ranges for both serum and red cell thiamin were determined on 505 healthy individuals aged 18 to 90 years in Australia prior to 1991. Mandatory enrichment of bread making flour in Australia since 1991 has increased the thiamin content of all breads to 0.45 mg/100 g (compared to 0.16 mg/100 g for white and 0.22 mg/100 g for wholemeal breads prior to thiamin fortification) with a concomitant increase in mean thiamin intake of 25% in men, and 20% in women in the Australian population. In those without diabetes and in the absence of excess alcohol, dietary intake of thiamin is the main determinant of thiamin status, therefore, it is reasonable to assume the reference levels used may now be too low. If this is so, it would not detract from the study results, merely increase the numbers of subjects with low serum and red cell thiamin levels.

5.2 Modifiers of thiamin status – non dietary

This study has shown reduced thiamin status in subjects with type 1 and type 2 diabetes. Thiamin deficiency may result from inadequate dietary intake of the vitamin as well as from decreased absorption, defective transport, increased requirements, and enhanced losses and various modifiers may impinge on any of these processes.

All known possible minor modifiers of thiamin status, age, duration of diabetes, BMI, physical activity, smoking status, diabetic control and urinary albumin excretion, were addressed in this study, with surprisingly little association found.
Age and duration of diabetes were found to have some initial positive association with thiamin status (age in type 2 subjects and duration in type 1 subjects) on preliminary testing but none on definitive testing with $\chi^2$ and Pearson’s correlation. There are conflicting reports in the literature as to the association of age and thiamin status, with biochemical evidence of thiamin deficiency being reported in both free living and institutionalized elderly people, but other studies have indicated that age has no appreciable impact on thiamin status, so this was not unexpected. Surprisingly, there was no association found between urinary albumin excretion and thiamin status. Thornalley found evidence of increased renal clearance of thiamin in diabetes, with a 24-fold increase in type 1 and a 16-fold increase in type 2 diabetes and plasma thiamin levels negatively correlating with this and it is thought that the development of thiamin deficiency with increased renal clearance and albuminuria in diabetic rats may indicate abnormal renal handling of thiamin. Also poor diabetes control was not significantly associated with reduced thiamin status, even when addressing both HbA1c and episodes of hypoglycemia. None of the other minor modifiers investigated were shown to have an association with thiamin status in these subjects with diabetes. This was not inconsistent with the literature as all had conflicting reports on their effect on thiamin status, with none being consistently reported as modifiers.

5.3 Modifiers of thiamin status – major

5.3.1 Alcohol

The effect of the major modifier of thiamin status, excess alcohol consumption, was eliminated by the exclusion criteria of the study. Low levels of alcohol intake appear to have no appreciable affect on thiamin status.

5.3.2 Dietary intake

As previously discussed, in those without diabetes, in the absence of excess alcohol, dietary thiamin intake is normally the major determinant of thiamin status. Dietary intake was investigated in the present study at two levels, physiological (food intake) and pharmacological (thiamin supplementation).
Assessment of dietary thiamin

A 24-h recall was used to assess dietary intake as this was a practical method of dietary assessment in a population such as this with varying literacy levels and sometimes poor English. The situation was conducive to taking a 24-h recall, with subjects available while waiting to see medical staff, few time constraints and a suitable interview room. Every effort was made to minimize systematic and random error to ensure accuracy of dietary data.

Systematic error in the 24-h recall was reduced by using a standard interviewing technique, having the same interviewer, using a standardized method and form for food data entry and an appropriate database for nutrient analysis (AUSNUT99). In addition, subjects were given study information, signed a consent and undertook the 24-h recall without prior warning, thus minimizing any subject-initiated changes to normal daily intake.

Random error in 24-h recall mainly originates in day-to-day variation in food intake which renders the data from a 24-h recall less representative of usual intake. This is reduced in estimating nutrients such as thiamin which are present in many foods, and therefore less likely to vary from day to day. Logically, thiamin is a suitable nutrient to assess intake by 24-h recall, as cereal products are a significant thiamin source and are generally eaten on a regular basis. Mandatory enrichment of bread-making flour in Australia since 1991 has increased the thiamin content of white, mixed grain and wholemeal breads to 0.45 mg/100 g (compared to 0.16 mg/100 g for white and 0.22 mg/100 g for wholemeal breads prior to thiamin fortification) with a concomitant increase in thiamin intake in all sub-groups of the population. It is assumed this increase is due to thiamin fortification of bread-making flour. This would increase the proportionate contribution of cereals to daily thiamin intake, and thereby tend to reduce day to day variation in thiamin intake. Supporting this, the 1995 National Nutrition survey showed breads and rolls contributed approximately 20% of total thiamin intake in the Australian diet. A number of studies have demonstrated acceptable data for thiamin intake using 24-h recalls, although not all, with a study by Basiotis asserting 41 to 46 days of dietary data were required to enable thiamin intake to be accurately expressed as a yearly average.
Although direct evidence is lacking, it is thought that a relationship exists between thiamin requirement and energy metabolism arising from the role of thiamin as thiamin pyrophosphate in the metabolism of carbohydrate in glycolysis and in the citric acid cycle. Energy was assessed in order to be used in a secondary measure of thiamin adequacy, i.e. thiamin/1000 kJ. Similarly carbohydrate was assessed to determine contribution to energy intake, as where carbohydrate intakes are high (contributing greater than 55% of energy intake) there may be an increased need for thiamin.

Reproducibility of the 24-h recall in this study was assessed by repeat 24-h recalls on 31 subjects (34.8% of the initial sample). There was no significant difference between those completing repeat recalls and the rest of the sample in basic demographics (age, gender, duration of diabetes, BMI, activity and smoking status). Reproducibility for total thiamin, carbohydrate and energy was assessed.

Preliminary assessment using continuous variables showed good correlation between original and repeat values of thiamin, carbohydrate and energy (all significant at 0.01 level). Correlation does not necessarily equate with agreement, however in this study, where we are examining the association of dietary intake of thiamin and serum and red cell thiamin levels, a good correlation in thiamin intake between repeat 24-h recalls implies a ranking of thiamin intake between subjects that relates to their propensity to attain normal serum and red cell thiamin levels.

Reproducibility was also assessed by means of a Bland-Altman plot for each nutrient, where differences are plotted against means and mean bias and limits of agreement calculated. The results of this for thiamin showed little variation in accuracy of assessment according to the quantity of thiamin assessed. Limits of agreement indicated that we can be confident that 95% of the time, the value of thiamin intake as assessed by repeat 24-h recalls will be within 0.6 mg of the original estimate across the range of measurement. This is approximately 50% of the RDI for thiamin (1.1 mg/day females, 1.2 mg/day males). As an RDI incorporates generous factors to accommodate variations in absorption and metabolism and exceeds the actual nutrient requirements of practically all healthy persons, a limit of agreement of 0.6 mg was considered acceptable.
The limits of agreement for carbohydrate and energy intake (123.3 g and 4000 kJ respectively) although having little variation with varying carbohydrate and energy values, were wider, mooting less than ideal repeatability by 24-h recall for both these nutrients. Assessed carbohydrate and energy values, although useful were interpreted with some caution.

Overall, the evidence was that the 24-h recalls gave acceptable accuracy of data for the purposes required in this study.

**Dietary thiamin – adequacy of intake**

a) Direct measure of thiamin intake

Thiamin intake in this study had a non normal distribution thus median as well as mean values were calculated. Mean thiamin intake in this group was well above mean intakes in the general population, with median intakes considerably lower. Mean thiamin intakes recorded in the National Nutrition Survey in 1995 were 1.94 ± 1.2 mg for adult males and 1.35 ± 0.76 mg for adult females. In both types of diabetes males had a higher median intake of thiamin than females, consistent with the trend noted in the 1995 National Nutrition Survey that males consumed larger quantities of vitamins and minerals than females. With the exception of females with type 2 diabetes, median thiamin intake in all groups was ≥ than the RDI (1.2 mg M, 1.1 mg F). In females with type 2 diabetes 87.6% (78 subjects) had a median dietary thiamin intake above the RDI, which is still highly acceptable. Overall there was no evidence of low thiamin intake with respect to the reference population, consistent with a study by SV showing 98% of subjects with type 2 diabetes had an intake of thiamin above the RDI and a study in subjects with type 1 diabetes showing similar results.

b) Adequacy of thiamin intake measured by thiamin energy ratio

Energy intake was normally distributed and mean energy intakes were less than the general population, possibly due to dietary education emphasising low intakes of fat and sugars in this population, less than ideal repeatability of energy assessment by 24-h recall or under-reporting. Under-reporting is well documented in assessments of energy intake by 24-h recall. Mean energy intake was greater in those with type 1 diabetes than those with type 2, which is not surprising as people with type 1 diabetes were generally both younger and thinner than those with type 2 diabetes, and would generally have higher energy needs. In addition more type 2 subjects were
overweight, increasing the chance of their adherence to a weight loss regime. However, given that a larger proportion of this group were overweight and that under-reporting is more common among overweight and obese individuals, then this data may be less reliable. Information was not collected which would allow differentiation between under-reporters and those justifiably restricting energy intake for the purposes of weight reduction or improvement of control of diabetes. Males had higher mean energy intakes than females, consistent with the 1995 National Nutrition Survey results with mean daily energy intake of 11,050 ± 4,278 kJ for men compared to 7,481 ± 2,917 kJ for women. Thiamin intake per 1000 kJ was highly adequate in all subjects, and even in the context of possible understated values of energy, supports the thesis that almost all subjects had an adequate thiamin intake, as the lowest mean intake (for male subjects with type 2 diabetes) was 4+ times greater than 0.1 mg/1000 kJ (the previous recommended dietary intake). c) Adequacy of thiamin intake - increased need due to high carbohydrate Carbohydrate intake had a similar pattern with males in both groups having a higher mean carbohydrate than females, consistent with the National Nutrition Survey mean figures for the general population (males 301 ± 129 g, females 211 ± 86.9 g). Mean intakes were considerably lower in all subjects than the general population, possibly reflecting recommendations to decrease sugar intake in the diet for diabetes, but under-reporting can also be an issue when assessing carbohydrate intake. Contribution of carbohydrate to total energy intake was interesting (all mean % values less than 46%), being lower than the sometimes recommendation of 50 – 60% for people with diabetes, but consistent with another study by SV. It was also considerably lower than the estimated 55% postulated to increase need for thiamin. In summary, dietary intake of thiamin in these subjects was equal to and often greater than the reference population for adequacy of intake.

5.4 Dietary thiamin intake and thiamin status

The relationship between thiamin intake and thiamin status was addressed in two ways:

a) Direct correlation between dietary variables and indices of thiamin status
In people without diabetes, blood thiamin levels correlate with dietary intake; a rise and fall in dietary thiamin intake in normal volunteers has been shown to be associated with a similar rise and fall in blood thiamin levels.\textsuperscript{209} In the 70 initial subjects who were not on thiamin supplementation there was no correlation between thiamin intake and serum or red cell thiamin. Scatterplots clearly show variables with little relationship to each other. In fact the two subjects with the lowest dietary thiamin intakes had red cell thiamin levels in the middle of the normal range and two subjects with dietary intakes almost double the RDI had red cell thiamin levels below the normal range. Similarly there was no correlation between serum or red cell thiamin and energy intake, carbohydrate intake or thiamin intake per 1000 kJ. The only comparable study is one by Saito in 1987, where prepared diets of predetermined quantities of thiamin, carbohydrate and energy were given to people with diabetes and blood thiamin levels measured. The length of time diets were given and methodology were not specified. This study found no significant correlation between blood thiamin levels and dietary thiamin, energy or carbohydrate; but did, however find a correlation between thiamin per 1000 kJ.\textsuperscript{209} A small study by SV in type 2 subjects showed red cell and serum thiamin did not correlate with any dietary variables.\textsuperscript{6}

b) Non parametric testing with categorical variables to address certain endpoints. This was definitive. On non parametric testing, dietary thiamin intake above the RDI was not significantly associated with having normal red cell or serum thiamin. Red cell thiamin correlated with serum thiamin in both groups and results did not differ significantly between those with type 1 and type 2 diabetes.

5.5 Thiamin supplementation

Thiamin supplements were taken by 19 out of 89 initial subjects (21.3%) This is lower usage than the general adult Australian population (43%).\textsuperscript{210} The highest median intake of vitamin supplements in the general population is by women over 60,\textsuperscript{210} thus it is rather surprising that type 2 subjects who had a mean age of 62 ± 10.2 years had a rate of supplementation equivalent to the younger type 1 subjects. This population with type 2 diabetes is often highly medicated. A recent study in Melbourne on 50 subjects with type 2 diabetes showed they took 7 ± 2.97 different types of medications per day. The dose frequency included twice a day, three times a
day and four times a day. One can only surmise that in a population with such a high intake of medications, both for diabetes and related conditions, that extra medication is avoided.

After extension of the study, supplemented subjects totalled 43. Of these, 25 were supplemented with thiamin nitrate and 18 with thiamin hydrochloride, forms of thiamin which are synthetic and not naturally present in food. Supplemented subjects had a median total thiamin intake of 12.96 mg (plus dietary intake), well above the physiological range compared to median total thiamin intake of 1.1 mg in the unsupplemented group (equivalent to the RDI for women). The minimum total intake in the supplemented group was 4.01 mg of thiamin, almost four times the RDI, and equivalent to the maximum intake in the unsupplemented group (4.44 mg). The maximum in the supplemented group was 122.60 mg (plus dietary intake), over 100 times the RDI.

Therefore, although there was some overlap in total thiamin intake between the upper end of the unsupplemented group and the lower end of the supplemented group, total thiamin intake in the supplemented group was largely pharmacological, being in a different form and with a quantity generally in excess of the unsupplemented group.

**Thiamin supplementation and thiamin status**

After extension of the study, supplemented subjects totalled 43, and were compared with the 70 unsupplemented subjects for statistical testing. Statistical analysis with \( \chi^2 \) and Pearson’s correlation showed dietary thiamin intake above the RDI (n = 37) was not significantly associated with having normal red cell thiamin. Supplementation to total thiamin intake > 4 mg/day, however, was significantly associated with having normal red cell thiamin (p = 0.025), with 2.3% (n = 1) of subjects having low red cell thiamin. Similarly, supplementation to total thiamin intake > 4 mg/day was significantly associated with having a serum thiamin above the upper limit of the normal range (p < 0.001) (44.2% of subjects, n = 17) compared to 0% of subjects in the unsupplemented group. This association was just as significant in those who had taken last supplement 24 hours previously (p < 0.001), with 45% of these subjects having elevated levels. Red cell thiamin correlated with serum thiamin (r = 0.463, p < 0.01; r = 0.426, p < 0.01) in both groups. Results did not differ significantly between those with type 1 and type 2 diabetes.
The subjects in the supplemented group were effectively thiamin loaded, and it is generally accepted that at higher concentrations, intestinal uptake of thiamin is predominately by diffusion\textsuperscript{23} rather than via the saturable carrier-mediated transport mechanism that predominates at physiological concentrations. This is in agreement with the very high levels of serum thiamin found in 44.2% of the supplemented group. Type of supplement (thiamin hydrochloride or thiamin nitrate) was not significantly associated with serum thiamin, which is concordant with animal studies which have found little difference between absorption of different water-soluble supplemental thiamin salts, indicating that they have similar potency.\textsuperscript{49} There was a highly significant correlation ($r = 0.59$, $p < 0.001$) between total thiamin intake and serum thiamin in the supplemented group which was not present in the unsupplemented group. This is in agreement with literature reports that absorption of thiamin hydrochloride and thiamin nitrate is dose-dependent\textsuperscript{15} but conflicts with others which suggest that humans have a rate-limited capacity to absorb water soluble thiamin supplements,\textsuperscript{48} that the proportion absorbed of water soluble supplements is very low,\textsuperscript{18} that at a supplementation level of 2.5 – 5 mg of thiamin, the majority is largely unabsorbed,\textsuperscript{18} and that daily absorption is usually limited to a maximum of 8-15 mg. The subject with the highest thiamin intake in this study (122.60 mg plus dietary intake) had a serum thiamin of 93.7 nmol/L (almost 60 nmol/L above the upper limit of the normal range) which appears to mitigate against an upper level of absorption. It could be postulated that the levels have accumulated over time, but the presumption would be that a large proportion would be excreted. In addition, levels of serum thiamin remained high in 45% of subjects who ingested their last supplement $\geq$ 24 hours previously, which is surprising against the background of literature reports of rapid excretion of high dose thiamin\textsuperscript{44, 57} and increased levels of thiamin excretion in diabetes with excretion correlating inversely with plasma thiamin\textsuperscript{2} but in agreement with a literature report of only 2.5% of an oral dose of 50 mg of thiamin being recovered in the urine in non-diabetics.\textsuperscript{57}

This study showed no significant association between subjects having a physiologically adequate intake of thiamin and attaining normal levels of red cell thiamin. It has been suggested that mild thiamin deficiency in diabetes may induce increased expression of the thiamin transporters THTR1 and THTR2, to increase
tissue scavenging of thiamin, and a decrease in the expression of transporter RFC-1, to retain tissue thiamin pyrophosphate, however these mechanisms appeared insufficient to ensure adequate thiamin status in these unsupplemented subjects. Supplementation with thiamin, however, was significantly associated with normal red cell thiamin levels, which seems to indicate a difference in transport into the red cell. *In vitro* studies of uptake of thiamin by erythrocytes in humans show it is probably transported by facilitated diffusion, by a low capacity carrier mechanism. Thus it is proposed that uptake has two different mechanisms, saturable and nonsaturable with the saturable mechanism dominating at physiological concentrations. Presumably the nonsaturable is dominating in the situation where thiamin supplementation has resulted in high serum thiamin levels, which may partly explain why supplemented subjects attained normal red cell levels of thiamin and unsupplemented subjects did not.

Finally, the results of the present study thus discussed did not differ significantly between those with type 1 and type 2 diabetes which is possibly surprising as type 1 and 2 diabetes generally have different origins, and are, to some extent different diseases, although, as has been discussed (see section 2.2.5), they may co-exist and also have some genetic overlap. Both types of diabetes, however, are diagnosed by a level of blood glucose which defines a group of people with a significantly increased risk of premature mortality and microvascular and cardiovascular complications and both types of diabetes cause gross aberrations of carbohydrate metabolism, in which thiamin is involved.

### 5.6 Summary

Overall there is agreement in the literature that mild thiamin deficiency is prevalent in diabetes, characterized by reduced blood thiamin levels in those with diabetes compared to people without diabetes however the literature differs in the fractions of blood affected and the proposed causes of the deficiency. A recent review on the role of thiamin in diabetes stated that thiamin malnutrition ‘is probably not caused by dietary deficiency of thiamin’. There is, however a dearth of definitive studies addressing dietary intake and thiamin status, with the bulk of research concentrating on the potential role of thiamin and its derivatives in amelioration of some diabetic
complications. This study differs from others in having dietary intake data and has definitively shown in these subjects with diabetes that thiamin supplementation to total thiamin intake $> 4$ mg (approximately 3 times recommended intake) was significantly associated with having normal red cell thiamin levels but an adequate dietary intake of thiamin within the physiological range was not. Thiamin supplementation to total thiamin intake $> 4$ mg (approximately 3 times recommended intake) was also significantly associated with having a serum thiamin above the normal range, with 45% of subjects taking last supplement 24 hours previously having elevated serum thiamin. Poor diabetes control was not implicated.
CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The aim of this study was to clarify the relationship between red cell thiamin levels and dietary intake and diabetes control using direct dietary intake data and adequate numbers of subjects to provide sufficient power. To a certain extent this has been accomplished and the conclusions are:

- The usual relationship between dietary thiamin and red cell thiamin does not exist in people with diabetes.
- In diabetes an adequate intake of dietary thiamin does not ensure red cell thiamin level within the normal range.
- Contrary to some reports in the literature serum thiamin does not appear to be affected to the same extent, but this may be an artefact of the thiamin assay used in this study.
- Thiamin supplementation to total intake greater than 4 mg/day is significantly associated with a normal red cell thiamin level.
- Thiamin supplementation to total intake greater than 4 mg/day is significantly associated with an elevated serum thiamin level.
- Elevated serum thiamin levels persist 24 hours after thiamin supplementation in 45% of subjects.
- Poor diabetic control is not related to reduced blood thiamin levels.

This study shows thiamin supplementation appears necessary to ensure acceptable thiamin status in diabetes. This raises questions as to actual thiamin requirements in diabetes and adds to evidence that thiamin deficiency in diabetes is not primarily due to dietary deficiency.

6.2 Recommendations

This was a cross-sectional study primarily looking at factors affecting thiamin status in diabetes. It has demonstrated that an adequate dietary intake is not sufficient to ensure adequate thiamin status in diabetes and that thiamin supplementation is
necessary. It would seem to be of immense benefit to follow this with an intervention study which could chart the changes in thiamin status with institution of thiamin supplementation, especially at different levels of supplementation. The present study showed the benefit of thiamin supplementation to a total thiamin intake of greater than 4 mg, however levels of supplementation varied greatly, and although the effect on red cell thiamin levels did not appear to vary, higher levels of supplementation were associated with much higher serum thiamin levels. It would be useful to quantify this relationship, while looking at the effect on red cell thiamin levels. Lipid soluble thiamin derivatives such as benfotiamin have greater bioavailability but are not easily obtained in Australia at present. In the longer term, a comparison study between this and water soluble thiamin supplements, as is normally used would be interesting. It would also be interesting to examine the effects of dietary intake to 4 mg (perhaps via vegemite) to see if this ensured adequate thiamin status.

A limitation of this study was the lack of data on thiamin excretion. It primarily looked at thiamin between the mouth and the red cell. An intervention study incorporating excretion would give more complete data. Thiamin excretion in diabetes as compared with normals has been addressed by Thornalley, however that study did not quantify or control for dietary thiamin intake. The link between thiamin and complications is firmly established, so some data on complications seems appropriate. Diabetic retinopathy is classified by stages, and would possibly be appropriate.

Finally, the thorny question of thiamin assays. Many different standardized assays are currently used and accepted, but there is a general move towards universal use of high performance liquid chromatography, so this is the obvious method to be used in any future studies.

6.3 Summary

Therefore, the recommendations arising out of this study are for a follow up intervention study looking at the effect of different levels of thiamin supplementation, possibly dietary intake to 4 mg, on serum and red cell thiamin and
thiamin excretion, with thiamin being assayed by high performance liquid chromatography and linking this to progression of diabetic retinopathy.
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Appendix 1
Subject Information Sheet
Patient Information Sheet

Study Title:

In Diabetes, Does The Amount of Thiamine (Vitamin B1) Eaten, Or The Level Of Glucose In The Blood Affect How Much Thiamine Is Present In The Blood?

Study Summary
Thiamine (vitamin B1) is necessary for the body to use carbohydrate. Thiamine is present in some foods and when eaten is absorbed from the gut and enters the blood. People with diabetes tend to have lower levels of thiamine in their blood than people without diabetes. People usually only have low thiamine levels in their blood, if they do not eat enough thiamine. However this may not be the reason for low blood thiamine levels in diabetes, diabetes itself may be the cause. This study will measure the amount of thiamine eaten by a group of people with diabetes, and compare this to the level of thiamine in their blood. It will also compare a long term measure of diabetes control (haemoglobin A1c) to thiamine blood levels. This study has received ethical approval from Royal Perth Hospital Ethics Committee.

Your Role In This Study
If you consent to take part in the study, information will be obtained from you about the foods you have eaten over the last 24 hours. Information will be collected at this visit and no other appointment will be needed. This information will be compared with your blood levels of thiamine and HbA1c that are routinely tested with your other survey tests. There will be no other involvement by you, and no cost. You do not have to take part in this study if you do not want to.

How The Information Will Be Handled.
All identifying data will be deleted once your food intake and blood results have been compared. Your name will not appear on any document or publication.

Further Information
If you have any further questions, you can contact the dietitian in Diabetic Clinic on 92242162.
Appendix 2
Consent Form
Study Title:

In Diabetes, Does The Amount Of Thiamine (Vitamin B1) Eaten, Or The Level Of Glucose In The Blood Affect How Much Thiamine Is Present In The Blood?

Study Summary

Thiamine (vitamin B1) is necessary for the body to use carbohydrate. Thiamine is present in some foods and when eaten is absorbed from the gut and enters the blood. People with diabetes tend to have lower levels of thiamine in their blood than people without diabetes. People usually only have low thiamine levels in their blood, if they do not eat enough thiamine. However this may not be the reason for low blood thiamine levels in diabetes, diabetes itself may be the cause.

This study will measure the amount of thiamine eaten by a group of people with diabetes, and compare this to the level of thiamine in their blood. It will also compare a long term measure of diabetes control (HbA1c) to thiamine blood levels.

This study has received ethical approval from Royal Perth Hospital Ethics Committee.

Your Role In This Study

If you consent to take part in the study, in formation will be obtained from you about the foods you have eaten over the last 24 hours. Information will be collected at this visit and no other appointment will be needed. This information will be compared with your blood levels of thiamine and HbA1c that are routinely tested with your other survey tests. There will be no other involvement by you, and no cost.

You do not have to take part in this study if you do not want to.

How The Information Will Be Handled.

All identifying data will be deleted once your food intake and blood results have been compared. Your name will not appear on any document or publication.

Further Information

If you have any further questions, you can contact the dietitian in Diabetic Clinic on 92242162.

If you are happy to take part in the study you should now sign the CONSENT FORM.

Thank you for considering participating in this study.

CONSENT TO PARTICIPATION IN A STUDY TO ASSESS THE AFFECT OF THIAMINE INTAKE ON BLOOD THIAMINE LEVELS

I, ................................................................. agree to participate in the above study. I have read and understood the attached Information Sheet and I have retained a copy of the signed document. I have been given the opportunity to ask questions about the study by the investigator. I understand that I may withdraw from the study at any time without affecting any future medical treatment, or the treatment of the condition which is the subject of the trial.

Signed................................................................. Date...........................

Signature of Investigator............................................................. Date...........................
Appendix 3
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<td></td>
<td></td>
</tr>
</tbody>
</table>
# THIAMIN INTAKE

Date ________________________________

<table>
<thead>
<tr>
<th>STICKY LABEL</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Thiamin – 24 hour recall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiamin - supplements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiamin rich foods (av)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutritional Supplements</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| TOTAL DIETARY THIAMIN |   |   |   |
Appendix 4

24-hour Food Recall Form
### 24 HOUR FOOD RECALL

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Food</th>
<th>Quantity</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>Thiamin Supplement</td>
<td>amount</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nutritional Supplement</td>
<td>amount</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vegemite/Marmite</td>
<td>amount</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nuts (Type)</td>
<td>amount</td>
<td></td>
</tr>
<tr>
<td>Between Breakfast &amp; Lunch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Lunch &amp; Dinner</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Dinner &amp; Breakfast</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 5
Vitamin Supplement Questionnaire
Diabetic Clinic                             Vitamin Supplement Survey

Name______________________________

Dear Survey Attendee,

I am doing a short survey of vitamin supplementation in people attending the Diabetic Survey.
- Could you please fill in the three questions on the survey sheet below.
- Could you return to the nurse when you attend the Diabetic Survey

Many thanks for your participation.

Sally Vindedzis
Dietitian
Endocrinology and Diabetes
Royal Perth Hospital
(08) 64775213
sally.vindedzis@health.wa.gov.au

VITAMIN SURVEY

1. Do you normally take vitamin supplements?
   YES/NO   (Please circle one)

2. If yes, When did you last take your vitamin supplements?
   On survey morning / The night before survey / The morning of the day before survey
   (Please circle one).

3. Which vitamin supplements do you take (see below)

1st Vitamin Supplement

Name of Vitamin Supplement______________________________________________
Manufacturer____________________________________________
No of tablets taken per day______________________________________________

2nd Vitamin Supplement
<table>
<thead>
<tr>
<th>Name of Vitamin Supplement</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td></td>
</tr>
<tr>
<td>No of tablets taken per day</td>
<td></td>
</tr>
</tbody>
</table>

PLEASE ADD OTHER VITAMIN SUPPLEMENTS ON BACK OF SHEET IF NECESSARY.