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The Role of Vitamin D in Controlling and Managing Glycaemic Control in Patients with Type 2 Diabetes (D4D)

by XueFei Yu

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

under the supervision of
Principal Supervisor: Associate Professor Hui Chen,
Co-Supervisors: Professor Chi Eung Danforn Lim and
Associate Professor Christopher Zaslawski

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Certificate of Authorship and Originality

I, Xuefei Yu declare that this thesis, is submitted in fulfillment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

Signature:

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Xuefei Yu

Date:

12/08/2021

Dedication

To my grandfather,

understanding science and respecting nature.

“For I am abstracted from the world, the world from nature, nature from the way，and the way from what is beneath abstraction.”

– 《Tao Te Ching》

“人法地、地法天、天法道、道法自然”。

– 《道德经》

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2020 has been a very challenge year for all of us,
may we be a rainbow in someone's cloud,
to embrace the first ray of tomorrow's sun.

With love, October 2020
Canberra, Australia

List of Publications During the Candidature

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Education brochure: **Healthy Eating and Breast Cancer (Chinese Version),** https://www.bcna.org.au/media/4787/healthy-eating-breast-cancer-booklet-chinese-web.pdf Breast Cancer Network Australia & CanRevive

Yu, X & Lim, Chi Eung Danforn & Cheng, Ncl. (2016). **Use of CAM for inflammatory bowel disease: a poor adherence to conventional therapy.** Focus on Alternative and Complementary Therapies. 21. 187-188. 10.1111/fct.12277.

Yu X, & Lim, Chi Eung Danforn **&** Zaslawski, Chris. (2016). **Moxibustion for diarrhoea predominant irritable bowel syndrome: what is the current evidence?** Focus on Alternative and Complementary Therapies. 21. 118-119. 10.1111/fct.12259.

Yu X, & Lim, Chi Eung Danforn & Cheng NCL. (2016). **Role of Compound Danshen Dripping Pill in Early Diabetic Retinopathy: A RCT**. Focus on Alternative and Complementary Therapies. 21(1): 38-39 March 2016

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Abbreviation

25(OH)D	*25-hydroxy-Vitamin D*	**2h-PG**	*2-hour Plasma Glucose*
ABS	*Australian Bureau of Statistics*	**ADA**	*American Diabetes Association*
AFCD	*Australian Food Composition Database*	**AHEI**	*Alternative Healthy Eating Index*
AI	*Adequate Intake*	**AIHW**	*Australia Institute of Health and Welfare*
ANOVA/ ANCOVA	*Analysis of Variance*	**AMED**	*Alternative Mediterranean Diet*
ANZCTR	*Australian New Zealand Clinical Trials Registry*	**APD**	*Accredited Practising Dietitian*
AUSNUT	*AUStralian Food and NUTrient Database*	**AUSDRISK**	*Australian Type 2 Diabetes Risk Assessment Tool*
BMC	*Bangor Medical Centre*	**BMI**	*Body Mass Index*
CRF	*Case Report Form*	**CVD**	*Cardiovascular Disease*
DASH	*Dietary Approach to Stop Hypertension*	**DBP**	*Vitamin D Binding Protein*
DCP	*Delta C-peptide*	**DPP4-2**	*Dipeptidyl Peptidase 4*
DM	*Diabetes Mellitus*	**EMC**	*Earlwood Medical Centre*
EPIC	*European Prospective Investigation of Cancer*	**ESA**	*Endocrine Society of Australia*
EVITA	*Effect of Vitamin D on All-cause Mortality in Heart Failure*	**EVOO**	*Extra Virgin Olive Oil*
EU	*European Union*	**FFQ-VDQ**	*Vitamin D specific Food Frequency Questionnaire*
FSG	*Fasting Serum Glucose*	**FPG**	*Fasting Plasma Glucose*
FSANZ	*Food Standards Australia New Zealand*	**GCP**	*Good Clinical Practice*
GDM	*Gestational Diabetes Mellitus*	**GI**	*Glycaemic Index*
GL	*Glycaemic Load*	**GLP-1RA**	*Glucagon like Peptide-1 Receptor Agonists*
GLP-1	*Glucagon-like Peptide 1*	**Glut-4**	*Glucose Transporter 4*
GP	*General Practitioner*	**GWAS**	*Genome-Wide Association Study*
HbA1c	*Glycated Hemoglobin or Haemoglobin A1c*	**HDL**	*High-Density Lipoprotein*
HOMA-IR	*Homeostasis Model Assessment-Insulin Resistance*	**HOMA-B**	*Homeostasis Model Assessment - Beta Cell Function*
IA	*Islet Amyloid*	**IDF**	*International Diabetes Federation*
IOF	*International Osteoporosis Foundation*	**IOM**	*Institutes of Medicine*
IRS-1	*Insulin Receptor Substrate 1*	**IRS-2**	*Insulin Receptor Substrate 2*
IU	*International Unit*	**KCNQ1**	*Potassium Voltage-Gated Channel Subfamily Q Member 1*

LDL	*Low-Density Lipoprotein*	**LOCF**	*Last Observation Carried Forward*
MBS	*Medicare Benefits Schedule*	**MED**	*Minimal Erythemal Does*
MUFA	*Monounsaturated Fatty Acids*	**NHANES**	*National Health and Nutrition Examination Survey*
NHMRC	*National Health and Medical Research Council*	**NIH**	*National Institutes of Health*
NUTTAB	*NUTrient TABles for use in Australia*	**NRV**	*Nutrient Reference Value*
OCCGS	*Osteoporosis Committee of China Gerontological Society*	**POE**	*Parent-of-Origin Effects*
PPG	Post Prandial Glucose	**PTH**	*Parathyroid Hormone*
PUFA	*Polyunsaturated Fatty Acid*	**QALY**	*Quality Adjusted Life Year*
QUICKI	*Quantitative Insulin Sensitivity Check Index*	**RACGP**	*Royal Australian College of General Practitioners*
RCT	*Randomised Clinical Trial*	**RDA**	*Recommended Dietary Allowance*
ROS	*Royal Osteoporosis Society*	**SFA**	*Saturated Fatty Acids*
SGLT-2i	*Sodium Glucose CoTransporter-2 inhibitors*	**SU**	*Sulfonylureas*
T1DM	*Type 1 Diabetes Mellitus*	**T2DM**	*Type 2 Diabetes Mellitus*
THADA	*Thyroid Adenoma Associated*	**VDD**	*Vitamin D Deficiency*
VDR	*Vitamin D Receptor*	**UL**	*Upper Level*
US	*United States*	**USDA**	*United States Department of Agriculture*
UV	*Ultraviolet*	**UVB**	*Ultraviolet B*
VITAL	*The Vitamin D and Omega-3 Trial*	**WHO**	*World Health Organization*

Abstract

Background: There is growing evidence indicating the link between serum 25-hydroxy-vitamin D (25(OH)D) level and risk of Type 2 Diabetes Mellitus (T2DM). Several large cohort observational studies have confirmed an inverse association between serum 25(OH)D level and T2DM risks, while Randomised Control Trials (RCTs) have presented inconsistent results. It was therefore decided to conduct a literature review of current RCTs to identify these limitations and assist in designing and conducting a more rigorous RCT, i.e. D4D trial, to evaluate the association between serum 25(OH)D level and T2DM.

Method: D4D trial was a long-term, multi-centre, single-blind, four-arm parallel-group trial. Eligible participants were randomised to receive vitamin D of, 1) 10-15 µg/day (400-600 IU/day) from food, 2) 12.5 µg/day (500 IU/day) from sunlight exposure, 3) 12.5 µg/day (500 IU/day) from commercial supplements or, 4) waitlist without intervention, for nine months. Serum 25(OH)D level, HbA1c and lipids levels were measured at baseline, 3rd, and 9th month after the intervention.

Results: 60 participants were recruited in the D4D trial, with 15 participants per group. Results showed serum 25(OH)D was inversely associated with HbA1c level albeit without statistically significance. At 9th month, serum 25(OH)D in the Diet group was significantly increased compared to its baseline (p=0.0166) and 3rd month (p=0.0268). Serum 25(OH)D level in the Sun exposure group at 3rd month and in Supplement group at 9th month was significantly increased compared to their baselines (p=0.0361 and p=0.0319). In addition, at 9th month, serum 25(OH)D level in the Diet group was significantly higher than that in the Waitlist (p=0.0068) and Supplement groups (p=0.0392). HbA1c level in the Diet group (p=0.0039) and Supplement Group (p=0.0332) at 3rd month, and Diet group (p=0.0299) at 9th month also significantly decreased compared to their baselines. Furthermore, blood HbA1c level in the Diet group at 9th month was significantly lower than that in the Waitlist group (p=0.0279). Blood lipid levels were not significantly changed by any interventions.

Conclusion: Dietary vitamin D is the most effective source to increase serum 25(OH)D levels and improve glycaemic control among T2DM patients. Vitamin D from sun exposure and supplements requires a large dosage and a long intervention time to show the effects. Future dietary recommendations can emphasise natural vitamin D sources for their benefits on glycaemic controls in patients with T2DM.

Abstract in Full Version

Background

There is growing evidence indicating the link between serum 25-hydroxy-Vitamin D (25(OH)D) level and Type 2 Diabetes Mellitus (T2DM). Many large cohort observational studies have confirmed an inverse association between serum 25(OH)D level and T2DM risks, while Randomised Control Trials (RCTs) have presented inconsistent results. Many RCTs have failed to report such associations due to study limitations. It was therefore decided to conduct a literature review of current RCTs to identify these limitations and assist in designing and conducting a more rigorous RCT, i.e. D4D trial, to evaluate the association between serum 25(OH)D level and T2DM.

Stage One: Literature Review

Method: A literature search was performed in PUBMED from its inception up to September 2017. The Jadad scale and the Van Trudle scale were used to assess the quality of the eligible RCTs.

Results: Sixteen RCTs were identified. All RCTs had a small sample size (8-45 participants in each group), with subjects aged between 48.5±8 to 66.9±3.1 years. In a total of sixteen studies, Vitamin D_3 was used in an oral form in twelve studies, in the injection form in two studies, and in fortified yoghurt form in two studies. Four studies concluded that vitamin D improved insulin sensitivity and glycaemia status among T2DM patients, while eleven studies reported no improvements. One study suggested that using calcium and vitamin D supplementation may benefit T2DM management.

Stage Two: D4D Trial

Hypothesis: An adequate serum 25(OH)D level could benefit glycaemic control in T2DM

patients.

Primary Objectives: To determine the role of vitamin D (through dietary intervention, sun exposure and vitamin D oral supplementation, compared to waitlist) in glycaemic control (as measured by HbA1c) in T2DM patients.

Secondary Objectives: 1) To determine the prevalence of Vitamin D Deficiency (VDD) among T2DM patients and, 2) To determine the differences between heterogeneous vitamin D sources and their impact on T2DM glycaemic control.

Methodology: Eligible participants were randomised into one of the four groups: Diet, Sun exposure, Vitamin D Supplement, and Waitlist control group for a total period of nine months. The dietary group was asked to follow a meal plan to obtain 10-15 µg/day (400-600 IU/day) of vitamin D from food. The Sun exposure group was required to expose 15% of the body surface to natural sunlight for a certain time to obtain 500 IU/day of vitamin D. The Vitamin D Supplement group was required to ingest commercial vitamin D supplementation at a dosage of 500 IU/day. No interventions were given to the Waitlist control group. Follow-up sessions were performed at baseline and on a monthly basis. Serum 25(OH)D, T2DM biomarker (HbA1c) and lipids levels were measured at baseline, 3rd month, and at the 9th month sessions.

Results: A total of 60 participants were recruited in the D4D trial with 15 participants per intervention group. Results suggested serum 25(OH)D was inversely associated with HbA1c level but was not statistically significant. Detailed results as following: 1) Serum 25(OH)D level in Diet group at 9th month was significantly increased compared to Waitlist group ($p=0.0068$) and Supplement group ($p=0.0392$), 2) Serum 25(OH)D in Diet group at 9th month was also significantly increased compared to its baseline ($p=0.0166$) and 3rd month ($p=0.0268$); 3) Serum 25(OH)D level in Sun exposure group at 3rd month and in Supplement group at 9th month was significantly increased

compared to their baseline (p=0.0361 and p=0.0319); 4) HbA1c level in Diet group (p=0.0279) at 9th month was significantly reduced compared to the Waitlist group, 5) HbA1c level in Diet group (p=0.0039) and Supplement Group (p=0.0332) at 3rd month and Diet group (p=0.0299) at 9th month also presented significant decreases compared to their baseline, 6) Total Cholesterol (TC), Triglyceride (TG), High Density Lipoproteins(HDL) and Low Density Lipoproteins (LDL) levels were not significantly changed by the interventions.

Conclusion: Dietary vitamin D is the most effective source to increase Serum 25(OH)D levels and improve glycaemic control among T2DM patients. Vitamin D from sun and supplements requires a large dosage and a medium to long intervention time to show the effects on the T2DM biomarker. Future well-designed clinical trials should assess the vitamin D from natural lifestyle interventions, e.g. sunlight and diet, to distinguish vitamin D's effect on glycaemic control from the confounding factors, and generate feasible and patient-oriented suggestions to T2DM patients.

Chapter 1. **Introduction**

1.1 Type 2 Diabetes Mellitus

1.1.1 General Information

Definition & Complications

Diabetes Mellitus (DM) is a group of metabolic diseases characterised by hyperglycaemia resulting from impairment in insulin secretion, insulin effectiveness defects, or both (1). The continuing hyperglycaemia can cause a series of long-term damage to both the microvascular and macrovascular systems. Such damage can affect organ functions, including the eyes, kidneys, nerves, heart and blood vessels, and can lead to severe medical complications, including loss of vision, renal failure, increased risk of foot ulcers, limb amputation, autonomic neuropathy (1, 2), and cardiovascular disease. The latter is also the leading cause of mortality and morbidity among Type 2 Diabetes Mellitus (T2DM) patients (3).

Types of Diabetes

Diabetes is currently classified into four different types, the main categories being Pre-diabetes, Type 1 Diabetes Mellitus (T1DM), Type 2 Diabetes Mellitus (T2DM), and Gestational Diabetes Mellitus (GDM) (4). T2DM is defined as a chronic hyperglycaemic condition either by body cells ineffectively using insulin with a background of insulin resistance and/or a relatively insulin secretory defect due to progressive loss of β-cells (5, 6). Currently, T2DM accounts for 90-95% of the diagnosed diabetes cases globally (1, 6) and 85-90% of total diabetes cases in Australia (5).

Diagnostic Criteria of T2DM

Four T2DM diagnostic criteria are currently recommended by the World Health Organization (WHO) (7) and the International Diabetes Federation (IDF) (8), which have been confirmed by The Royal Australian College of General Practitioners (RACGP) (9). These criteria include Glycated Haemoglobin (HbA1c), Fasting Plasma Glucose (FPG) level or Fasting Blood Glucose (FBG) level, 2-hours Postprandial Glucose (2-h PG) level, and Random Plasma Glucose (RPG) level (at least 2 hours postprandial) (Table 1.1).

Table 1.1
Diagnostic Criteria for Type 2 Diabetes Mellitus

WHO & IDF Diagnostic Criteria
HbA1c • HbA1c ≥ 6.5% (48 mmol/mol) OR FPG • FPG ≥ 7.0mmol/L (126 mg/dL) **Fasting is defined as no caloric intake for at least 8 h.* OR OGTT • Two-hour postprandial plasma glucose ≥ **11. 0 mmol/L** (200 mg/dL) during an OGTT **The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.* OR RPG • A random plasma glucose > 11.0 mmol/L (200 mg/dL), in a patient with signs and symptoms considered to have diabetes
RACGP Diagnostic Criteria
HbA1c • HbA1c ≥ 6.5% (48 mmol/mol) OR FBG • FBG <5.5 mmol/l: Diabetes unlikely • FBG 5.5–6.9 mmol/L: May need to perform an OGTT • FBG ≥7.0 mmol/L (>11.0 non-fasting): Diabetes likely; repeat fasting blood sugar on a separate day to confirm • Impaired fasting glucose is diagnosed on the basis of a result between 6.1 and 6.9 mmol/L OR OGTT • Measure the plasma glucose before (fasting) and two hours after a 75 g glucose load is taken orally. Diabetes is diagnosed if fasting plasma glucose is ≥7.0 mmol/L or two-hour plasma glucose is ≥11.0 mmol/L. If the two-hour plasma glucose is between 7.8 and 11.0 mmol/L, there is impaired glucose tolerance. A two-hour result <7.8 mmol/L is considered normal

WHO, World Health Organization; IDF, International Diabetes Federation; HbA1c, Glycated Haemoglobin or Haemoglobin A1c; FBG, Fasting Blood Glucose; FPG, Fasting Plasma Glucose; OGTT, Oral Glucose Tolerance Test; RPG, Random Plasma Glucose.

1.1.2 The Mechanism of T2DM

The specific aetiology and molecular mechanism associated with the onset of T2DM remain unknown. The disease development process is heterogeneous, but insulin resistance and β-cell dysfunction are well-acknowledged as two critical factors in the T2DM pathogenesis (10) (Figure 1.1).

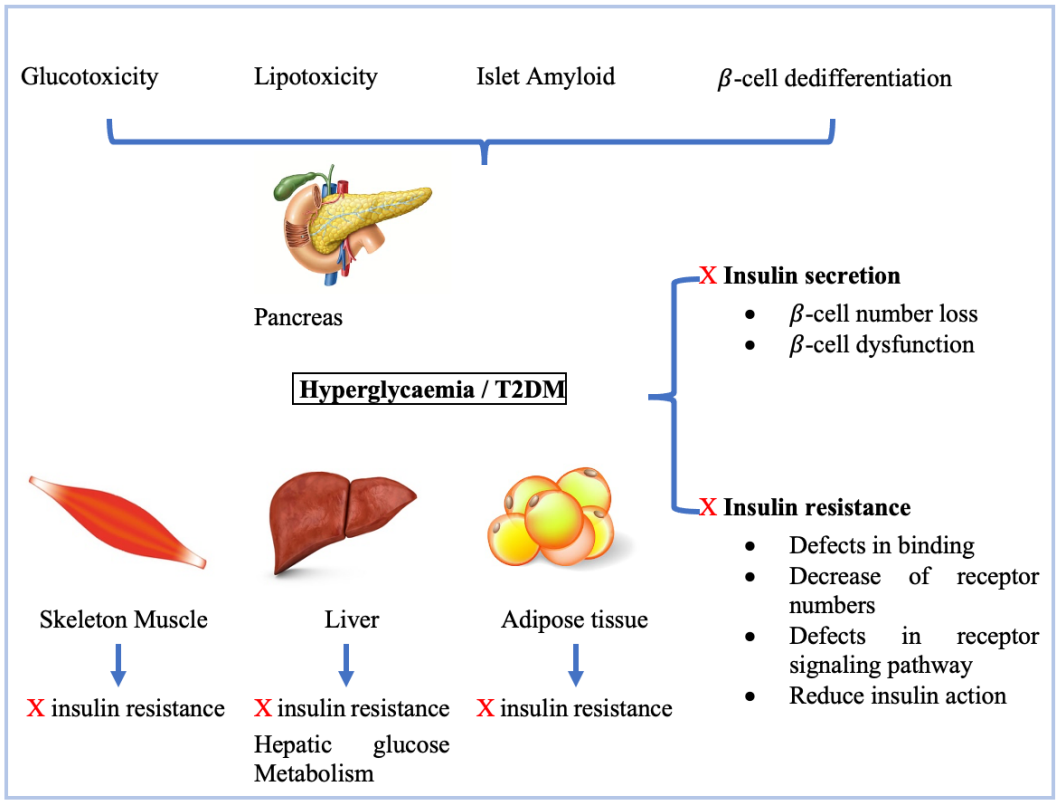


Figure 1.1
Pathogenesis of Type 2 Diabetes Mellitus

Insulin Resistance

Insulin resistance is the earliest detectable defect in the development of T2DM; it is defined as a subnormal biological response to normal insulin concentration (11) that may occur in skeletal muscle, fat and liver. The possible explanations of insulin resistance are the reduction in insulin receptor numbers, defects in the binding of insulin receptors

(Glucose Transporter Glut-4), and a decrease in the insulin receptor signalling activities, including phosphatidylinositol-3-kinase and the Insulin Receptor Substrates (IRS) - the IRS-1 and IRS-2 (12).

Insulin Secretion

The progressive defects in insulin secretion resulting from β-cell loss, β-cell dysfunction, or both (13), and may be explained by Islet Amyloid (IA), β-cell dedifferentiation, glucotoxicity and lipotoxicity (14).

The compensatory hypersecretion of insulin due to insulin resistance will simultaneously increase amylin as they are co-stored and co-secreted. The increased amylin leads to increased formation of amylin fibrils and IA, which are toxic to β-cells (15) and can cause β-cell loss and β-cell dysfunction (16). Furthermore, β-cell dedifferentiation has been observed as another factor contributing to β-cell function defects, in which the β-cells degenerate back to the progenitor-like state and convert into α-cells and δ-'like' cells hence lost β-cell identity and function (17). Glucotoxicity and lipotoxicity could also affect β-cells numbers and secretion by creating chronic hyperglycaemia (18) and islet lipid deposition (19), therefore also contributing to the pathogenesis of T2DM.

Other Contributors

Glucagon-like Peptide 1 (GLP-1) is one of the gut hormones (20) that can simulate glucose-dependant insulin secretion, decrease glucagon secretion and β-cell apoptosis and is related to β-cell proliferation and regeneration (21). Although T2DM patients present with a normal GLP-1 level, they usually have a decreased GLP-1 action which could possibly cause the β-cell defects (22).

The abnormal hepatic glucose metabolism is also linked with hyperglycaemia among T2DM patients. Insulin resistance or insulin deficiency, or both, can cause decreases in insulin action, lead to hyperglucagonemia and increases in liver gluconeogenesis, hence contributing to hyperglycaemia and T2DM (23).

1.1.3 T2DM Prevalence in World and Australia

Worldwide Prevalence

T2DM is a growing global public health issue with a worldwide prevalence (Figure 1.2). Data from 2019 showed an estimated 463 million (9.3%) of the world's population aged 20-79 years suffer diabetes, with 90% diagnosed with T2DM (6). VDD prevalence has significantly increased over the past two decades from 151 million (4.6%) in 2000 (24) and is estimated to increase to 578 million (10.2%) by 2030 and to 700 million (10.9%) by 2045 (6).

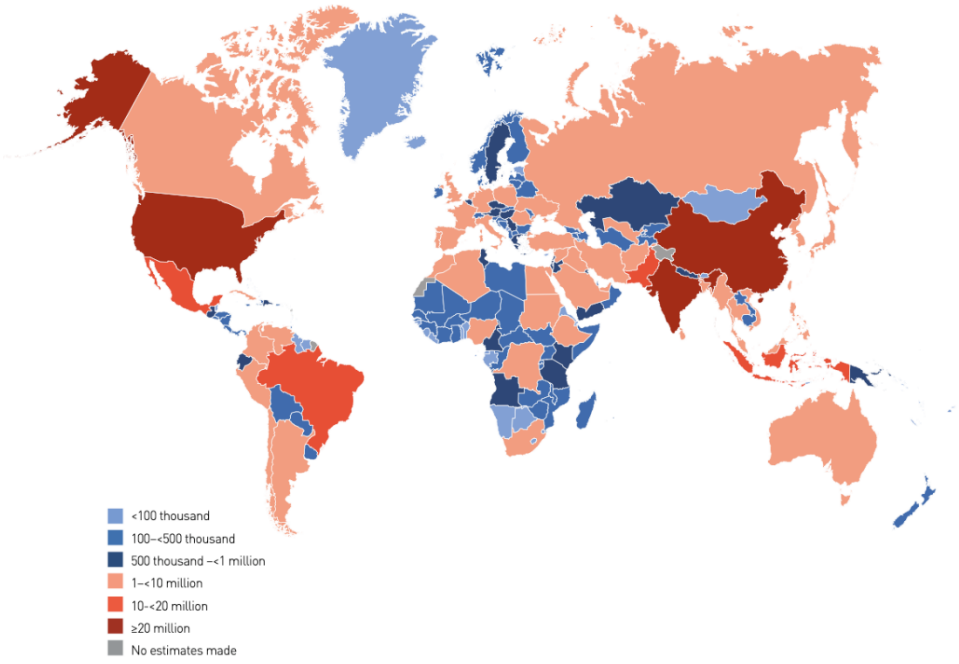


Figure 1.2
Estimated Total Number of Adults (20-79 years) with Diabetes in 2019, World Atlas form International Diabetes Federation (6).

Concerning T2DM, the Middle East and North Africa regions have the highest T2DM prevalence globally (12.2%), followed by Western Pacific (11.4%), South-East Asia (11.3%), and North America and Caribbean regions(11.1%). The North America and Caribbean areas is forecasted to become the third-highest region by 2030 and the second-highest region by 2045 (6). Figure 1.3 shows the numbers of people with diabetes in the top 10 countries or regions in 2019.

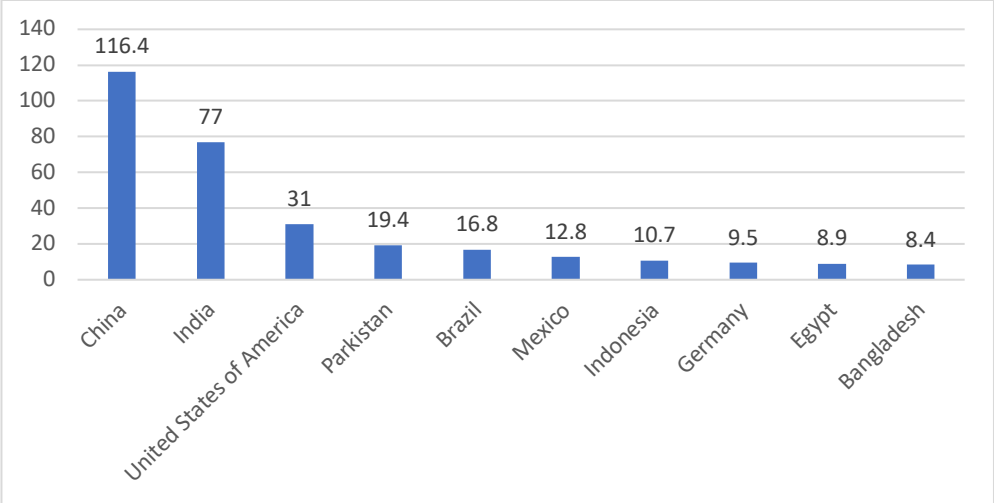


Figure 1.3
Number of People with Diabetes in 2019 in Million, the Top Ten Countries / Regions, data sourced from the World Atlas form International Diabetes Federation 2019.

VDD Prevalence in Australia

In Australia, approximately 4.1% (almost one million) Australians were living with T2DM in 2017-18 (25). Prevalence increments of all types of diabetes in Australia seem smaller than the global number as it only increased from 4.2% in 2011-2012 to 4.4% in 2017-2018 (26). However, this prevalence could be largely underestimated due to the limitation of the self-report data that were used in the current survey.

Australia's T2DM prevalence shows a significate difference in socioeconomic levels for those affected. The prevalence among those living in the lowest socioeconomic regions (7.0%) was two times higher than those in high socioeconomic areas (3.3%). People living in the lowest socioeconomic areas also had two times higher hospitalisation and mortality rates compared to those living in high socioeconomic areas (26). There was no significant difference in T2DM prevalence between men and women in Australia, and the prevalence of T2DM is similar across all major cities and regional areas (25).

Cause to Death and Disability

Diabetes directly increases the risk of heart attack or stroke, and is likely to trigger certain conditions or risk factors, such as high blood pressure or high cholesterol, that increase the chances of getting heart disease and stroke (27). Due to the strong relationship with Cardiovascular Disease (CVD), diabetes was the 9th leading cause of disability in 1990 and emerged as the 4th leading cause of disability in 2017 (28). In Australia, diabetes is the 7th leading cause of death and causing approximately 1.6 million deaths according to 2016 data (29).

1.1.4 Risk Factors of T2DM

T2DM is the result of a complex interaction between genetic, epigenetic, clinical factors (age, sex, family history, Body Mass Index (BMI), blood pressure, triglycerides, FPG, etc.) and environmental factors (diet, physical activities, lifestyle, etc.) (6).

Genetic

Genetic factors are a contributing cause for the onset of T2DM. The lifetime risk of developing T2DM is about 40% for individuals with one parent with T2DM and can

increase to 70% if both parents have T2DM (30). Studies, including the CODIAB and Botina study, suggested that the mother's link could be stronger - twice as common as the paternal one (31, 32). They found that the male offsprings of diabetic mothers have a reduced incremental insulin response to glucose compared to male offsprings with a diabetic father. Recent genetic evidence also observed the Parent-of-Origin Effects (POE) from the KCNQ1 and THADA loci, explaining the maternal link from a molecular level (33).

Increased types and numbers of genes and loci have been identified as linked to T2DM; however, it is still not yet fully understood. According to GWAS (Genome Wide Association Studies) in 2015, there were over 150 variants including TCF7L2, KCNJ11, and PPARG, mapping to >120 loci for T2DM, but this could only explain 20% of the heredity (33). In different studies, the variance of heritability could range from 20-80% (34), and the risk seemed to increase with the study's follow-up time. Therefore, a measurement of genetic factors at an early life stage is more meaningful in predicting the future T2DM risk (35).

Age

The risk of developing T2DM increases with age. T2DM prevalence presented the lowest at 1.9% among the age group 20-24 years, prevalence increased to 10% in the age group 45-49 years and peaked at around 20% in the age group 65 to 79 years (6). In terms of the number of diabetes patients, the age group 40-59 years currently has the greatest number of people with T2DM globally. However, it is expected to shift to the age group 60-79 years by 2030 considering the effects from urbanisation and ageing (36).

Diet

- ***Marco-nutrients – Carbohydrate and Fat***

Carbohydrate quality measured by Glycaemic Index (Gl) and Glycaemic Load (GL) shows a significant association with T2DM development as a diet contains high GI and GL carbohydrates can increase the T2DM risks (37). This association may help explain the increased T2DM risks by a large intake of white rice (38) and potato (39), as both of them are classified as high GI foods.

Total fat intake was not associated with the risks of T2DM compared with carbohydrates when providing the same amount of energy. However, the types of fat seem to have a different impact on T2DM. Saturated Fatty Acids (SFA) and Monounsaturated Fatty Acids (MUFA) were not associated with T2DM risks, but trans-fat intake appears to be positively associated with the T2DM risks while increased Polyunsaturated Fatty Acid (PUFA) intake has shown to have a protective effect in reducing T2DM risks (40).

- ***Food Groups - Fruits and Vegetables***

Total fruit and vegetable intake is not associated with T2DM, but green leafy vegetables are strongly associated with a reduction in the T2DM risks. Even a minor increase in the green leafy vegetable of one serve/day can contribute to a moderate decrease in T2DM risks (41). However, in terms of fruit, most studies suggested that whole fruit may have a minor benefit or even nil association to the T2DM risks, in contrast to fruit juice which is linked to an increased risk of T2DM due to high concentration of sugar (41-43).

- ***Food Groups - Red and Processed Meat***

A large intake of red meat, especially processed red meat, can increase the T2DM risks.

An increased intake of 50g/day (one serve/day) of both unprocessed and processed red meat can significantly increase the T2DM risks (44). Cohorts studies have observed that an increase of ≥ 0.5 servings per day in overall red meat consumption over four years can increase the risk by up to 30% after adjusting for anthropometric factors (45). This may be explained by dietary glycation and lipoxidation end products that present in processed food, as the lipid modification could affect insulin output (46), or it could be the heme iron in the red meat that may interfere with glucose metabolism and create oxidative stress, affecting β-cells and their function (47).

- ***Food Groups - Wholegrain Food***

Cohort studies suggest that increased consumption of wholegrain foods, including wholegrain breakfast cereal, oatmeal, dark bread, brown rice, added bran and wheat germ were inversely associated with the T2DM risks (48). Wholegrain foods are rich in dietary fibre, antioxidants, magnesium and phytochemicals, which may help to reduce the risks of T2DM (49, 50). A meta-analysis also confirmed the association between wholegrain food and T2DM, and suggested that replacing refined grain with wholegrain choices for at least two servings per day can reduce the T2DM risks (51).

- ***Food Style***

The western-style diet that is high in calories, processed meat, sweets and dessert, deep-fried food and refined grains has been associated with a 49% increase in the risk of developing T2DM (52). Mediterranean diet, especially when consumed with Extra-Virgin Olive Oil (EVOO), has been shown to reduce the T2DM risks (53). Furthermore, the Alternative Healthy Eating Index (AHEI), the Dietary Approach to Stop Hypertension (DASH) diet and the alternative Mediterranean (aMED) diets are all strongly associated

with a lower risk of T2DM (54).

Women whose diet that are low in GI, trans fats, and high in cereal fibre and balanced ratio of PUFA and SFA, have a lower risk of T2DM than those with imbalanced nutrient combinations (55). The regular eating pattern is also linked with the T2DM risks. An eating pattern of often skipping breakfast or eating irregularly with only 1-2 meals per day showed a stronger link to T2DM compared to the eating pattern of a regular three meals per day pattern (56).

- ***Individual Nutrients***

Individual nutrients that have been assessed as lowering the T2DM risks include heme iron (57), Docosahexaenoic Acid (DHA) & Eicosapentaenoic Acid (EPA) and α-inolenic acid (58), fibre from vegetable, fruit and cereal (59), magnesium (60), and vitamin D (61) and the Glycaemic Index (GI), Glycaemic Load (GL) of foods (37),

Physical Exercise

Physical exercise can significantly reduce the risks of T2DM, even without weight loss (62). In terms of the intensity and duration of exercise, studies suggest that both aerobic exercise and resistance training are effective in preventing T2DM (63). Exercise intensity can have a wide range from moderate levels such as walking (64) to intense levels such as cycling (65). The American Diabetes Association (ADA) (66) recommended that: 150 min/week or 30 min/day for five days a week of moderate to vigorous physical exercise will help prevent T2DM. Moreover, there is a strong association between sedentary time and diabetes incidence, which appears to be independent of physical activities. A recent study found every 2 hours/day increment of sitting and television watching was associated

with a 14% and 7% increase in the T2DM risks, respectively (67).

Other Risk Factors – Smoking & Urbanisation

Smoking has also been found to have a positive association with the T2DM risks. The number of cigarettes smoked per day, past smoking history and the length of quitting time were all strongly associated with the T2DM risks (68).

The number of T2DM people who live in urban areas is twice as much as the number of people who live in rural areas (IDF 2019). Rapid urbanisation has led to a more sedentary lifestyle, unhealthy diet, increased sugar consumption and obesity; hence is another risk factor for T2DM. A recent study argues that the uncontrolled growth of the large urban agglomerates, rather than urbanisation itself, is linked to the globally increased T2DM prevalence (69).

1.1.5 Current Treatment of T2DM and The Medical Burden

Screening at the Early Stage

Although currently there is no direct evidence of whether T2DM screening at an early stage will or will not benefit individuals, it has been demonstrated that early-stage detection can reduce the T2DM incidence and future T2DM development in high-risk groups (70).

Assessing T2DM risks by administering questionnaires followed by a blood glucose measurement test (including urine glucose test, blood glucose test and HbA1c) could improve the diagnostic accuracy and performance (70). Questionnaires, including the ADA Type 2 Diabetes Risk Test that was published by the American Diabetes

Association in '2017 Standards of Care', are commonly used for the initial screening (71). In Australia, the RACGP suggests using the Australian Type 2 Diabetes Risk Assessment Tool (AUSDRISK) for T2DM initial screening. The RACGP also suggests that patients over 40 years old with a high risk of T2DM should be screened for T2DM every three years, Aboriginal and Torres Strait Islander peoples from 18 years old should also have a T2DM screen every three years, and the T2DM screening should be part of a CVD assessment (72).

Lifestyle Intervention – Diet & Exercise

Lifestyle intervention, including physical exercise improvement and diet modification, is significantly more effective than medication (73), especially for people younger than 60 years (74). In general, physical activity with increased levels and healthy diets rich in fresh fruit and vegetable, whole grain, cereal fibre and healthy fats such as omega-3, will reduce long-term risks of CVD for people with diabetes (6).

A Randomized Controlled Trial (RCT) suggested that physical exercise, over a range of intensities, can significantly improve glycaemic control, lower triglyceride and reduce visceral adipose tissue in the T2DM group, even without weight loss (62). A recent study suggested that starting an exercise program from the low intensity and slowly increasing to a moderate level with daily maintenance, would be preferential than starting exercise at an intense level but with periodic maintenance (75).

Appropriate calories intake from diet is also important for maintaining a healthy BMI or achieving a healthy body weight if overweight. Weight reduction in obese individuals can improve insulin sensitivity and benefit glycaemic control (76). The IDF 2017 (77)

suggested that the low-calorie diet (i.e. 800-1200 calories/day) can benefit the needed weight loss, and other recommended guidelines include: avoiding confectionary food such as sugar, sweets and sweetened beverages, increasing high-fibre and low GI food and preferring a special dietary pattern such as the Mediterranean diet (78).

Oral Medication & Insulin Treatment

The current treatment of T2DM is designed to reduce the development or progression of DM complications with good glycaemic controls (79). Metformin is often used as the first-line treatment for most T2DM patients. If the HbA1c target has not been reached in three months, second-line treatment including Sodium-GlucosebTransporter-2 inhibitors (SGLT-2i), Dipeptidyl Peptidase-4 Inhibitors (DPP4I), Sulfonylureas, Glucagon-Like Peptide-1 Receptor Agonist (GLP-1RA) and insulin should be introduced (80).

Medical Burden and Future Direction

According to the IDF 2019 (6), the annual global health expenditure on diabetes was USD $760 billion in 2019 and estimated to rise to USD $825 billion by 2030 and USD $845 billion by 2045. The cost of diabetes in the United States (US) had been largely increased by 33% in the six years since 2012 (USD $245 billion) to 2018 (USD $327 billion) (81).

In Australia, T2DM accounted for 2.2% of the total disease burden in 2015 and is the 12th largest contributor to disease burden (28). Together with T1DM and pre-diabetes, diabetes contributed 4.7% of the total disease burden with a total annual cost of AUD $2.68 billion in 2015-2016 (82, 83).

The current T2DM treatment of oral medication and insulin injection aims to reduce the

metabolic syndrome risks and slow the development from pre-diabetes to diabetes, but not ameliorate the disease incidence and pharmacological needs as they are still steadily growing. Medical and pharmacological treatment usually has a good controlling outcome when there is a good compliance; however, lifestyle interventions may be more beneficial as they show a better health outcome and cost much less, hence reducing the medical burden. Studies showed that lifestyle interventions only cost USD $1,100 per quality-adjusted life-year (QALY) than the metformin intervention that was estimated to cost $31,300 per QALY (84). Therefore, as suggested by the Australian Institute of Health and Welfare (AIHW), more effective and innovative treatment methods including lifestyle changes, advanced screening and monitoring, and the improved medication adherence (85), are promising for the future to decrease the medical burden and improve T2DM management (86).

1.2 Vitamin D

1.2.1 Background Information on Vitamin D

Vitamin D is a fat-soluble vitamin that acts as a steroid hormone. It has two forms, vitamin D_2 and D_3. Vitamin D_2 is generated from the plant sterol ergosterol and is found naturally in mushrooms, especially the sunlight-exposed varieties. The major vitamin D form for humans is vitamin D_3. Traditionally, it is believed that very few foods contain much vitamin D_3, with the best food sources of vitamin D_3 in egg yolk, fish such as barramundi and salmon, and the fortified margarine spread (mandatory fortified in Australia). The majority of the vitamin D needs (90- 95%) are fulfilled through skin synthesis following sun exposure (87).

Metabolism of Vitamin D

Ultraviolet B (UVB) light (290-315nm) can convert 7-dehydrocholesterol in the skin to vitamin D_3. The sunlight-induced vitamin D_3, together with a small amount of dietary vitamin D (D_2 and D_3), will be hydrolysed in the liver by D-25-hydroxylase (25-OHase) and further hydrolysed in the kidney by 25(OH)D-1-OHase (CYP27B1) to form the biologically active form of vitamin D – 1,25-dihydroxy-Vitamin D (1,25(OH)D) (88).

The primary function of 1,25(OH)D is to maintain calcium homeostasis in conjunction with parathyroid hormone (PTH), serum calcium and phosphorus. For example, low serum calcium concentration will stimulate the renal secretion of 1,25(OH)D and increase the calcium absorption in the intestine (89), hence decrease PTH secretion and increase the serum calcium concentration (Figure 1.4). Inversely, inadequate vitamin D levels can reduce calcium absorption in the intestine and result in the serum PTH increase, which leads to calcium mobilisation from the bone tissue (90).

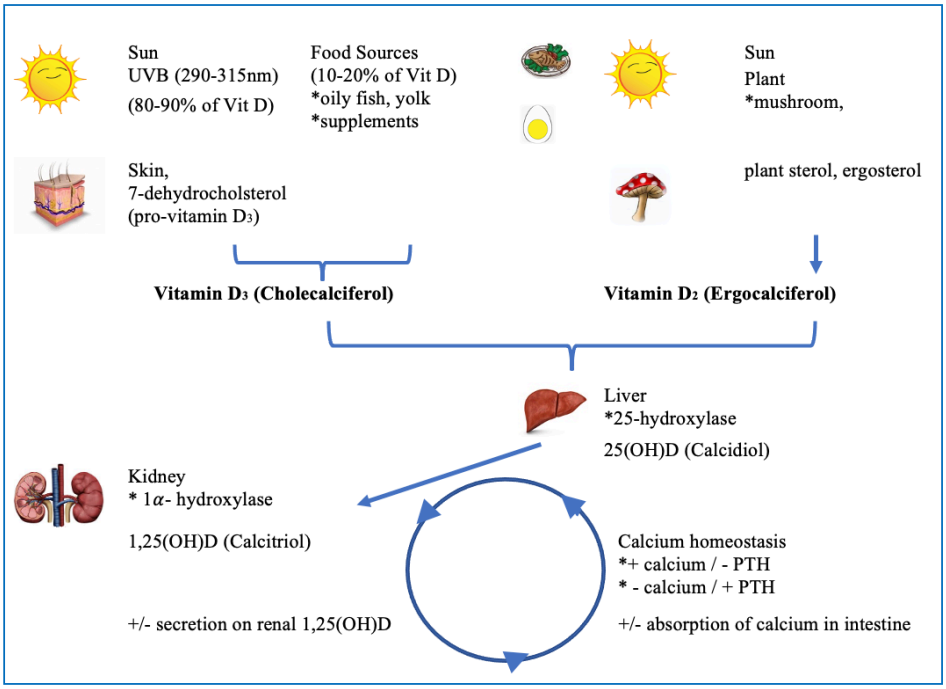


Figure 1.4
Vitamin D Sources and Metabolism Pathway, UVB, Ultraviolet B, nm, nanometre, Vit D, Vitamin D, PTH, Parathyroid hormone, 25(OH)D, 25-hydroxy-Vitamin D, 1,25(OH)D, 1,25-dihydroxy-Vitamin D.

Sources of Vitamin D

The primary source of vitamin D for people living in Australia is natural sunlight. However, sun exposure can be negatively affected by weather, geographic differences, skin pigmentation, sunscreen application and over-covered traditional or religious cloth wearing, that inhibit the required sun exposure time to obtain a sufficient amount of vitamin D (87). Prolonged sun exposure may also increase skin cancer risks. Therefore, balanced consideration of sun exposure time to maintain an adequate vitamin D level from the 'just enough' sun exposure and to limit skin cancer risks from excessive sun exposure is required.

The traditional understanding is that dietary vitamin D sources are limited. Based on current dietary patterns, the diet can only contribute 5-10% of the daily vitamin D needs for most adults (87). However, a recent study has reported a large discrepancy between dietary intake (71%) and the serum vitamin D level (19%) (91) and pointed out the discrepancy could come from the uncounted dietary 25(OH)D. Dietary 25(OH)D is two to five times more efficient in raising serum 25(OH)D concentration than its parent compounds - vitamin D_2 and D_3 (92); hence the inclusion of dietary 25(OH)D can be very important when calculating the vitamin D intake from food.

Nowadays, fortified foods are a very important source of dietary vitamin D, and different countries applied different fortification regulations. In the United States, milk is voluntarily fortified with vitamin D at 40 IU/100ml; other fortified foods may include breakfast cereal, juice, yoghurt, cheese and oil spreads. In Canada, milk is mandatorily fortified with vitamin D of 30-45 IU/100ml and margarine is fortified with at least 530 IU vitamin D per 100g (93). In Australia, mandatory vitamin D fortification is only

required for margarine and oil spreads, and the table margarine should contain no less than 22 IU/100g (55µg/kg) of vitamin D (94).

Recommended Dietary Intake (RDI) of Vitamin D

The current Australian and New Zealand's guidelines (2006) (95) for the Recommended Dietary Intake (RDI) of vitamin D is as follow: Adequate Intake (AI) of 200-400IU/day (5-10µg/day) for children and adult less than 70 years, and 600 IU/day (15µg/day) for 70 years and older. However, these data are out-of-date and need a review (87). The most recent revised Recommended Daily Allowances (RDAs) of vitamin D is from the United States and suggests 600 IU/day (15µg/day) for children and adults aged 1–70 years, 800 IU/day (20µg/day) for those older than 70 years, with an upper limit (UL) of 4,000 IU/day (100µg/day) for people older than 9 years of age (96).

Definition of Vitamin D Deficiency

There is no universal cut-off point to determine Vitamin D Deficiency (VDD). Different organisations have slightly different definitions of VDD (see Table 1.2).

Table 1.2
Definition of Vitamin D Deficiency (97-99).

Deficiency Level	ESA & OA, AUS	MBS, AUS	ROS, UK	Endocrine Society, USA	IOM , USA	Mayo Clinic, USA	OCCGS, CHN
Severe	<12.5 nmol/L*	<12.5 nmol/L	Deficiency <25nmol/L	Deficiency <50nmol/L	Deficiency <30nmol/L	< 25 nmol/L	Deficiency <30nmol/L
Moderate	12.5-29nmol/L	12.5–24.9 nmol/L					Inadequate 30-50 nmol/L
Mild	30-49 nmol/L	25–49 nmol/L	Inadequate 25-50 nmol/L	Insufficient 50-74.9 nmol/L	Insufficient 30-50 nmol/L	26-60 nmol/L	
Sufficient	≥50nmol/L	≥50nmol/L	>50nmol/L	n/a	n/a	n/a	≥50nmol/L
Optimal				≥75nmol/L	≥ 50 nmol/L	61-200 nmol/L	50-75 nmol/L

ESA, The Endocrine Society of Australia, Sydney Australia; OA, Osteoporosis Australia, Sydney, Australia; MBS, Medicare Benefits Schedule, Department of Health, Australia Government, Canberra, Australia; ROS, Royal Osteoporosis Society, Bath, England; IOM, Institute of Medicine, Washington DC, USA; OCCGS, Osteoporosis Committee of China Gerontological Society, Beijing, China.

*vitamin D unit conversion factor: ng/ml * 2.496 = nmol/L

The Institute of Medicine sets 50 nmol/L as the sufficient cut-off point of VDD when considering optimal bone health as the only causal association established with vitamin D. This cut-off point was confident to cover 97.5% of the US population (96). Other organisation like Mayo clinic, has suggested a different or a higher cut-off point of VDD when considering further causal associations such as secondary hyperparathyroidism and osteoporosis (100). However, current evidence indicates the inconsistencies in the optimal serum 25(OH)D level due to the PTH concentration plateau (101).

1.2.2 Health Impact of Vitamin D

Vitamin D influences many organs and body systems including bones, intestines, immune and cardiovascular systems, pancreas, muscles, brain, and has possible benefits for preventing many diseases such as cancer, heart disease, falls and fracture, autoimmune diseases, influenza and T2DM (102). Based on systematic reviews, narrative reviews and RCTs, Haye reported the following summarised associations between vitamin D levels and disease risks in the 2016 Health Technology Assessment (103) (see Table 1.3).

Table 1.3
Associations between serum 25(OH)D and Diseases

Serum 25(OH)D's Association	Risk of Disease
Harmful	Cancer mortality in men
Positive	Bone health Cardiovascular health Type 2 diabetes Colorectal cancer Ovarian cancer All-cause mortality
Unclear (inconsistent)	Cancer other than colorectal or ovarian cancer
Insufficient	Obesity Gestational diabetes Multiple sclerosis Depression and mood disorders

Bone Health

Vitamin D's major function is maintaining appropriate blood calcium and phosphorus level and supporting skeleton and muscle development and maintenance. A severe and prolonged VDD can cause bone demineralization diseases such as rickets in children and osteomalacia in adults (104). A sufficient vitamin D level can help prevent muscle weakness (105), bone fracture and reduce morbidity and mortality, especially in elderly groups (106).

Cardiovascular Health

Epidemiological studies generally have shown that lower serum 25(OH)D levels are associated with an increased risk of heart disease, stroke and hypertension (107, 108). A meta-analysis of prospective studies presented a linear, inverse association between serum 25(OH)D ranging from 20-60 nmol/L and CVD risks (109) and concluded that a sufficient serum 25(OH)D concentration (50-62.5nmol/L) was associated with the lowest risk of a total CVD event and CVD mortality (110).

RCTs suggested that 1,000 IU/day of Vitamin D supplementation can significantly improve High-Density Lipoprotein (HDL), apoA-I concentrations and Low-Density Lipoprotein (LDL) in overweight and obese women hence reducing CVD risks (111). Amongst people with a low baseline vitamin D level, vitamin D supplementation of 3,000 IU/day could significantly lower blood pressure (112).

Although few RCTs showed an inverse association between vitamin D and CVD risks, most RCTs and meta-analyses of vitamin D supplementation have shown a null or non-significant association (113). The EVITA trial (Effect of Vitamin D on All-cause

Mortality in Heart Failure) reported that vitamin D supplementation of 4,000 IU/day did not reduce mortality in patients with advanced heart failure (114). The latest large trial VITAL (The Vitamin D and Omega-3 Trial) using vitamin D supplementation of 2,000 IU/day also failed to show preventative effects on CVD (115).

The current association between vitamin D and CVD is similar to T2DM aetiology as most large epidemiological studies suggested the inverse association between serum 25(OH)D level and CVD risks, but the conclusions were inconsistent within RCTs. The inconsistencies may be due to the difference in dose regime, intervention duration, and the sensitivity of selected biomarkers.

Cancer and Mortality

Epidemiological studies, observational studies, RCTs, and molecular studies have supported vitamin D's cancer preventative effects (116) and its role in cancer treatment (117). Studies suggested that vitamin D works as an anti-cancer agent and can function in cancer prevention and treatment through proliferation, differentiation, apoptosis and anti-inflammatory effects (118).

The anti-cancer effect of vitamin D has been established in several cancer types, including colorectal cancer (119), breast cancer (120), and prostate cancer (121). A higher serum vitamin D concentration was associated with a decreased risk of cancer incidence and mortality rate, particularly cancers in the digestive and endocrine systems (122). A study showed that serum 25(OH)D level at 50 nmol/L or higher could reduce the risk of colon cancer by 75-80% (123), and an extra intake of vitamin D supplements >1,000 IU/day could reduce the incidence of breast cancer by 50% (124).

T2DM

Evidence suggests a possible link between vitamin D and T2DM. A large Finnish observational study showed that males with the highest serum 25(OH)D level had the lowest risk of developing diabetes (125), with a similar result also observed amongst women (126). An RCT conducted among non-diabetic insulin-resistant South Asian women living in New Zealand reported a significant improvement in insulin resistance by vitamin D supplementation (127). Another RCT indicated that vitamin D could benefit the T2DM risk group as it slowed down the increase in fasting glucose among individuals with impaired glucose metabolism (128).

Anti-inflammation and COVID-19

Vitamin D's anti-inflammatory effects have been observed consistently in the in vitro studies, with its effects observed in both blood mononuclear cells and human cell lines (129). A review of RCTs concluded that vitamin D could decrease most inflammation markers under the condition of a highly inflammatory status (130). The latest research has reported that an optimal vitamin D level and vitamin D supplementation can reduce the influenza risks and COVID-19 infections and death, as it may lower the viral replication rates and reduce the infection risk through its anti-inflammatory effects (131).

1.2.3 Prevalence of Vitamin D Deficiency

It is estimated that one billion people worldwide may have VDD or an insufficient vitamin D level (132). According to the Vitamin D Deficiency Map developed by the International Osteoporosis Foundation (IOF), Asia, the Middle East, parts of Europe and South Africa generally have a higher VDD prevalence than other regions (Figure 1.5).

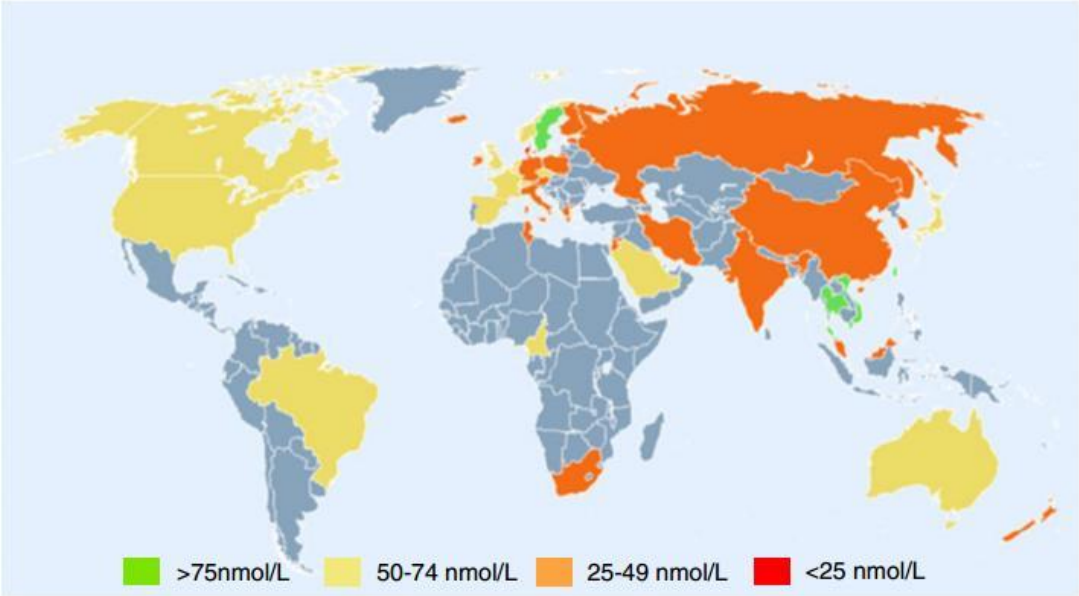


Figure 1.5
Vitamin D Status in Adults (>18 years) Around the World (133), winter values were used to calculate the mean 25(OH)D levels.

The US National Health and Nutrition Examination Survey (NHANES) from 2011-2014 found that 23.3% of Americans (age ≥ one year) had VDD with a cut-off point of 50 nmol/L. The prevalence rate was significantly higher in Black (53.3%), Asian (34.0%) and Hispanic groups (32.2%) than Caucasians (13.9 %) (134).

The Australian Bureau of Statistics (ABS) (5) has reported that the majority of Australian adults have sufficient serum (OH)D levels (> 50 nmol/L), with only 23% (4 million) having VDD and less than 1% having a severe deficiency (<13 nmol/L). No significant difference was observed between men and women. Supplements were commonly used among the elderly group; hence the elderly were less likely to have VDD than the younger group.

1.2.4 Risk Factors of Vitamin D Deficiency

Ageing

Both ageing (135) and skin pigmentation (melanin) (136) can affect vitamin D synthesis in the skin. Although the young age group of 18-34 years was reported with a similar VDD prevalence (31%) when compared to the senior age group at 65 plus years (35%) (5). It was argued that the elderly group generally had a higher risk of VDD due to decreased sun exposure time and reduced ability of intestinal absorption due to ageing (137). Those who live in aged care institutions reported a high VDD prevalence that could reach as high as 79% (138).

Ethnicity, Darker Skin and Cloth Covers

Asians also have a higher prevalence of VDD. India reported approximately 94.3% of the population could have VDD (< 50 nmol/L) (139), while China reported 55.9% of the population could have VDD and women have a significantly higher VDD rate than men (140).

Individuals with darker skin plus extra clothing coverage are most likely to have VDD (87), as the VDD was exacerbated by limited or no sun exposure due to the use of traditional or religious cloth covering (141). Statistical reports have shown that VDD was much more common among immigrants from Southern and Central Asia (67% on average and 60% in summer), North-East Asia (64%), South East Asia (58% on average and 50% in summer) and North Africa and the Middle East (50%) (5).

An Australian study found that 80% of dark-skinned and veiled women had VDD with an average serum vitamin D level under 22.5 nmol/L (142). Among them, 66% of women had a moderate VDD in summer, and this number could worsen to 90% in winter. Another study in the city of Oslo compared five different immigrant groups from the Middle and

South-East regions (Turkey, Sri Lanka, Iran, Pakistan and Vietnam) also reported a high VDD prevalence (64.9%) among women from Pakistan due to darker skin pigmentation, covered clothes and limited time of outdoor activities (143).

Skin pigmentation has also been shown to decreased vitamin D synthesis in the dark-skinned population (144). A study reported that 51% of African-American adolescents have a low serum vitamin D level (< 37.5 nmol/L), especially in winter (145), while another study (146) from Melbourne, Australia, revealed a similar percentage (53%) of VDD among African immigrants.

Other Risk Factors

A reduction in sun exposure time can increase the risk of VDD. The reduction could be caused by lifestyle changes such as limited outdoor activities (147), sunscreen usage (148) and environmental changes such as air pollution (149).

Besides, people with certain medical conditions, including osteoporosis, liver failure, renal diseases, and malabsorption problems such as Crohn's disease and bariatric surgery, are at a higher risk of VDD. Medications including isoniazid, rifampicin, and anticonvulsants may increase the risk of VDD as these medications could affect vitamin D synthesis and absorption in the digestive tract and alter the endocrine action of vitamin D (150). Moreover, breastfed infants are also at risk of VDD if they are fed by a vitamin D deficient mother (98).

1.3 Current Evidence of the link between Vitamin D and T2DM

1.3.1 Cohort studies

The meta-analysis of cohort observational studies generally has had a consistent conclusion of the association between vitamin D and T2DM. A lastest meta-analysis (151) of twenty-eight prospective cohort and nested case-control studies and eighty-three cross-sectional and case-control investigations concluded that serum 25(OH)D level was reversely associated with the T2DM risks, each 25 nmol/L increment of serum 25(OH)D was associated with 12% reduction in T2DM risks. Similarly, a meta-analysis of twenty-one prospective studies also found a significant inverse association between serum 25(OH)D level and T2DM risks across the whole population (152). It concluded that each 10 nmol/L increase in serum 25(OH)D level could lead to 4% lower risk of T2DM, and reported that the sufficient baseline 25(OH)D level was significantly associated with a lower T2DM risk.

Another systematic review and meta-analysis (153) analysed data from eighteen long-term prospective studies also suggested optimal baseline serum 25(OH)D level may protect from developing future T2DM, and observed that individuals' baseline serum 25(OH)D levels within the top one-third of the cohort had approximately 19% lower risks in incident T2DM compared with those in the bottom third. Similarly to these findings, Mitri et al. (154) found that individuals with a higher baseline serum vitamin D level (higher than 62.5 nmol/L) had a 43% lower risk of developing T2DM compared to those with a lower baseline serum 25(OH)D level (less than 35 nmol/L). They also concluded that taking vitamin D supplements of more than 500 IU/day can decrease the risk of T2DM by approximately 13%.

Although most of the cohort studies confirmed that both vitamin D supplements and sufficient baseline serum 25(OH)D levels could reduce the risk of developing T2DM, confounding factors have always been a drawback in previous publications on vitamin D research. The optimum baseline vitamin D levels are commonly observed in individuals with a healthier lifestyle or of a younger age compared to those with lower baseline vitamin D levels (155). One can argue that the lifestyle and age themselves are associated with lower T2DM risks in this population, while the optimal blood vitamin D levels are also the result of such confounders. Other factors, including sun exposure, outdoor physical activities, dietary intake and medication or insulin usage, can also influence both vitamin D and glucose levels (156). Therefore, if such factors have not been adjusted for, studies would have significant limitations that make the conclusion not sound.

1.3.2 Randomised Clinical Trials

The therapeutic effects of vitamin D supplements have not been well supported by previous clinical trials either. Meta-analysis of randomised clinical trials has not established the association between vitamin D and glycaemia control in T2DM patients. A meta-analysis conducted in 2012 including fifteen trials concluded that vitamin D supplements have no significant impact on fasting glucose, HbA1c levels, or insulin resistance index compared with placebo after combining all studies; however, small but non-significant beneficial effects were observed on fasting glucose and insulin resistance among people with diabetes or impaired glucose tolerance (157). A 2018 systematic review and meta-analysis (158) including twenty trials found that vitamin D supplements failed to improve fasting blood glucose, HbA1c and fasting insulin levels. However, this study also found that vitamin D supplements in large dosage (more than 2000 IU/day) for a short period (less than or equal to three-month) may be able to improve insulin

resistance, especially in T2DM patients with normal body weight, Asian background especially Middle Easterners, vitamin D deficiency, and an optimal fasting glucose level. This suggests that any complications can diminish the effect of vitamin D supplements.

Single randomised clinical trials have also presented inconsistent results regarding the association of vitamin D supplementation and glycaemic control in patients with T2DM. Some have reported that vitamin D supplements may benefit people with impaired glucose as vitamin D slowed down the increase in fasting glucose and insulin resistance (128, 157); many others reported a null association (159-163). A trial conducted among non-diabetic but insulin-resistant South Asian women in New Zealand (127) suggested that keeping an optimum 25(OH)D level (i.e. 50-80 nmol/L) in the long-term can benefit insulin sensitivity. However, two clinical trials that used a large injection dosage of 300,000 IU/day had observed inconsistent results; one study indicated that vitamin D3 improved insulin sensitivity by increasing the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and HbA1c (164), while in the other, vitamin D_3 had failed to improve the glycaemic control (159). Oral supplements generally used a lower dosage from 500 to 6,000 IU/day, and most of them failed to affect glycaemic control in T2DM (160-163).

Two trials with a large sample size also reported some marginal effects of vitamin supplementation (165, 166). In one study (165), 511 prediabetic adults were randomised with vitamin D 20,000 IU/week or placebo and followed up for 3.3 years for the incident of T2DM. The incident was lower in the vitamin D group compared to placebo, however, it was not statistically significant. Another recently completed large randomised clinical trial - Vitamin D and Type 2 Diabetes (D2D) Trial (166), compared the vitamin D

supplemented at a dosage of 4,000 IU/day with the placebo. The study analysed data from more than 2,400 participants with prediabetes for over 2.5 years and also found a non-statistically significant reduction (12%) in the risk of diabetes. However, other studies suggested that a possible but non-significant benefit in glycaemic control (162), insulin secretion (163) or insulin sensitivity (167).

It was noticed that some well-designed large studies such as D-HEALTH and VITAL (Vitamin D & Omega-3 Trial) projects are ongoing and would help to explore the association between vitamin D and T2DM once they are completed. D-HEALTH trial in Australia is the second-largest trial of high dosage vitamin D supplementation ever conducted (168). It is designed to compare 60,000 IU/month supplementation with placebo followed up for five years to explore the association between vitamin D supplements and health outcomes, including Cardiovascular Diseases, cancer, and diabetes (168). The VITAL study has included 25,871 Americans using vitamin D of 2000 IU/day or omega-3 fatty acids 1000mg/day and follows up for five years to assess the effects on cardiovascular diseases and cancer. This ancillary study will use incident diabetes as a secondary outcome (169).

A few limitations need to be noted when interpreting inconsistent results from existing randomised control trials, including relatively small sample sizes, short study duration, poor research design, vitamin D sources and dosage, and other confounding factors such as sun exposure, dietary intake, supplement and medication usage (170). Such limitations led to the decision to undertake a literature review on current RCTs and perform a more rigorous designed RCT to examine the effects of vitamin D intervention on glycaemic control among T2DM participants.

Chapter 2. **Literature Review**

First assessed was the association between vitamin D deficiency and glycaemic control among T2DM patients in published RCTs. The information presented in this Chapter has been recently published in (171),

> Yu, X & Lim, Chi Eung Danforn & Zaslawski, Chris. (2016). **Vitamin D on glycaemia control in type 2 diabetes patients: A systematic review of randomised clinical trials.** Journal of the Australian Traditional-Medicine Society, Winter 2018, vol. 24 Issue 2, p82-88. 7p.

2.1 **Background and Introduction**

Diabetes has become a major health issue for many developed and developing countries. There are 347 million people affected by diabetes globally, and this number is expected to increase to 366 million by 2030 (172). Among those affected by diabetes, over 90% people are diagnosed with T2DM based on data from World Health Organization. There were 999,000 Australians (4.6%) reported to have diabetes in 2012, the majority (85.3%) of whom were T2DM. The prevalence of diabetes in Australia is expected to grow to 3.5 million by 2033 (173).

Diabetes mellitus is a chronic disease characterised by hyperglycaemia resulting from defects in insulin secretion, insulin function or both. It significantly affects an individual's health and is associated with many complications, including damage to several organs, especially the eyes, kidneys, heart, nerves and blood vessels.

The treatment of T2DM involves achieving an optimal blood glucose level and of other known risk factors that damage blood vessels or organs (174). The management of T2DM

can be very challenging (175), as it requires medication and insulin use as well as lifestyle changes such as a healthy diet (176, 177) and increased physical exercise (178). Haye reported in the 2016 Health Technology Assessment (103) which summarised systematics reviews, narrative reviews and clinical trials, concluded that (OH)D had a protective association with T2DM.

2.1.1 Cohort Studies and Limitations

The meta-analysis of cohort studies generally have had a consistent conclusion regarding the association between vitamin D and T2DM. One meta-analysis found a significant inverse association between serum 25(OH)D level and T2DM risks across the whole population (152). It summarised that each 10 nmol/L increase in serum 25(OH)D level can lead to 4% lower risk of T2DM and the sufficient baseline 25(OH)D level was significantly associate with a lower T2DM risks.

Another systematic review and metanalysis (153) analysed data from 18 long-term prospective studies and also suggested a significant inverse association of baseline vitamin D level and incident T2DM. Similarly, Mitri et al (154) found that persons with a higher baseline serum vitamin D level (higher than 62.5 nmol/L) had a lower risk then those with serum 25(OH)D level less than 35 nmol/L. They also concluded that Vitamin D >500 IU/day decreased 13% of the T2DM risks.

Confounding factors or residual confounders are always problematic within vitamin D research. Optimum vitamin D level usually indicated a healthier lifestyle and a younger age, which may associate with lower T2DM risks independent from vitamin D (155). Besides, the dietary consideration may not be sufficient as we cannot exclude the possible

effect from unmeasured or unknown food component which may influence the result accuracy (156).

2.1.2 Clinical Trials & Limitations

Meta-analysis of RCTs has not established the association between vitamin D and glycaemia control in T2DM patients however some have reported that vitamin D might benefit people with impaired glucose as vitamin D slowed down the increase in fasting glucose (128).

A meta-analysis included 15 RCTs in the health population reported that vitamin D has no significant improvement in T2DM control when compared with placebo. T2DM control markers selected in these RCTs including fasting glucose, HbA1c and insulin resistance.

Although no association was found with vitamin D and T2DM control markers, this study showed there was a small effect on fasting glucose and insulin resistance among people with diabetes or impaired glucose levels (157).

Single RCTs usually reported null association. Two RCTs used large dosage 300,000 IU/day injections and observed inconsistent results, one study indicated that vitamin D_3 improved the insulin sensitivity by increasing the HOMA-IR and HbA1c (164), while the other failed to prove the association (179). Oral supplement intervention generally used lower dosage from 500 to 6,000 IU/day and was failed to report the association between vitamin D and glycaemic control in T2DM, however it was suggested a possible positive link between vitamin D and glycaemia (162), insulin secretions (163) or insulin

sensitivity (164). A recent-completed large RCT, Vitamin D and Type 2 Diabetes (D2D) Trial, compared the vitamin D supplemented at a dosage of 4,000 IU/day with a compatison of a placebo group. The study analysed data from more than 2,400 participants with prediabetes for over 2.5 years and also found a non-statistically significant reduction (12%) in the risk of diabetes.

It was noticed that some well-designed large studies are ongoing and may help to explore the association including D-HEALTH and VITAL (Vitamin D & Omega-3 Trial) projects. For example, D-HEALTH trial in Australia is the second-largest trial of high dosage vitamin D supplementation ever conducted. It is designed to compare 60,000 IU/month supplementation and with placebo followed up for five years, to explore the association between vitamin D and health outcomes including CVD, T2DM and cancer (168). The trial is due to finish in June 2020, with additional follow up planned through to 2024.

2.1.3 Possible Mechanism

Changes in serum vitamin 25(OH)D level can affect β-cell function, insulin secretion and sensitivity, insulin resistance and is related with inflammation. Current studies suggest several possible pathways that vitamin D may affect the glucose level and T2DM.

Vitamin D can act directly on islet β cells and regulate insulin synthesis and secretion. It stimulates insulin secretion by increasing the intracellular calcium concentration via voltage-gated calcium channels or may mediate activation of β cells calcium-dependent endopeptidases to assist conversion of proinsulin to insulin. Vitamin D may also improve insulin function directly through stimulated expression of the insulin receptors and regulation of insulin-mediated intracellular processes via calcium regulation (180).

Vitamin D affects insulin sensitivity mainly mediated via increasing vitamin D_3 receptor (VDR) numbers, stimulating metabolism of fatty acid in skeleton muscle and fatty tissues and inhibiting the expression of inflammatory factor (181).

Another study found that 25(OH)D can be combined with VDR on β cells, increasing insulin inhibiting inflammatory factors, alleviating a chronic inflammatory process of the pancreas to improve the function of islet β cells (182). Furthermore the substance not only works through VDRs, 25(OH)D can also combine with the vitamin D-dependant calcium-binding protein (calbindin-D28k). The expression of calbindin-D28K has been shown to protect beta-cells from cytokine-mediated cell death (183), thereby reducing the risks of T2DM. Based on the above information and evidence, we have conducted a review specifically assessing RCTs.

2.2 Methodology of Literature Review

We performed a search in PUBMED from its inception up to September 2017. The main aim was to identify eligible RCTs that assessed the efficacy of vitamin D supplementation on glycaemic control in T2DM patients.

2.2.1 Inclusion Criteria

Included were all RCTs on human subjects who had been diagnosed with T2DM. Studies had to be published in English as a full-text original article. There was no restriction on vitamin D intervention type: studies using supplement interventions such as vitamin D_2 (ergocalciferol) or D_3 (cholecalciferol), orally or through injection, or by lifestyle change such as sun exposure or dietary resources were all included. In addition, eligible studies had to report at least one of the following outcomes of glycaemia control: fasting plasma

glucose (FPG); post-prandial glucose (PPG); haemoglobin A1c (HbA1c), insulin sensitivity (Quantitative Insulin Sensitivity Check Index, QUICKI), insulin secretion (homeostasis model assessment, HOMA-IR or beta cell function, HOMA-B). If there were more than one publication from the same research group that included similar or the same interventions, we included only the study with the longer intervention period.

2.2.2 Exclusion Criteria

Excluded were studies with a short trial period, that is less than or equal to one month. Research designs other than RCTs such as reviews, cross-section studies and retrospective studies were also excluded. Studies involving children, pregnant women and/or participants with pre-diabetes, type 1 diabetes and gestational diabetes and/or participants with conditions such as chronic renal diseases and hyperparathyroidism were also excluded.

2.2.3 Data Search and Extraction

'Vitamin D', 'diabetes', 'glycaemic control' and related terms were used in the search. Vitamin D related terms including 'vitamin D' or 'vitamin D_3' or 'cholecalciferol' or 'vitamin D_2' or 'ergocalciferol', and diabetes-related terms including 'diabetes' or 'type 2 diabetes' or 'T2DM' or 'hyperglycaemia' or 'hyperglycaemia'. 'Human research' and 'clinical trials' were added to the search criteria. Thirty-eight articles were initially retrieved and then following an examination of the articles' title and abstract based on the selection criteria 16 eligible articles remained.

The full text of these articles were retrieved. Study characteristics such as location of study, study type, age of participants, intervention dosing, sample size, intervention

pathway, study duration and targeted biomarker [FPG, PPG, HbA1c, insulin residence (HOMA-IR), beta cell function (HOMA-B), delta C-peptide (DCP), Quantitative insulin sensitivity check index (QUICKI)], positive biomarker which had a significant increase or decrease, and the general conclusion were extracted from the eligible studies and listed in a table.

2.2.4 Quality Assessment

The Jadad scale and the Van Trudle scale were used to assess the quality of the eligible RCTs. The Jadad scale has five questions related to randomisation, blinding and related research design. The Van Trudle scale was designed to make assessments of RCTs on 11 components including randomization, allocation concealment, baseline characteristics, patient blinding, caregiver blinding, observer blinding, co-intervention, compliance, dropout rate, end-point assessment time point, and intention-to-treat analysis.

For both scales, the reviewer marked 'Y' (YES) and 'N' (NO) or 'NK' (NOT KNOWN) to answer each question. If the answer was 'Y', a score of 1 was given; 'N' or 'NK' received zero. The total score presents the quality of research. A total score >3 for the Jadad scale or >5 for the Van Tulder scale indicates a high-quality study.

Sixteen RCTs were identified based on the inclusion and exclusion criteria. Among them, fifteen studies were randomised placebo-control studies, thirteen (159, 161-164, 167, 179, 184-189) were double-blind design, two (190, 191) were single-blind design and one (192) was unclear. All RCTs had a small sample size (8-45 each group), with subjects aged between 48.5±8 years to 66.9±3.1 years. Vitamin D_3 (cholecalciferol) was used as an oral vitamin D supplement in most of the studies (12/16), two used vitamin D_3 via injection,

two used vitamin D fortified yoghurt, and there were no studies that used vitamin D_2 (ergocalciferol) or a simple lifestyle intervention such as dietary resources or sun exposure.

2.3 Results

2.3.1 Characteristics of Studies

Sixteen RCTs were identified based on the selection criteria. Four studies concluded that vitamin D had improved insulin sensitivity and glycaemia status among T2DM patients while eleven studies reported no improvements. One study suggested a T2DM benefit of a conjoint calcium and vitamin D supplementation.

All studies were randomised placebo-control studies, with thirteen being double-blind design, two being a single blind design (190, 191) and one unclear (192) . All RCTs had a small sample size ranging from 8 to 45 per group, with subjects aged between 49.6±6.1 years to 66.9±3.1 years. Vitamin D_3 (cholecalciferol) was used as an oral vitamin D supplement in most of the studies, two used vitamin D_3 via injection (159, 164) and two used vitamin D fortified yoghurt (187, 190). The results are shown in Table 2.1.

Table 2.1

Characteristics and General Conclusion of Selected RCTs

Author, year, country	Research design	Age (year)	Intervention and sample size	Duration	Related target biomarker	Positive biomarker	General conclusion
Shab-Bidar, Neyestani & Diazavery. 2015, IRAN (190)	Single blind, randomized, placebo-controlled trial	Mean age of intervention group: 54.1 years (SD 8.0) vs. placebo group 51.3 years (SD 7.7)	Fortified yoghurt contains 500IU vitamin D3/250 ml x 2/d (N=31) vs. plain yoghurt contains no vitamin D/250 ml x 2/d (N= 29), oral	3 months	Fasting serum glucose, HbA1c and QUICKI	Significant change in HbA1c (-0.71, P<0.001), QUICKI (-0.009, P<0.001)	QUICK improved, HbA1c decreased but no significant changes between groups, 500IU vitamin D not enough to improve insulin resistance
Sadiya et al. 2015, UAE (185)	A randomized controlled double-blinded clinical trial	Intervention: 49±8 years vs. placebo: 48.5±8 years	6000 IU vitamin D3 /d followed by 3000 IU vitamin D3 /d in phase 2 (N=45) vs. placebo group (n = 42) received matching placebo, oral	6 months with 2 phases	Fasting blood glucose, HbA1c, C-peptide	n/a	No improvement in glycemic control including fasting blood glucose, HbA1c and C-peptide.
Elkassaby et al. 2014, AUSTRALIA (162)	Randomized, double blind placebo-controlled clinical trial	54 (48-58)	Vitamin D3 10,000 IU/d x 2wks then 6,000 IU/d x 6 months (n=26) vs. placebo (N=24), oral	6 months	Delta C-peptide (DCP), FPG; PPG, HbA1c and HOMA-IR	FPG lower at 3 months (D –0.40 vs placebo +0.1mmol/L, p=0.006); PPG lower at 3 months (D –0.3 vs placebo +0.8mmol/L, p=0.005)	Vitamin D has a transient improvement in glycaemia, but little to no therapeutic benefit in T2DM
Ryu et al. 2014, KOREA (188)	Prospective, randomized, double blind, placebo-controlled trial	Intervention: 56.7±7.9;placebo: 54.5±7.4	Vitamin D3 2,000 IU/ + calcium 200 mg/d (N=30) vs. placebo (calcium 200 mg/day) (N=32), oral	6 months	Fasting glucose, HbA1c, insulin, HOMA-IR and high-sensitivity C-reactive protein [hsCRP]	n/a	No improvement in HbA1c, HOMA-IR or glycemic control
Kampmann et al. 2014, DANMARK (163)	Double blind, randomized, placebo controlled trial	Intervention: 61.6 ± 4.4;placebo: 54.7±4.5	Vitamin D3 11,200 IU/d x 2wks then 5,600 IU/d x10wks (N=8) vs. placebo (N=8), oral	3 months	IVGTT, hyperinsulinemic euglycemic clamp, assessment of baseline high-frequency insulin pulsatility, glucose-entrained insulin pulsatility, DXA scans and fasting blood samples	C-peptide boarderline increased at 12 weeks (D 245±111vs placebo 112±98 pmol/L, p=0.07)	No improvement in insulin resistance or HbA1c, might increase insulin secretion in T2DM patients.
Al-Sofiani et al. 2015, SAUDI ARABIA/USA (184)	A double blind, randomized clinical trial	Intervention: 54.8±9.16 years vs. placebo group: 55±11.99 years	Vitamin D3 5,000IU/d (N=10) vs. placebo (N=10), oral	3 months	HbA1C, FBG, insulin, HOMA-IR and HOMA-%B	HOMA-%B increased significantly by 35.9% [-3.11, 42.67] (P = 0.03) and insulin increased by 1.82 [-0.78, 7.61] μU/mL (P = 0.11) compared with baseline	Vitamin D repletion improved β-cell activity in vitamin D – deficient T2DM with no significant changes in HbA1c or insulin sensitivity.
Tabesh et al. 2014,, IRAN (186)	Parallel designed, double blind, randomized placebo-controlled clinical trial	group 1: 50.2±6.6; group 2: 53.7±5.7; group 3: 49.8± 6.1; group 4: 51± 6.1;	Group 1: 50,000 IU/wk vitamin D3 + calcium placebo (N=29), oral; Group 2: 1,000 mg/d calcium + vitamin D placebo (N=29), oral; Group 3: 50,000 U/wk vitamin D + 1,000 mg/d calcium (N=30), oral; Group 4: vitamin D placebo + calcium placebo (N=30), oral	2 months	FPG, HbA1c, HbA1c, Insulin, HOMA-IR, QUICKI and HOMA-B	A significant decrease in serum insulin (–14.8±3.9 pmol/L, p=0.01) and HbA1c (–0.70±0.19%, p=0.02), significant increase in QUICKI (0.025±0.01, p=0.004) and HOMA-B (11.8±12.17, p=0.001) in calcium & vitamin D group	Joint calcium and vitamin D supplementation might improve the glycemic status of vitamin D insufficient people with T2DM.
Jehle et al. 2014, SWITZERLAND (164)	Prospective, randomized, double-blind, placebo-controlled pilot study	Intervention: 66.9±3.1; placebo: 63.7±3.5	Vitamin D3 300,000 IU / (N=29) vs. placebo (N=26), injection	6 months	HbA1c and HOMA-IR	Reduced HbA1c (2.9±1.5% vs +6.9± 2.1%, p=0.041); reduced HOMA-IR (-12.8±5.6% vs +10± 5.4%, p = 0.032)	Vitamin D3 improved insulin sensitivity (HOMA-IR) and affected the course of HbA1c in patients with T2DM.
Yousefi et al. 2014, IRAN (167)	Randomized double-blind placebo-controlled clinical trial study	Intervention: 50.03; placebo: 49.90	Vitamin D 4000 IU /d (N=28) vs. placebo group (N=30), oral	2 months	HbA1c, insulin and HOMA-IR	A significant decrease in HbA1c (from 7.29 ± 0.22 % to 6.76 ± 0.18 %, P<0.001) and insulin concentration (from 8.24 ± 0.97 µIU/mL to 6.55 ± 0.28 µIU/mL, P=0.048)	Vitamin D supplementation has beneficial effects on glucose homeostasis and can increases insulin sensitivity in T2DM patients.

Nasri et al. 2014, IRAN (179)	Randomized, double-blind, placebo controlled clinical trial	55 (10.7)	Vitamin D3 50,000IU/wk (N=30) vs. placebo (N=30), oral	3 months	HbA1c	HbA1c in male diabetic patients in interventional group was less than that of control group (p=0.0068)	Weekly vitamin D supplementation had beneficial effect on glycemic parameters in male T2DM patients.
Heshmat et al. 2012, IRAN (159)	Double blind, randomized clinical trial	Intervention: 56.2±9.3; placebo: 56.2±11	300,000 IU of vitamin D3 (N=21) vs. placebo group (N=21), injection	3 months	Fasting blood sugar, HbA1c, HOMA and insulin	n/a	Injection of vitamin D3 did not improve on control of diabetes and its risks.
Nikooyeh et al. 2011, IRAN (187)	Double-blind, randomized clinical trial,	50.7±6.1	DY group: 500 IU vitamin D3 + 150 mg Cal /250 mL) twice/d (N=30), oral; DCY group: 500 IU vitamin D3 + 250 mg Cal/250 mL) twice/day (N=30), oral; PY group (no vitamin D + 150mg calcium / 250ml) twice/d (N=30), oral	3 months	FSG, HbA1c and HOMA-IR	Significant decrease in in serum glucose, insulin, HOMA-IR, and HbA1C:DCY & DY FSG –12.9±33.7 mg/dL (P=0.015) vs PY –9.6±46.9 mg/dL (P=0.035); HbA1c –0.4±1.2% vs –0.4±1.9% (P<0.001); HOMA-IR –0.6±1.4 (P=0.001) vs –0.6±3.2 (P< 0.001); an inverse correlation between changes in serum 25(OH)D and FSG (r=–0.208, P=0.049), FM (r= –0.219, P=0.038), and HOMA-IR (r=–0.219, P=0.005)	Fortified yoghurt contains vitamin D significantly improved the glycaemia status including FSG, HbA1c and HOMA-IR in T2DM patients.
Strobel et al. 2014, GERMANY (161)	Placebo-controlled, randomized, double-blind study	60(30-78)	Vitamin D3 1,904 IU/d (N=34) vs. placebo (N=43), oral	6 months	Fasting glucose, insulin, HOMA-index, delta C-peptide and HbA1c	Fasting insulin positively correlated with 25OHD after 6 months in both groups (verum: r=0.23, p= 0.492; placebo: r=0.41, p=0.013)	No significant effects of vitamin D in DM biomarkers
Soric, Renner & Smith 2012, USA (191)	Prospective randomized single-blind placebo-controlled study	Intervention: 53.8±9.2; placebo: 55.3±7.8	2000 IU/d vitamin D3 (N=19) vs. placebo vitamin C (N=12), oral	3 months	HbA1c	Significant reduce in HbA1c -1.4 (-2.4 to -0.4) vs 0.2% (-0.2 to 0.6%) in baseline HbA1c >9.0% group (p=0.013)	Non-significant changes of HbA1c between groups, significant HbA1c reduction found in subgroup (baseline HbA1c >9.0%)
Patel, Poretsky & Liao 2010, USA (192)	Pilot prospective randomized trial, Nil control group	58.4±2.5	Vitamin D3 400 IU/d (N=13) vs. vitamin D3 1,200 IU/d (N=11), oral	4 months	Fasting plasma glucose, HbA1c and QUICKI	N/a	No improvement in the glycaemia and insulin sensitivity
Witham et al. 2010, UK (189)	Randomized, double-blind, parallel group, placebo-controlled trial	Placebo 66.7(9.7); 100,000 IU 65.3 (11.1); 200,000 IU 53.3 (9.3)	Group 1:Vitamin D3 100,000 IU (N=19), oral; Group 2: vitamin D3 200,000 IU (N=18), oral; Group: placebo (N=21), oral	4 months	HOMA-IR, glucose and HbA1c	N/a	No improvement in insulin resistance or HbA1c

2.3.2 Main Results

Eligible studies were generally long-term studies with duration ≥ 3 months, with only two considered a short-term study (167, 186). Among them, four studies (164, 167, 179, 187) showed a positive change in glycaemia control, specifically in insulin sensitivity (HOMA-IR) (164), glycaemia status including serum insulin, FPG, HbA1c and HOMA (167, 179, 187). Tabesh et al. (186) used calcium and/or vitamin D as oral supplements and reported a positive result from conjoint supplementation of calcium and vitamin D in improving glycaemia status among T2DM patients. Nikooyeh et al. (187) chose vitamin D and/or calcium fortified yoghurt as a supplementary intervention and Jehle et al. (164) used 300,000 IU vitamin D via injection: Authors of both studies concluded that vitamin D improved the glycaemic status and insulin sensitivity of T2DM patients.

Of the remaining eleven studies, none showed a significant improvement for glycaemia control (159, 161-163, 184, 185, 188-192).

Five of the included studies, however, showed a temporary improvement or suggested an association between vitamin D and glycaemic control. Kampmann et al. (163) suggested a possible link between an adequate vitamin D level and insulin secretion, and it was noted that it was the only research study that used a hyperinsulinemic euglycaemic clamp to quantify beta cell response (163). Al-Sofiani et al. (184) suggested that vitamin D improved beta-cell activity in the vitamin D-deficient T2DM group with no significant changes in HbA1c or insulin sensitivity. Another study showed a temporary improvement on FPG and PPG at the 3-month period but failed to show a difference again at the 6-month period (162). A significant reduction in HbA1C was shown but only among patients who had a baseline of HbA1c > 9.0 % (191), and a positive link of 25(OH)D and

fasting insulin was reported by Strobel et al. (161).

A 300,000 IU vitamin D injection was the highest dosing of vitamin D_3 intervention among the 16 RCTs, and it indicated that vitamin D_3 improved the insulin sensitivity by increasing the HOMA-IR and HbA1c (164, 179). However, another study with large dosing (up to 20,000 IU, Oral) found no improvement in glycaemic control (189).

2.3.3 Quality Assessment

According to the JADAD score, eleven RCTs were assessed as high quality with Jaded score ≥ 3. Details are shown in Table 2.2. Thirteen RCTs were considered as high-quality studies based on the Van Tulder scale with a total score >5; the remaining three studies all scored 5/11. Details are shown in Table 2.3.

Table 2.2
Quality Assessment of RCTs as assessed by JADAD Scale

Author, Year, Country	Was the study described as random?	Was the randomization scheme described and appropriate?	Was the study described as double blind?	Was the method of double blinding appropriate?	Was there a description of dropouts and withdrawals?	JADAD SCORE
Shab-Bidar, Neyestani & Djazayery 2015, IRAN (190)	Y	Y	N	N	N	2
Sadiya et al. 2015, UAE (185)	Y	Y	Y	Y	Y	5
Al-Sofiiani et al. 2015, SAUDI ARABIA / USA (184)	Y	Y	Y	N	Y	4
Yousefi et al. 2014, IRAN (167)	Y	Y	Y	N	N	3
Elkassaby et al. 2014 AUSTRALIA (162)	Y	Y	Y	Y	Y	5
Nasri et al. 2014, IRAN (179)	Y	Y	Y	N	N	3
Jehle et al. 2014, SWITZERLAND (164)	Y	Y	Y	Y	N	4
Kampmann et al. 2014, DANMARK (163)	Y	Y	Y	Y	Y	5
Strobel et al. 2014, GERMANY (161)	Y	N	Y	Y	N	2
Tabesh et al. 2014, IRAN (186)	Y	Y	Y	Y	Y	5
Ryu et al. 2014, KOREA (188)	Y	Y	Y	Y	Y	5
Soric, Renner & Smith 2012, USA (191)	Y	N	N	N	N	1
Heshmat et al. 2012, IRAN (159)	Y	N	Y	N	N	2
Nikooyeh et al. 2011, IRAN (187)	Y	N	Y	N	Y	3
Patel, Poretsky & Liao 2010, USA (192)	Y	N	N	N	Y	2
Witham et al. 2010 UK (189)	Y	Y	Y	Y	Y	5

*Y=yes (1 score); N=No (0 score);
**Total score > 3 indicate a high-quality RCT.

Table 2.3
Quality Assessment of RCTs as assessed by VAN TULDER Scale

Author, year, country	Randomization adequate	Concealed treatment allocation	Similarity between groups at baseline	Patient blinding	Care giver blinding	Observer blinding	Outcome assessment time similar	Co-intervention avoided/ similar	Compliance acceptable in all group	Drop out description & acceptable	Include 'intension to treat' analysis	Total score
Sadiya et al. 2015, UAE (185)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	**10/11**
Shab-Bidar, Neyestani & Djazayery 2015, IRAN (190)	N	N	N	Y	N	NK	Y	Y	Y	Y / nil	Y	**6/11**
Elkassaby et al. 2014 AUSTRALIA (162)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	**11/11**
Ryu et al. 2014, KOREA (188)	Y	Y	Y	Y	NK	NK	Y	Y	Y	Y	N	**8/11**
Kampmann et al. 2014, DANMARK (163)	Y	N	N	Y	Y	Y	Y	Y	Y	Y	N	**9/11**
Al-Sofiiani et al. 2015, SAUDI ARABIA / USA (184)	Y	N	N	Y	Y	NK	Y	Y	Y	Y	N	**7/11**
Tabesh et al. 2014, IRAN (186)	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	**9/11**
Jehle et al. 2014, SWITZERLAND (164)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	**11/11**
Yousefi et al. 2014, IRAN (167)	Y	N	N	Y	NK	NK	Y	Y	Y	N	Y	**6/11**
Nasri et al. 2014, IRAN (179)	Y	N	Y	Y	NK	NK	Y	Y	Y	N	Y	**7/11**
Heshmat et al. 2012, IRAN (159)	NK	NK	Y	Y	NK	NK	Y	Y	Y	N	Y	**6/11**
Nikooyeh et al. 2011, IRAN (187)	NK	N	N	Y	NK	NK	Y	Y	Y	N	Y	**5/11**
Strobel et al. 2014, GERMANY (161)	N	N	Y	Y	NK	NK	Y	Y	N	Y	N	**5/11**
Soric, Renner & Smith 2012, USA (191)	N	Y	Y	Y	N	N	Y	Y	Y	N	Y	**7/11**
Patel, Poretsky & Liao 2010, USA (192)	N	N	Y	Y	NK	NK	Y	Y	NK	N	N	**5/11**
Witham et al. 2010, UK (189)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	**10/11**

*Y=yes (1 score); N=No (0 score); NK=Not know (0 score);
**Total score > 5 indicate a high-quality RCT.

2.4 Discussion

The current review analysed selected RCTs based on 'evidence based' criteria. However, several features may have caused inconsistent findings associated with the reviewed RCTs. These features include joint intervention of calcium and vitamin D, different dosing, vitamin D from sun exposure and diet sources, small sample size, baseline similarity, methodology and research design.

2.4.1 Confounding Effects of Vitamin D & Calcium

There were conflicting results among the four studies that used vitamin D and calcium as an intervention. A Korean study that used cholecalciferol and calcium as the intervention found no improvements on HOMA-IR, HbA1c or glycaemia control (188). However, Tabesh et al. (186) observed a significant decrease in serum insulin, HbA1c and a significant increase in QUICKI and HOMA-B in calcium + vitamin D group [calcium (1,000mg) and vitamin D (50,000 IU)]. However, no significant change was found between the vitamin D and the placebo group.

Using vitamin D fortified yoghurt as an intervention, Shab-Bidar, Neyestani & Djazayery (190) observed a significant change in HbA1c and QUICKI, but no significant change between groups. Similarly, Nikooyeh et al. (187) found a significant decrease in serum Fasting Serum Glucose (FSG), HbA1c and HOMA-IR in the vitamin D and/or vitamin D + calcium groups. They also observed an inverse correlation between serum 25(OH) D and FSG and concluded that vitamin D improved the glycaemic control in T2DM patients. It was noted that these two studies had a minimum vitamin D intake (500 IU/day), and the quality of these two studies was generally lower (scored 2 and 3 respectively, Jaded) than the previous two (scored 5, Jaded).

A study using calcium supplementation with 1,500mg/day for 8 weeks showed calcium had no effect on an insulin parameter (193). However, other studies showed a positive effect of vitamin D on beta–cell function and glucose tolerance which may have been due to the correction of calcium level and secondary hyperparathyroidism (194). Therefore, with calcium as the co-intervention, calcium's effects on glycaemia should be considered when discussing the link between vitamin D and glycaemic control.

2.4.2 Vitamin D Dosage

Vitamin D of different dosing showed inconsistent results. The largest dose used among the 16 RCTs was the 300,000 IU vitamin D injection which occurred in two studies. One reported that vitamin D_3 improved the insulin sensitivity by increasing the HOMA-IR and HbA1c (164), while the other failed to prove the association (179). Another study using oral vitamin D supplements of 100,000 IU and 200,000 IU found no improvement in glycaemic control (189). Most of the studies selected 500 to 6,000 IU vitamin D as the intervention dosing, the majority of which reported a negative link between vitamin D and glycaemic control in T2DM patients, but suggested a possible positive link between vitamin D and glycaemia (162), insulin secretions (163) or insulin sensitivity (164).

2.4.3 Vitamin D Sources

The most common source of vitamin D in daily life is sun exposure, however there were no RCTs using sun exposure as an intervention. A current systematic review concluded that there is a significant gap between sun exposure and glycaemic outcomes (195). It also indicated that clinical trials using vitamin D supplementation may fail to capture the additional benefits of sun exposure. A recent study (196) showed a moderate level of evidence that sun exposure may prevent the development of T2DM. Hence more studies

and RCTs are needed to explore the link between vitamin D via sun exposure and its impact on glycaemic control.

The National Health and Medical Research Council (NHMRC) suggested that sunlight is still the main approach for obtaining sufficient vitamin D and that dietary resources can replenish the level to a limited extent. More recently a study has suggested that the current data may underestimate the level of vitamin D_3 in food since there is a discrepancy of vitamin D deficiency between calculation based dietary data (71%) and actual serum 25(OH)D test data (19%) (91). This study also suggested that if we take 25(OH)D in animal-based food into consideration, there will be 2-18 times higher level of vitamin D content, depending on the food type. Thus, more studies are encouraged to explore the impact of dietary vitamin D on insulin sensitivity and glycaemic control.

2.4.4 Limitations

Database bias is a limitation in the current review. Only one database (PUBMED) was used to conduct the article search and it is possible that some existing research studies may not have been included. Another criticism of this review is objective bias. Articles were selected and analysed by only one researcher thus there may have been subjective bias regarding article selection and analysis. A third limitation was the type of vitamin D intervention. Most of the studies used vitamin D_3 through oral supplements and there were two studies that used injection. There were limited data of vitamin D_2 or vitamin D_3 gained via sun exposure or dietary resources to compare the differences. The fourth limitation was related to data extraction. Data such as ethnicity, gender, baseline fat mass index, outdoor physical exercise time, calcium intake from diet or supplements, vitamin D intake from diet or supplements could all affect the level of vitamin D, however, these

features were not reported in all of the studies. The fifth limitation was the limited range and variety of biomarkers used. HbA1c and HOMA were commonly used in most of the studies. However, there was only one study that used a hyperinsulinemic euglycemic clamp technique; three studies used QUICKI. More studies which use these measurement techniques such as clamp and QUICKI are required for future analysis. Finally, this was a narrative review and we did not undertake a statistical analysis or meta-analysis of the data. Consequently, only assessment of the quality of the RCTs was used.

2.5 **Conclusion**

The current review was aimed to evaluate the link between vitamin D and glycaemia control within T2DM patients, specifically as evidenced by RCTs. It was concluded that currently RCTs do not support an association between vitamin D and glycaemia control in T2DM patients. More large, long-term and better-designed studies using heterogeneous vitamin D sources are required for future studies.

Chapter 3. **D4D Trial**

3.1 **Study Background**

The recent-published review (171) presented in **Chapter 2** assessed the association between vitamin D and glycaemic control among T2DM patients from sixteen eligible RCTs and concluded that the current RCTs did not support the association between vitamin D and T2DM. Considering the study limitations mentioned above, next-stage RCT is proposed with a larger scale, longer-term, and better design using heterogeneous vitamin D sources.

3.2 **Study Hypothesis and Significance**

Based on the current evidence, the D4D trial hypothesised that adequate serum vitamin D would assist with glycaemic control in T2DM patients.

It is hoped that the D4D trial could provide evidence-based recommendations to use a complementary strategy that effectively manages blood glucose in patients with T2DM and help to reduce the medical burden.

3.3 **Study Objectives**

Primary Objective

- The primary objective was to determine the role of vitamin D (through dietary intervention, sun exposure and vitamin D oral supplementation, compared to waitlist) in glycaemic control (as measured by HbA1c) among T2DM patients.

Secondary objectives

- To determine the prevalence of VDD among T2DM patients and,
- To determine the differences between heterogeneous vitamin D sources and their impact on glycaemic control.

Chapter 4. **Methods of D4D Trial**

4.1 **Ethical Consideration**

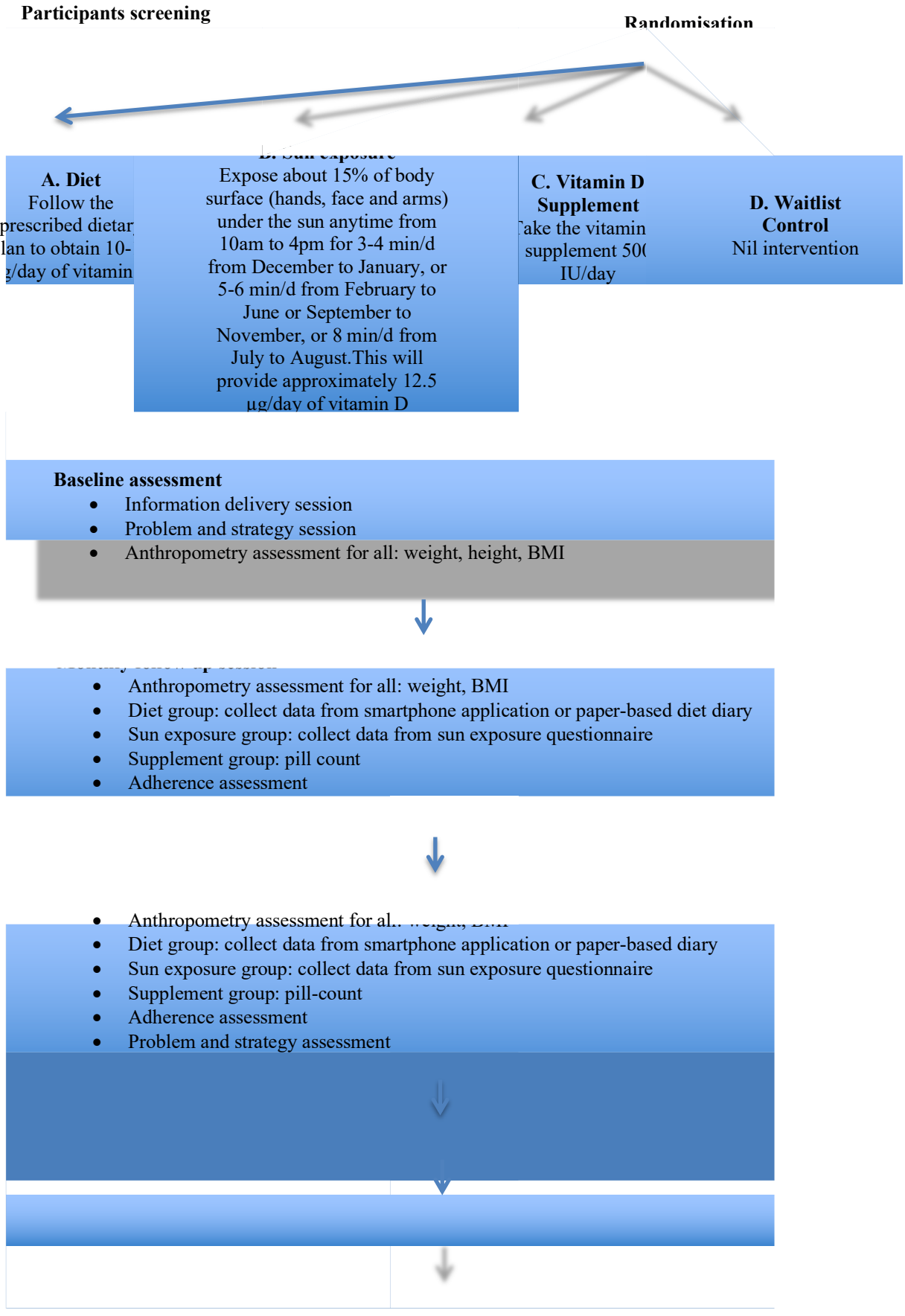
The D4D study was approved by the Human Research Ethics Committee of The University of Technology Sydney (ETH16-0640) and was registered in the Australian New Zealand Clinical Trials Registry (ANZCTR) (ACTRN12620000121965). The research followed the Declaration of Helsinki, and the written informed consent was obtained from all patients.

4.2 **Research Design**

The D4D trial was designed as a multi-centre, single-blind, randomized, four-arm, parallel-group, superiority study. It aimed to determine vitamin D's role and differences (obtained from the diet, sun exposure and oral supplement) in glycaemic control among T2DM patients.

4.2.1 Study Framework

Below is the framework of the D4D trial.


Participants screening
Randomisation
A. Diet
Follow the
prescribed dietar
plan to obtain 10-
g/day of vitamin
Expose about 15% of body surface (hands, face and arms) under the sun anytime from 10am to 4pm for 3-4 min/d from December to January, or 5-6 min/d from February to June or September to November, or 8 min/d from July to August.This will provide approximately 12.5 µg/day of vitamin D
C. Vitamin D Supplement
ake the vitamin
supplement 50(
IU/day
D. Waitlist Control
Nil intervention
Baseline assessment
Information delivery session
Problem and strategy session
Anthropometry assessment for all: weight, height, BMI
Anthropometry assessment for all: weight, BMI
Diet group: collect data from smartphone application or paper-based diet diary
Sun exposure group: collect data from sun exposure questionnaire
Supplement group: pill count
Adherence assessment
Diet group: collect data from smartphone application or paper-based diary
Sun exposure group: collect data from sun exposure questionnaire
Supplement group: pill-count
Adherence assessment
Problem and strategy assessment

4.2.2 Study Groups

Eligible participants were randomised in equal numbers into one of the four groups: Diet, Sun exposure, vitamin D Supplement and the Waitlist group.

4.2.3 Power Calculation & Number of Participants

A power analysis was performed using G*Power 3 (197). The F-test repeated measured ANOVA was chosen to test the within-between interaction (Figure 4.1). In order to detect changes with 95% power with an effective size of 0.25 and α err prob = 0.05, the sample size is required to be at least 60. With consideration of a 10-20% dropout rate, it is therefore required to recruit 66-72 participants.

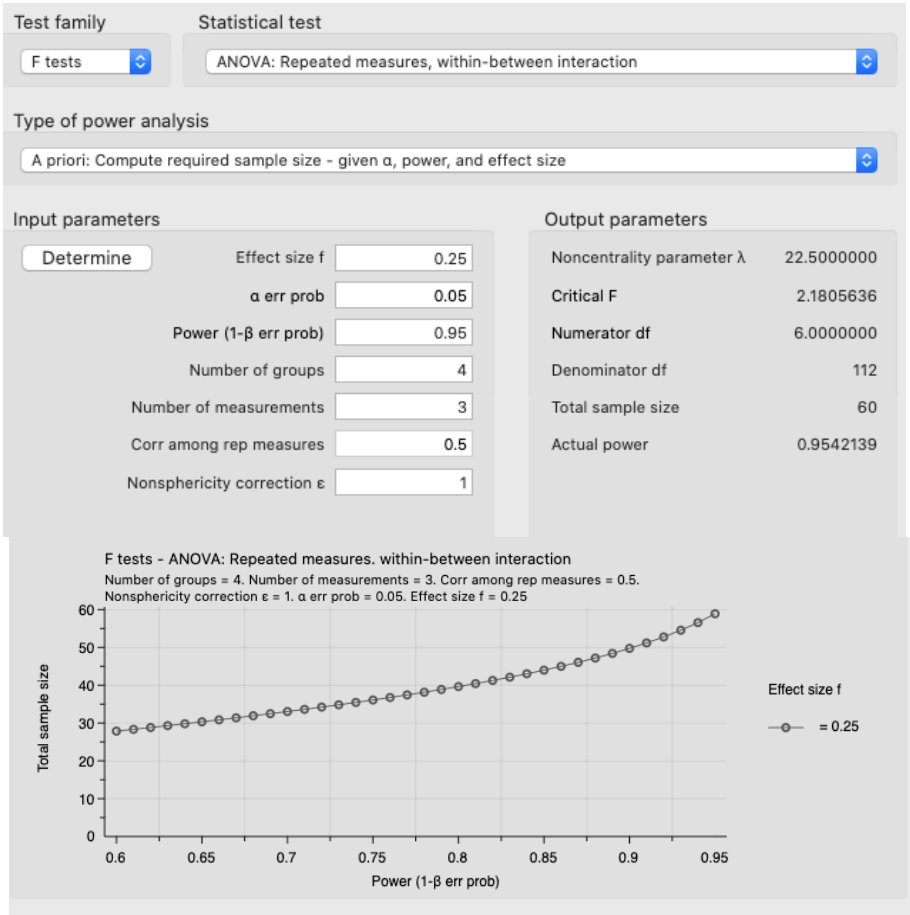


Figure 4.1
Power Calculation using G*Power 3, running with F-test measured ANOVA for within/ between interaction, 95% power with the effective size of 0.25 and α err prob = 0.05.

4.2.4 Duration and Location of the Study

The D4D research commenced in June 2019 and was completed in September 2020. Considering the limitations from pevious current RCTs and feasibility, the intervention duration of D4D trial was designed as nine months for each participant.

Participants were recruited from the Earlwood Medical Centre - a community medical centre located in south-central Sydney, and the Bangor Medical Centre - also a community medical centre located in south Sydney. All the following research visits, including assessment sessions and data collection, were performed at the Earlwood Medical Centre.

4.3 Selection Criteria

Participants were approached for recruitment if they meet the following inclusion criteria:

1) Aged ≥ 18 years old;
2) Diagnosed with T2DM in the preceding 12 months, and not using diabetic medication or insulin treatment;
3) Serum 25(OH)D between 25 nmol/L and 85 nmol/L and not using vitamin D supplements or any relevant medications, range was selected considering the unethical reason and study sensitivity (162);
4) Do not have thyroid diseases, liver impairment, renal failure, cancer, osteoporosis, dementia, mental diseases, or taking relevant medications;
5) Not pregnant or breastfeeding.

Participants were excluded from the trial if they were:

1) Unable to give written informed consent or follow the instructions;

2) Already on vitamin D supplementation or relevant medications;
3) Have liver impairment, renal failure (eGFR <50 ml/min), cancer, osteoporosis, dementia, mental disease, and thyroid diseases including hyperparathyroidism, hypercalcaemia, or taking any relevant medications;
4) Pregnant or breastfeeding women;
5) Servere allergy history, vegan, vegetarian, and cannot ingest any animal product.

4.4 **Recruitment and Enrolment**

Trial information was provided through the website (*www.d4dresearch.com*), flyers and local advertising. The trial was also introduced to General Practitioners (GPs) and allied health professionals for cross-referral recruitment. Participants who expressed an interest through email, phone calls and personal attendance at clinics were recruited. These include self-referred participants and participants who were referred by a GP or other health professionals. However, due to the COVID-19 impact, we had suggested participants contact us via phone call, email or mail only (from 25/03/2020).

Diabetic patient lists (name only) in both medical centres were used to contact for an EOI (Expression of Interest). Responses to inquiries about participation in the current research were answered via phone call during business hours and answered by voicemail at all other times. A research assistant responded to each inquiry immediately, using a screening instrument to identify eligible participants and provided the related instructions.

Once patients showed their interests in the trial, they were directed to read the information through the website or paper-based information package. Then they were required to sign the consent form and prepare to attend the baseline assessment if eligible. Due to COVID-

19, from 25/03/2020 all consents were obtained virtually via phone calls or online tools such as facetime, zoom and skype. The research staff then conducted the eligibility screening and consent form confirmation and finally enrolled the participants into the D4D trial.

4.5 **Randomisation and Allocation**

Randomisation was performed as a block of eight participants with an equal number allocation through MINUM, a computer program that randomises and stratifies participants equally into the four different study arms. The block sizes were not disclosed to ensure the concealment.

Allocation concealment was also ensured as the centre did not release the randomisation code until all participants were recruited into the trial. An independent investigator (not the research student) conducted the MINUM randomisation and sent the sequence to study therapists who are only responsible for implementing the interventions.

Participants were randomly assigned to one of four intervention groups (Diet, Sun exposure, Supplement, and Waitlist group) referring to the permuted block randomisation. The study therapists prepared the closed envelopes with printed randomisation numbers inside (available in both recruitment centres) and used them to provide participants with the treatment information at the baseline assessment session. The randomisation list was stored in the two centres for the period of study.

4.6 Data Collection

4.6.1 Pre-Training Session

The study therapists and GPs who met Good Clinical Practice (GCP) were required to attend a compulsory training session. The GCP training was provided by PRAXIS Australia before the study commencement, which aimed to facilitate their understanding of the trial and their roles in implementation.

4.6.2 Participant Sessions and Data Collection

Information Session

This session was to inform the participants of several important information:

- The importance of following study guidelines and adhering to study intervention daily;
- Instructions on how to apply the dietary plan and how to record dietary intake on a daily basis by the smartphone application - 'Easy Diet Diary' or by a paper-based food diary and what to do if missed a record; and a notification of a dietary diary checking at the monthly study visit;
- Instructions on taking supplements including dosage, storage and what to do in the event of a missed dose, and a notification of a pill count at the monthly study visit;
- Instructions on clothing, timing, weather condition for sun exposure and how to record daily sun exposure time on the paper-based diary and what to do if missed a record; and a notification of a sun exposure diary checking at monthly study visit;
- The importance of notifying the clinic if they experienced problems related to the interventions, such as side-effects, upset symptoms, missed pills, and loss of

dietary plan or diary books.

Anthropometry Assessment

Anthropometry assessment was scheduled during each visit at the Earlwood Medical Centre. It took approximately 10 minutes to complete the following measurements:

- Body weight (kg) using a digital scale (Tanita UM-051);
- Calculation of the BMI using equation: BMI = body weight (kg)/height $(m)^2$;
- Due to COVID-19, from 25/03/2020 this session was changed to a self-report version, data were remotely obtained via phone calls or virtual tools (i.e., facetime, zoom, skype).

Data Collection: Dietary data, Sun Exposure Time and Supplement Pill Count

Data collection occurred during each visit at the Earlwood Medical Centre. It took approximately 20 minutes to conduct the following procedures:

- **Dietary Data:** Used a 24-hour food recall and a vitamin D specific Food Frequency Questionnaire (FFQ-VDQ) at the baseline to collect information on energy, macronutrients (protein, fat and carbohydrate) and the vitamin D intake. The FFQ-VDQ was validated to assess the habitual vitamin D intake (198). Used the 3-day food diary (two weekdays and one weekend day per week, four copies per month) for each monthly follow-up visit. The diet diary could be completed through either a paper-based diary or the smartphone application 'Easy Diet Diary'. If participants failed to complete the diary, a 24-hour food recall and FFQ-VDQ was required to be used to collect the missing information. An Accredited Practicing Dietitian (APD) was appointed to design the dietary plan for participants and to provide training to study therapists who conducted

the nutritional information collection. The nutritional data was later processed through the software 'FOODWORK' (version 8 & 9) using NUTTAB database.

- **Sun Exposure Time:** Sun exposure time was evaluated by the Sun exposure questionnaire (adapted from 45 and Up Questionnaire) at the baseline. For monthly follow-up visits, the study therapists checked scores from the Sun exposure diary (7-day per dairy, four diaries per month) to assess the sun exposure status (199). If participants missed recording the information, they needed to compensate with extra entries.
- **Supplement Pill Count:** Participants returned the unused tablets and bottles at each monthly follow-up visit. Unused tablets were counted and recorded on the appropriate Case Report Form (CRF).
- Due to COVID-19, this session was changed to remote contact via phone calls or virtual tools (i.e., facetime, zoom, skype) from 25/03/2020. Study therapists contacted participants to collect self-reporting data, and to provide assistance via remote contact if required.

Trouble-shooting Session

The trouble-shooting session occurred during each visit at the Earlwood Medical Centre. It took approximately 5 minutes. The participants were asked about any problems regarding taking supplements or using the food diaries and phone application. There was a brief discussion of reasons for missed doses and simple strategies for enhancing adherence, e.g., linking supplements to meals or other daily activities for adherence, scheduling an outdoor time, efficiently recording the diet intake through the phone application. Due to COVID-19, this session was also changed to remote contact from 25/03/2020.

Adverse event assessment occurred during each visit. There was the possibility that in the dietary intervention group, some participants may have reported food intolerance or allergy to seafood or dairy products. If so, study therapists would record the incident. An Accredited Practising Dietitian (APD) was asked to provide dietary consultation and appropriate suggestions to minimise the risks. If the adverse effect continued, participants were excluded from the trial and provided with an allergy test and related treatment if necessary. Due to COVID-19, this session was changed to remote contact from 25/03/2020.

Blood Test

The blood test occurred at baseline, 3rd month and 9th month only at the Earlwood medical centre by an accredited pathology collection staff or registered nurses. The blood samples were sent to the National Association of Testing Authorities (NATA) accredited pathology laboratory to measure serum 25(OH)D and HbA1c.

Adherence Session (During the Trial)

A series of activities were implemented to accomplish the adherence:

- Maintaining the participants' interest in the study through mails and phone calls;
- Sending letters to participants before every monthly follow-up assessment, and calling on the previous day to remind the next day assessment;
- Providing timely assistance if participants had questions about the dietary menu or experienced any food intolerance and allergy;
- Providing participants with information about the current status of the study, plans for the next phase, as well as to acknowledge their cooperation;

Due to COVID-19, this session was changed to remote contact from 25/03/2020.

4.6.3 Early Termination

The vitamin D dosage administered in the D4D trial (500IU/day) was considered safe for daily usage and is the current recommendation level in Australia. Hence, it is unlikely to cause any side effects or symptoms. However, some participants may experience food intolerance or allergy by adding seafood or eggs into their daily meals even if they were cleared from the initial allergy history screening. Participants with any severe food intolerance or allergic reaction were excluded from the current study and provided with appropriate medical treatments, including an allergy test if necessary. The intervention plan of the D4D trial is shown in the table below (Table 4.1):

Table 4.1
Intervention Plan of the D4D Trial

List Interventions	Baseline assessment	3rd month visit	9th month visit	Other monthly visits
Training plans	Prior to baseline assessment	N/A	N/A	N/A
Information delivery session & Informed Consent	✓	N/A	N/A	N/A
Inclusion / Exclusion criteria	Prior to the initial visit	N/A	N/A	N/A
Anthropometry assessment	✓	✓	✓	✓
Dietary intake exam	✓	✓	✓	✓
Sun exposure exam	✓	✓	✓	✓
Pill count	N/A	✓	✓	✓
Adherence assessment with Problem & strategies session	✓	✓	✓	✓
Adverse Event Assessment	✓	✓	✓	✓
Blood test	✓	✓	✓	X

4.7 Blinding and Unblinding

The pathology technician conducting the blood collection was blind to the treatment allocation. Due to the nature of the intervention, neither participants nor study therapists were blind to the interventions but were informed not to disclose the allocation status and

participants' status in the follow-up assessments. An employee outside the research team put the data into the computer in separate datasheets; therefore, the research team could blindly analyse the data.

4.8 Confidentiality, Data Storage and Archiving

All reports, data, forms and documents were re-identified by a coded ID number to maintain participant confidentiality. We used the UTS data management system, 'STASH', and an external hard disk to store and manage the data of the D4D trial. All databases were securely encrypted, password protected and could only be accessed by the principal investigators. Participants' information was only be used for the purpose of this study project and were not be disclosed without the participants' permission, except if as required by law. The participant files, both hardcopy and digital format were planned to be maintained in storage indefinitely.

4.9 Statistical Analysis

Data were expressed as mean±SD. Initially, the Kolmogorov–Smirnov goodness-of-fit test was used to assess the normal distribution of the values. A two-way mixed-model repeated-measures Analysis of Variance (ANOVA) was used with factors of time and interventions. Tukey's post-hoc tests were applied to identify the differences between four groups at the 3^{rd} and 9^{th} months in the event of a significant interaction finding from the two-way ANOVA analysis. Confouding factors analysis of gender and racial impact and was used ANOVA followed by post-hoc Fisher's Least Significant Difference (LSD) tests. The correlations between the variables were evaluated by either a Pearson (r) (for data with normal distribution) or a Spearman (rs) (for data with no normal distribution) correlation.

A p-value <0.05 was considered to be statistically significant. All statistical analyses were performed using the Statistical Package for Social Science version 23 (SPSS version 9.0.0 (86), Chicago, IL, USA). Missing values were treated according to the Last Observation Carried Forward (LOCF) method and were assessed via sensitivity analysis.

Chapter 5. **Results**

5.1 **Study Flowchart**

Ninety-three participants were recruited out of one hundred thirty-four participants who were interested in the D4D study. A total of sixty participants eventually completed the nine-month intervention in the D4D trial. The progress of the D4D trial is shown in Figure 5.1.

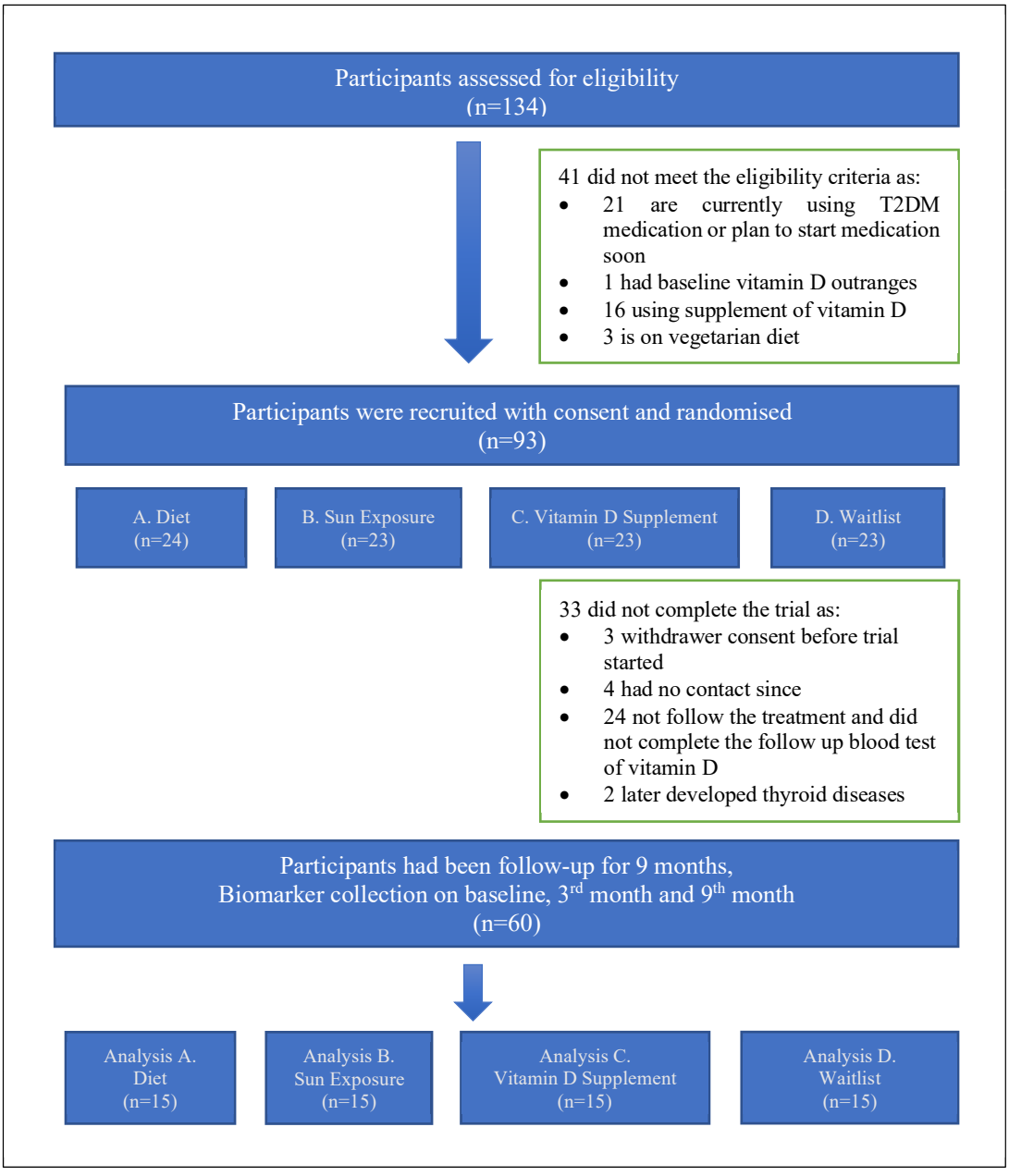


Figure 5.1 Study Flow of the D4D Trial.

5.2 Baseline Information: Demography and Biomarkers

There were no statistical differences for the baseline characteristics among the four groups. The baseline characteristics of all participants who had completed the study are shown in Table 5.1.

Table 5.1 Baseline Characteristics

Characteristics	Diet Group (n=15)	Sun Exposure Group (n=15)	Supplement Group (n=15)	Waitlist Group (n=15)	Total Participants (n=60)
Age, years	59.90±3.92	59.13±2.44	64.96±2.83	55.70±2.81	59.92 ±1.55
Weight, kg	72.03±6.67	69.37±3.24	67.01±2.62	76.41±4.17	71.21±2.23
Height, cm	163.53±2.08	160.87±1.48	163.13±2.43	167.63±2.21	163.79±1.06
BMI	26.48±1.89	26.91±5.44	25.16±2.87	27.10±4.92	26.41±0.68
Sex, No. (%)					
Male	8 (53.3%)	6 (40.0%)	7 (46.7%)	11 (73.3%)	31 (51.7%)
Female	7 (46.7%)	9 (60.0%)	8 (53.3%)	4 (26.7%)	29 (48.3%)
Race, No. (%)					
Caucasian	4 (26.7%)	2 (13.3%)	3 (20.0%)	4 (26.7%)	13 (21.7%)
Asian	11 (73.3%)	12 (80.0%)	10 (66.6%)	10 (66.6%)	43 (71.7%)
Black	0	0	1 (6.7%)	0	1 (1.7%)
Indian	0	1 (6.7%)	0	1 (6.7%)	2 (3.2%)
Hispanic	0	0	1 (6.7%)	0	1 (1.7%)
Smoking, No. (%)	2 (13.3%)	1 (6.7%)	2 (13.3%)	3 (20%)	8 (13.3%)

BMI, Body Mass Index.
Data presented as mean ± standard error of the mean (SEM) or percentage %.

Table 5.2 shows the baseline blood biomarkers that were collected from participants.

Table 5.2 Baseline Blood Biomarkers

Characteristics	Diet Group (n=15)	Sun Exposure Group (n=15)	Supplement Group (n=15)	Waitlist Group (n=15)	Total Participants (n=60)
25(OH)D, nmol/L	59.20±3.93	51.20±5.99	47.93±3.14	50.00±4.08	50.87±17.85
HbA1c, %	7.06±0.30	6.49±0.27	6.79±0.27	6.81±0.25	6.79±0.14
BGL, mmol/L	7.45±0.94	7.00±0.80	6.87±0.39	7.21±0.51	7.14±0.34
TC, mmol/L	4.99±0.24	4.96±0.38	5.18±0.25	5.09±0.26	5.05±0.14
TG, mmol/L	1.75±0.30	2.20±0.35	1.64±0.19	2.02±0.24	1.90±0.14
HDL, mmol/L	1.33±0.09	1.27±0.06	1.32±0.11	1.28±0.13	1.30±0.05
LDL, mmol/L	2.88±0.17	2.86±0.34	3.11±0.22	2.90±0.21	2.94±0.12

25(OH)D, 25-hydroxy-Vitamin D, HbA1c, Haemoglobin A1c, BGL, fasting blood glucose level, TC, Total Cholesterol, TG, Triglyceride, HDL, High-Density Lipoprotein, LDL, Low-Density Lipoprotein.
Data presented as mean ± SEM or percentage %.

5.3 Prevalence of Vitamin D Deficiency (VDD)

About 50% of participants (31 out of the total 60 participants) at the baseline experienced VDD with serum 25(OH)D level less than 50 nmol/L. VDD prevalence had decreased in Diet, Sun exposure and Supplement groups after nine-month intervention but remain the same in the Waitlist group (Figure 5.2).

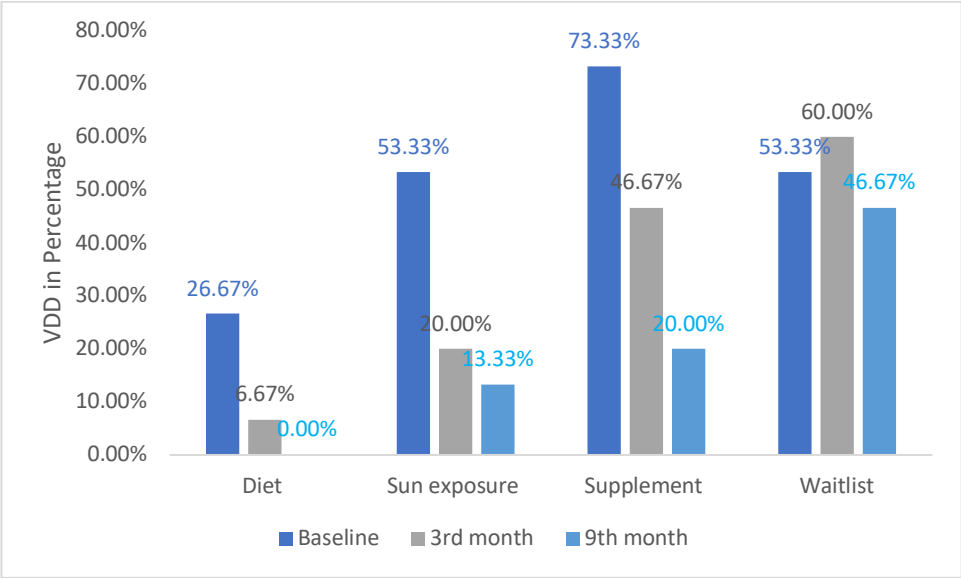


Figure 5.2 Vitamin D Deficiency (VDD) among Groups. Data are presented as percentage %.

Males presented a lower VDD prevalence at the baseline compared to females in all four groups (48.4% vs 55.2%). After the three-month intervention, VDD prevalence among males and females dropped to 35.5% and 31.0%, and continued to reduce to 19.4% in males and 20.7% in females after nine-month intervention.

Asian (n=43) and Caucasian (n=13) were the two major racial groups in our study. After excluding the limited cases of Indian (n=2), African (n=1), and Spanish (n=1), whom all presented with VDD at the baseline, the Asian group reported a higher VDD prevalence compared to the Caucasian group in general (51.2% vs 38.5%). The VDD prevalence

remained higher in the Asian group than the Caucasian group after interventions at the 3rd month (27.3% vs 22.2%) and 9th month (18.2% vs 0.0%).

5.4 Effects of time and interventions

Shown in Table 5.3, there was a significance in the time effect on both the serum 25(OH)D ($F(1.730, 96.89) = 12.68$, $p<0.0001$) and HbA1c ($F(1.671, 93.56) = 4.608$, $p=0.0172$). Intervention had a significant effect on serum 25(OH)D ($F(3, 56) = 3.556$, $p=0.0199$) but HbA1c level, and there was a significant effect from the interaction of time and intervention on HbA1c level ($F(6, 112) = 5.719$, $p<0.0001$).

Table 5.3 Effects of Time, Intervention and Time x Intervention on Serum 25(OH) and HbA1c Level

	Measure	p-value	F
Time	Serum 25(OH)D	<.0001**	12.68
	HbA1c	.0172*	4.608
Intervention	Serum 25(OH)D	.0199*	3.556
	HbA1c	.1341	2.150
Time x Intervention	Serum 25(OH)D	.2552	1.318
	HbA1c	<.0001**	5.719

25(OH)D, 25-hydroxy-Vitamin D, HbA1c, Haemoglobin A1c.
* P<0.05, ** P<0.01.

5.4.1 Changes in Serum 25(OH)D Level

Although the serum 25(OH)D level increased among all four groups, only the Diet group at the 9th month presented a significant increase in serum 25(OH)D level compared to the Waitlist group ($p=0.0068$), and the 25(OH)D level in the Diet group at 9th month was also significantly higher than Supplement group ($p=0.0392$) (Figure 5.3).

Within the group, serum 25(OH)D level in the Diet group was significantly increased at 9th month compared to its baseline ($p=0.0166$) and 3rd month ($p=0.0268$). Serum 25(OH)D level in the Sun exposure group was significantly increased at 3rd month

compared to its baseline (p=0.0361). In the Supplement group, serum 25(OH)D level at 9th month showed a statistical increase compared to its baseline (p=0.0319).

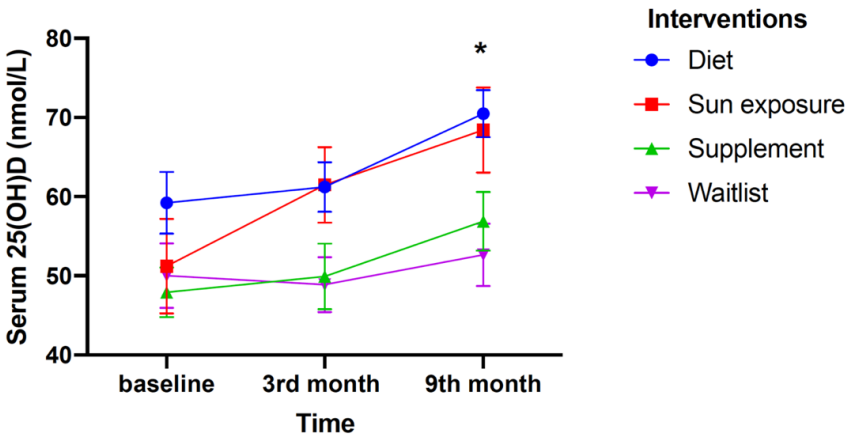


Figure 5.3 Serum 25(OH)D Level in Participants with Interventions of Diet, Sun exposure, Supplement and Waitlist at Baseline, 3rd month and 9th month. Data are presented as means ± SEM and analysed by two-way mixed ANOVA, * P=0.0068 Diet vs Waitlist group and, p=0.0392 Diet vs Supplement group at the same time point.

5.4.2 Changes in HbA1c Level

The HbA1c level was decreased in the Diet and Supplement group but was unchanged in the Sun exposure group and increased in the Waitlist group (Figure 5.4). Only the Diet group at 9th month showed a significant reduction in HbA1c level compared to the Waitlist group (p=0.0279).

Within groups, the HbA1c level in the Diet group showed a significant decrease at the 3rd month (p=0.0039) and 9th month (p=0.0299) than its baseline. HbA1c in the Supplement group at 3rd month also presented a significant decrease (p=0.0332) compared to its baseline. No change was found in the Sun exposure group, and the increase of HbA1c level in the Waitlist group was not statistically significant.

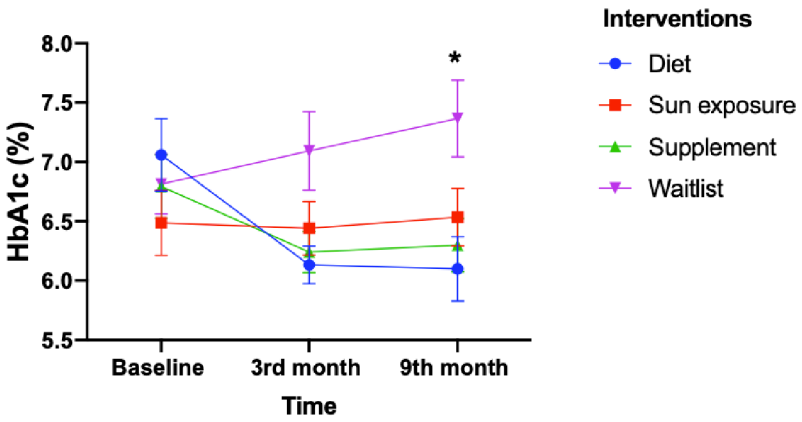


Figure 5.4 HbA1c Level in Participants with Interventions of Diet, Sun exposure, Supplement and Waitlist at Baseline, 3rd month and 9th month. Data are presented as mean ± SEM and analysed by two-way mixed ANOVA, * P=0.0279 Diet vs Waitlist group at the same time point.

5.5 Correlation between Serum 25(OH) D and HbA1c Level

A correlation analysis of serum 25(OH)D and HbA1c showed that during the 9-month intervention period, HbA1c was inversely associated with serum 25(OH)D in all four groups. However, the association was not statistically significant (Figure 5.5).

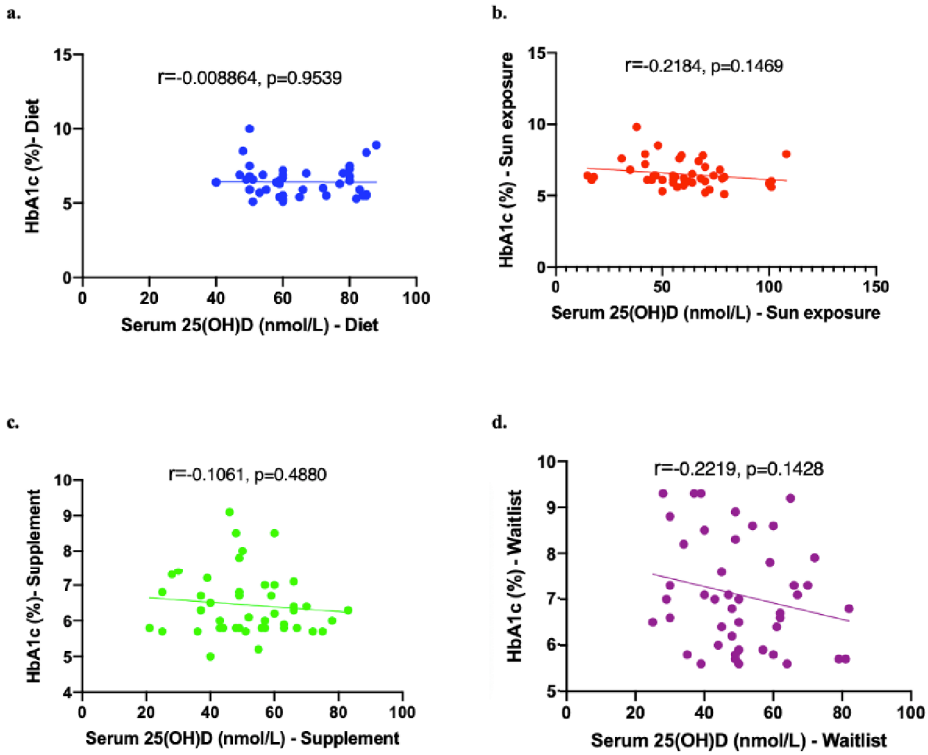


Figure 5.5 Association between Serum 25(OH)D and HbA1c in Four Experimental Groups. 25(OH)D, 25-hydroxy-Vitamin D, HbA1c, Haemoglobin A1c.

The correlation analysis of serum 25(OH)D and HbA1c divided by time and intervention also showed that the HbA1c level was inversely associated with serum 25(OH)D among all groups at baseline (Figure 5.6a) and 3rd month (Figure 5.6e). However, at the 9th month, the HbA1c level in the Sun exposure group and Waitlist group were weaker and HbA1c level in the Diet group (r=0.3845, p=0.1571) (Figure 5.6l) and Supplement group (r=0.09937, p=0.7246) were positively associated with serum 25(OH)D (Figure 5.6k).

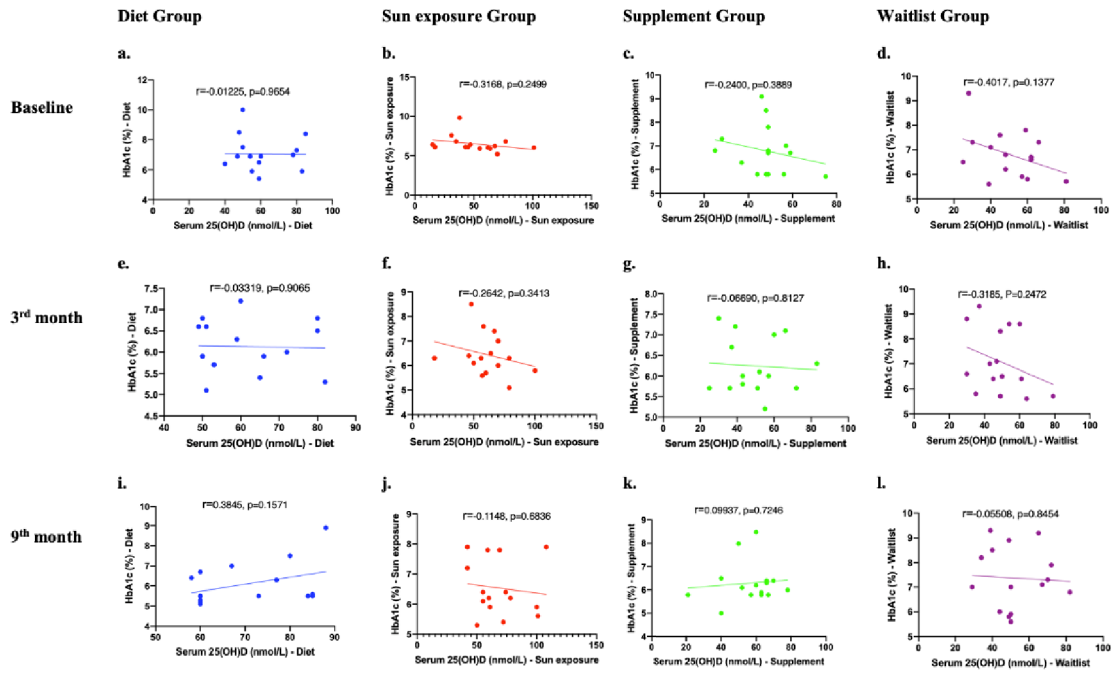


Figure 5.6 Correlation Analysis to assess the Association between Serum 25(OH)D and HbA1c with Interventions and Time Stages. HbA1c, Haemoglobin A1c, 25(OH)D, 25-hydroxy-Vitamin D.

5.6 **Lipids profile**

Changes in the blood lipids are shown in Table 5.4. As shown in Table 5.4, over the trial period, Total Cholesterol (TC), Triglyceride (TG), HDL and LDL levels were not significantly changed by the intervention or lack of intervention. There was a trend of reduction in TG levels by all three interventions, albeit without statistical significance (9th month, p=0.06 diet vs Waitlist).

Table 5.4
Lipids in Groups at Baseline, 3rd month and 9th month

	Time	Diet Group (n=15)	Sun exposure Group (n=15)	Supplement Group (n=15)	Waitlist Group (n=15)
Total Cholesterol	**Baseline**	4.99±0.24	4.96±0.38	5.18±0.25	5.09±0.26
	3rd month	5.08±0.21	4.35±0.30	4.90±0.29	5.32±0.28
	9th month	4.80±0.21	4.46±0.31	4.93±0.23	5.14±0.18
Triglyceride	**Baseline**	1.75±0.30	2.20±0.35	1.64±0.19	2.02±0.24
	3rd month	1.41±0.15	1.82±0.19	1.54±0.14	2.21±0.37
	9th month	1.42±0.14	1.78±0.24	1.53±0.14	2.08±0.34
HDL	**Baseline**	1.33±0.09	1.27±0.06	1.32±0.11	1.28±0.13
	3rd month	1.33±0.06	1.25±0.07	1.31±0.09	1.17±0.07
	9th month	1.27±0.07	1.27±0.08	1.34±0.11	1.25±0.07
LDL	**Baseline**	2.88±0.17	2.86±0.34	3.11±0.22	2.90±0.21
	3rd month	3.04±0.17	2.33±0.26	2.87±0.22	3.17±0.25
	9th month	2.86±0.18	2.49±0.28	2.88±0.16	3.12±0.20

HDL, High-Density lipoprotein, LDL, Low-Density lipoprotein.
The results are presented as mean ± SEM.

The correlation between lipids, serum 25(OH)D level and HbA1c level are shown in Figure 5.7. TC in the Diet group (r=-0.4142, p=0.005) and Waitlist group (r=-0.3087, p=0.0391), TG in the Supplement group (r=-0.4721, p=0.001), and LDL in the Diet group (r=-0.4492, p=0.002) were significantly correlated with serum 25(OH)D level. TG in the Diet group (r=0.3589, p=0.02) and the Waitlist group (r=0.40, p=0.004) were found significantly correlated with HbA1c level.

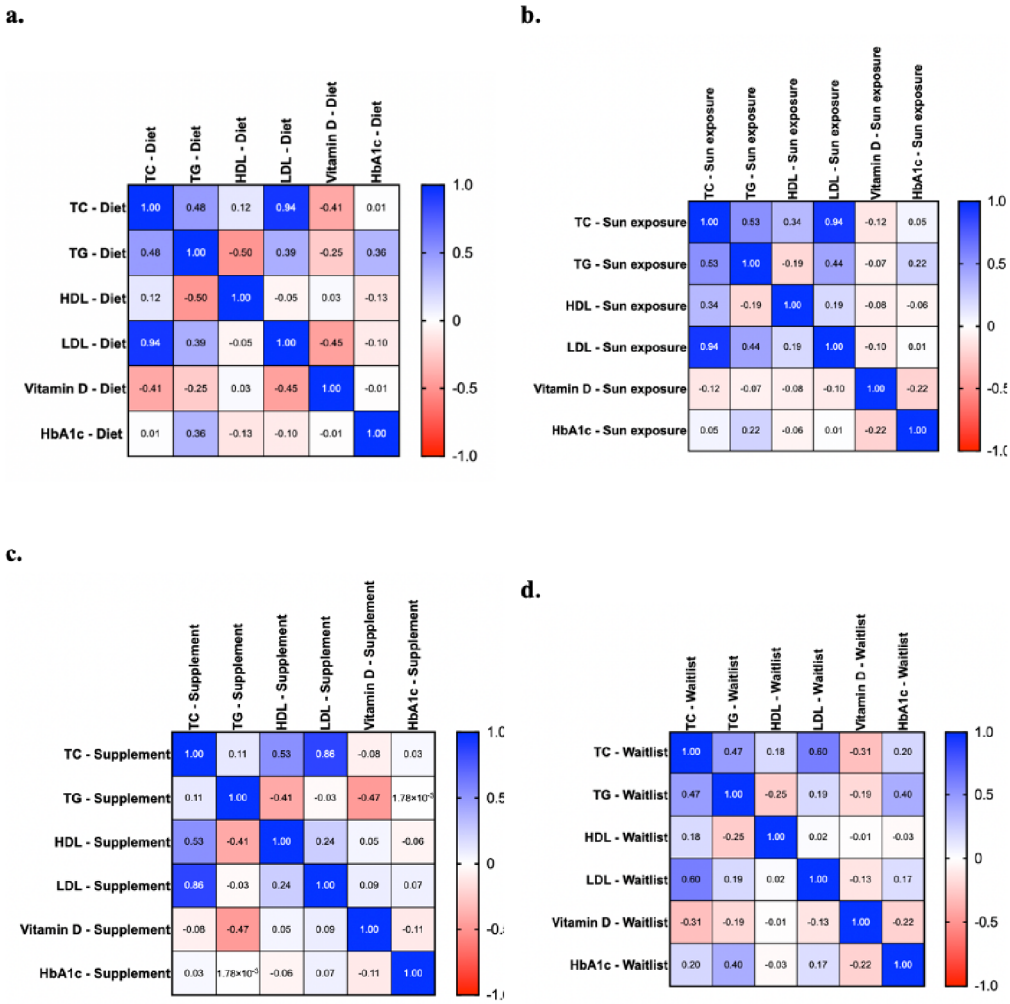


Figure 5.7 Correlation Matrix of Lipids, Serum 25(OH)D and HbA1c in Four Groups. TC, Total Cholesterol, TG, Triglyceride, HDL, High-density lipoprotein, LDL, Low-density lipoprotein, HbA1c, Haemoglobin A1c.

5.7 Confounding Factors

5.7.1 Age & BMI

Age at the baseline was not significantly associated with serum 25(OH)D (p=0.798) and HbA1c (p=0.948) levels.

As the Chinese group dominated the Asian cohort in this D4D trial, only the Chinese participants were compared with Caucasians. The Caucasian group presented a higher BMI and HbA1c level at the baseline than the Chinese group (Figure 5.8). However, the BMI did not significantly affect serum 25(OH)D (p=0.717) nor HbA1c (p=0.794) levels.

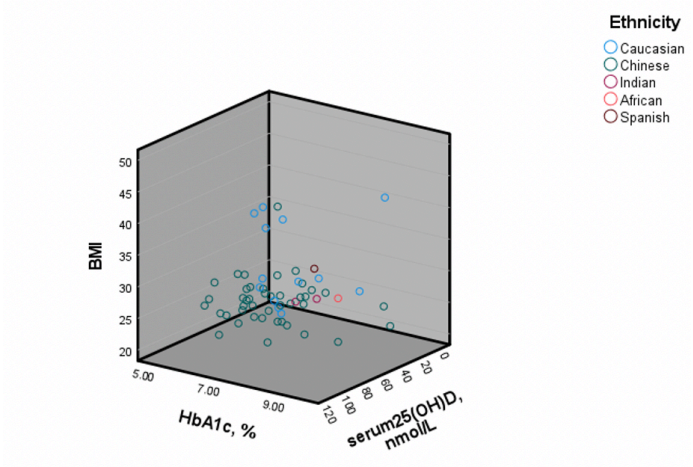


Figure 5.8 3D-Scartted Plot of Association between Body Mass Index and the Measures of Serum 25(OH)D and HbA1c according to Ethnicity. 25(OH)D, 25-hydroxy-Vitamin D; HbA1c, Haemoglobin A1c.

5.7.2 Body Weight

Only time showed a significant effect on body weight (p=0.009) (Figure 5.9a) and bodyweight change percentage (p=0.02) (Figure 5.9b). No significant effect was found from the interventions. Bodyweight percentage in the Diet group showed the largest reduction compared to other groups with 2.18% at the 3rd month and 2.81% at the 9th month, and followed by the Sun exposure group of 1.48% and Supplement group of 1.13% at the 9th month. However, the post hoc test failed to show any statistical significance.

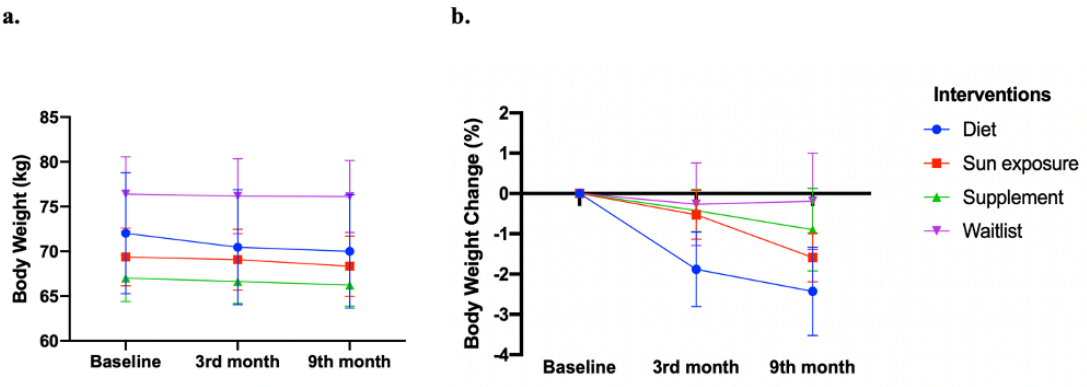


Figure 5.9 The Body Weight (a) and Body Weight Change in Percentage (b) at Baseline, 3rd month and 9th month. Data are analysed by two-way mixed ANOVA and presented as mean ± SEM, n=15 in each group.

5.7.3 Gender

5.7.3.1 Serum 25(OH)D Level

The result suggested no gender difference in serum 25(OH)D level in response to the treatment. However, a gender difference was observed in the HbA1c level in the Waitlist group (Figure 5.10). Serum 25(OH)D level of males in the Diet group was significantly increased at 9th month compared to baseline (p=0.0022) and 3rd month (p=0.0048), and was significantly higher than males in the Waitlist group (p=0.0155) and in the Supplement group (p=0.0381) (Figure 5.10a). Serum 25(OH)D level of males in the Supplement group was increased steadily and at 3rd month (p=0.0414) at the 9th month (P=0.0104) compared to its baseline (Figure 5.10c). In females, 25(OH)D level only in Sun exposure group at 9th month showed a significant improvement compared to the Waitlist group (p=0.0499) (Figure 5.10b).

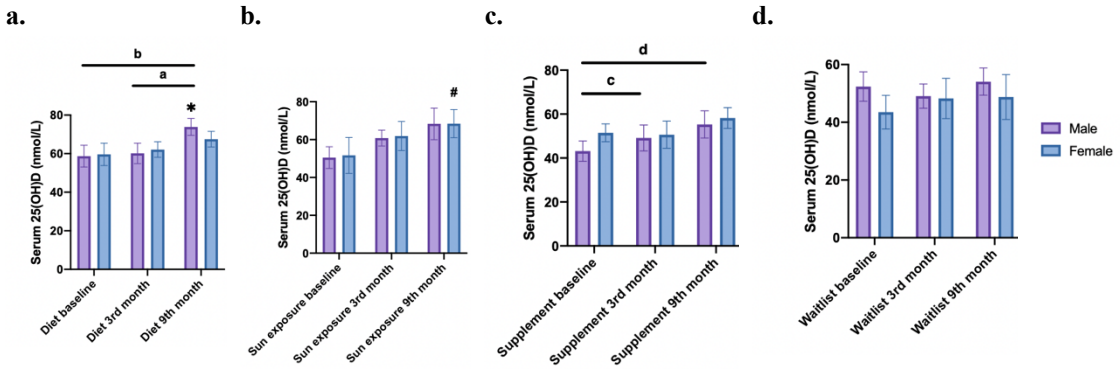


Figure 5.10 Serum 25(OH)D level of Male and Female in Diet group (a), Sun exposure group (b), Supplement group (c) and Waitlist group (d). Data are analysed by two-way mixed ANOVA and presented as mean ± SEM. In (a), a p= 0.0048 for males in Diet group 9th month vs 3rd month and b, p= 0.0022 males in the Diet group 9th month vs baseline, * p= 0.0155 males in the Diet group 9th month vs Waitlist and p= 0.0381 vs Supplement group at the same time point, in (b), # p= 0.0499 females in Sun exposure group 9th month vs Waitlist at the same time point, in (c), c p= 0.0414 males in the Supplement group 3rd month vs baseline, d p= 0.0104 males in the Supplement group 9th month vs baseline.

5.7.3.2 HbA1c level

Gender difference in the HbA1c level was only observed in the Waitlist group at 3rd month and 9th month (Figure 5.11).

In the Diet group (Figure 5.11a), the HbA1c level of females at 3rd month (P=0.0220) and 9th month (p=0.0310) was significantly reduced compared to its baseline, no other significant changes in females were observed. The level of males in the Diet group at 3rd month (p=0.0493) was significantly lower compared to its baseline and the Waitlist group (p=0.0135), the level at 9th month was also significantly lower compared to the Waitlist group (p=0.0196) (Figure 5.11a). HbA1c level of males in the Sun exposure group at the 9th month was reduced significantly compared to the Waitlist group (p=0.0292) (Figure 5.11b). In the Supplement group, HbA1c level of males at the 3rd month and 9th month was significantly lower compared to the Waitlist group at the same time point (p=0.0079 and p=0.0063) (Figure 5.11c). HbA1c level of males in Waistlist group at 9th month was increased significantly compared to its baseline (p=0.0432), and males at the 3rd month (p=0.0098) and 9th month (p=0.0252) were observed a significant higher HbA1c level compared to females at the same time point (Figure 5.11d).

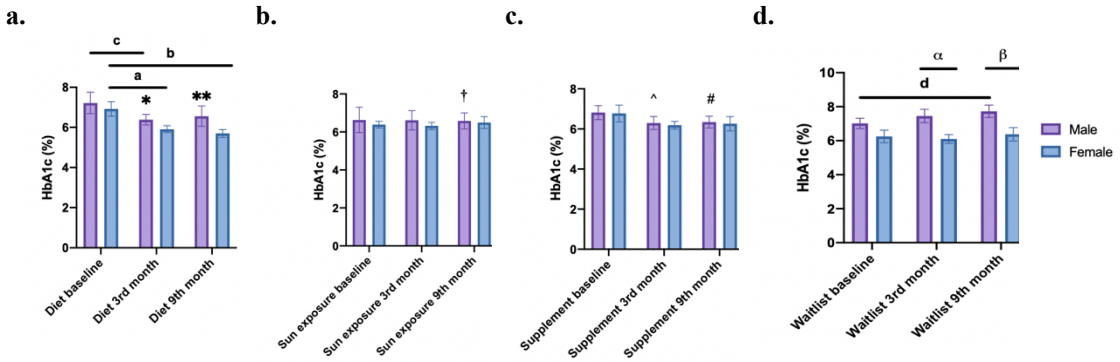


Figure 5.**11** HbA1c level of Male and Female in Diet group (a), Sun exposure group (b), Supplement group (c) and Waitlist group (d). Data are analysed by two-way mixed ANOVA and presented as mean ± SEM. In (a), a p= 0.0220 for females in Diet group 3rd month vs baseline, b p=0.0310 9th month vs baseline, and c p=0.0493 for males in Diet group 3rd month vs baseline. * p=0.0135 males in Diet group 3rd month vs Waitlist and ** p= 0.0196 9th month vs Waitlist, In (b), † P=0.0292 males in Sun exposure group 9th month vs Waitlist, in (c), ^ p=0.0079, males in Sun exposure group 3rd month vs Waitlist, # p=0.0063, males in 9th month vs Waitlist, in (d), d p=0.0432 males in Waitlist group 9th month vs baseline, α p=0.0098 Waitlist group 3rd month males vs females, and β p=0.0252 9th month males vs females.

5.7.4 Racial Impact

5.7.4.1 Serum 25(OH)D Level

Racial impact on Serum 25(OH)D level was showed in Figure 5.12. The serum 25(OH)D level of Chinese in the Diet group was significantly increased after nine-month intervention compared to baseline (p=0.0009) and 3rd month (p=0.0029), and is significantly higher than Waitlist group at 9th month (p=0.0367) (Figure 5.12a). Serum 25(OH)D level of Chinese in Sun exposure group at 9th month was significantly improved compared to baseline (p=0.0339), the level at 3rd month is also significantly higher than supplement group (Figure 5.12b). Serum 25(OH)D level of the Caucasian group in the Diet group at 3rd month also showed a significant improvement compared to the Waitlist group (p=0.0467), it was also significantly higher than the Chinese group (p=0.0320) (Figure 5.12a). Serum 25(OH)D level of Caucasian in Supplement group increased significantly at 9th month compared to its baseline (p=0.0170) (Figure 5.12c).

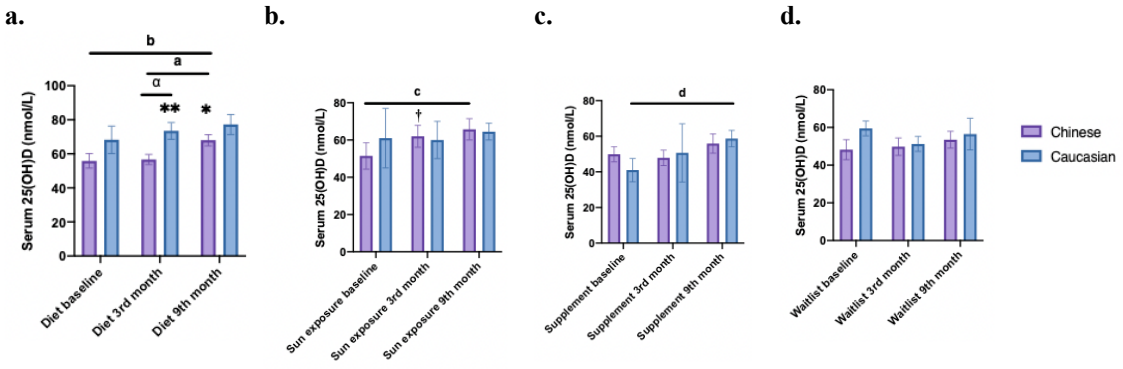


Figure 5.12 Racial impact on Serum25(OH)D Level in Diet group (a), Sun exposure group (b), Supplement group (c) and Waitlist group (d). Data are analysed by two-way mixed ANOVA and presented as mean ± SEM. In (a), a p= 0.0009 for Chinese in Diet group at 9th month vs baseline, b p=0.0029 vs 3rd month, * p=0.0367 for Chinese in Diet group at 9th month vs Waitlist, ** p=0.0476 for Caucasian in Diet group at 3rd month compared to Waitlist group at the same time point, α p=0.0320 for Chinese vs Caucasian in Diet group at 3rd month. In (b), c P=0.0399 Chinese at 9th month vs baseline, † p=0.0386 for Chinese group at 3rd month vs Supplement group, in (c), d p=0.0170 for Caucaian group 9th month vs baseline.

5.7.4.2 HbA1c Level

Racial impact on HbA1c level was shown in Figure 5.13. HbA1c level of Caucasians in Diet group at 9th month also showed a significant reduction compared to Supplement group (p=0.0358) and its baseline (p=0.0169) (Figure 5.13a). HbA1c level of Chinese and Caucasians in Diet group at 9th month both showed significant reduction compared to the Waitlist (p=0.0417 and p=0.0100) (Figure 5.13a). HbA1c level of Chinese in Diet group at 3rd month was reduced significantly compared to its baseline (p=0.0012) and Waitlist group at the same time point (p=0.0182) (Figure 5.13a). HbA1c level of Chinese in Sun exposure group at 9th month was significantly reduced compared to Waitlist group (p=0.0228) (Figure 5.13b). HbA1c level of Chinese group in Supplement group at 3rd month and 9th month were significantly reduced compared to Waitlist (p=0.0087 and p=0.0027) (Figure 5.13c). HbA1c level of Chinese in Supplement group at 9th month was also significantly less than Caucasian group (p=0.0169) (Figure 5.13c), no other racial difference was found in other intervention groups.

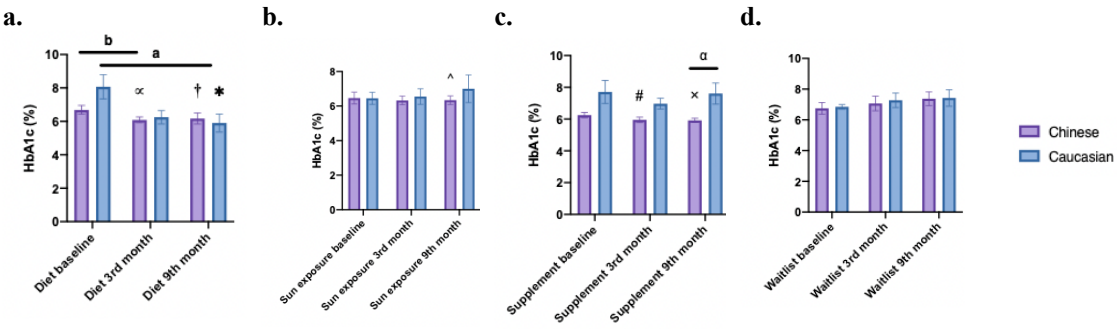


Figure 5.13 Racial impact on HbA1c Level in Diet group (a), Sun exposure group (b), Supplement group (c) and Waitlist group (d). Data are analysed by two-way mixed ANOVA and presented as mean ± SEM. In (a), a p= 0.0169 for Caucasians 9th month vs baseline, b p=0.0012 for Chinese 3rd month vs baseline, ∝ p=0.0182 Chinese 3rd month vs Waitlist group, † p=0.0100 Chinese 9th month vs Waitlist, * p=0.0417 Caucasians 9th month vs Waitlist andP=0.0358 vs Supplement. In (b), ^ p=0.0228 Chinese 9th month vs Waitlist. In (c), # p=0.0087 Chinese 3rd month vs Waitlist, x p=0.0027 Chinese 9th vs Waitlist, α p=0.0169 Chinese vs Caucasians at 9th month.

5.8 **Adverse effects**

No adverse events were reported and were linked to vitamin D supplementation, dietary changes and sun exposure.

Chapter 6. **Discussion**

The major finding in the D4D trial is that the HbA1c was inversely associated with serum 25(OH)D level. The dietary intervention may be the most effective way to improve serum 25(OH)D level and manage blood glucose in T2DM patients. A long period is required for interventions especially the supplements, to exert a significant effect on glycaemic control among T2DM patients. None of the interventions has shown significant blood lipid-lowering effects.

The inverse association between serum 25(OH)D level and HbA1c in D4D trial is consistent with previous large observational studies (200, 201). However, many RCTs have presented inconsistent results and failed to show the association due to study limitations such as small sample size, short intervention period, and relatively small intervention dosage (171). Similar to these RCTs, the D4D trial did not observe a statistical significance in the association between serum 25(OH)D and HbA1c. This weak association could be explained by small sample size and a high basal vitamin D level. The baseline serum 25(OH)D concentration among the participants was 50.87±17.85 nmol/L, and was above the VDD cut-off point (< 50 nmol/L). Therefore, this adequate basal level may not be sensitive enough to show the interventions' impacts on glycaemic control. Similarly, a study (202) conducted among participants with a high baseline serum 25(OH)D level (27 ng/mL or 67.5 nmol/L) failed to show the influence of vitamin D supplementation on the blood glucose level.

In this D4D trial, the serum 25(OH)D level was significantly affected by treatment lengths and the type of intervention. The serum 25(OH)D level was significantly increased in the Diet and Supplement groups at 9th month and was significantly increased in the Sun

exposure group at 3rd month compared to the baseline. Serum 25(OH)D level in the Diet group at the 9th month was also significantly higher than the Waitlist group and the Supplement group. The significant change of serum 25(OH)D level obvserved following dietary intervention suggests its superiority over the supplement, which could be explained by the lately improved understanding of the dietary vitamin D source, the higher bioavailability and retention of dietary vitamin D compared to the supplements.

The HbA1c level was also significantly affected by time lengths and the interaction of time and intervention. The HbA1c level in the Diet group at the 9th month was significantly decreased compared to the Waitlist group, and the HbA1c level in Diet group at the 3rd month and 9th month were statistically decreased compared to the baseline. Dietary sources may affect HbA1c level by increased intake of protein and low glycaemic index carbohydrate, in addition to the direct hypoglycaemic effects of dietary vitamin D. HbA1c level in the Supplement group also showed a significant decrease at the 3rd month compared to the baseline. The supplement dosage, nature and carrier, and treatment duration could all positively affect the HbA1c level in addition to the possible direct effect of vitamin D on glucose control.

6.1 Prevalence of Vitamin D Deficiency, Serum 25 (OH)D and HbA1c Status in Gender and Ethnicity

6.1.1 Gender

Although the Australian national data from ABS has reported that men and women have no difference in VDD levels, many small-scale studies reported a sex difference in VDD. An Indian study showed that men could sometimes present lower vitamin D levels than women ($p<0.02$) (203), and other studies observed a high prevalence of VDD in females

than males (204, 205). Studies also suggested that female diabetic patients tend to have significantly lower serum vitamin D levels than healthy female patients (206, 207). Like these studies, our trial observed a slightly higher prevalence of VDD in females than males, but the average serum 25(OH)D level was similar between males and females.

There was no gender difference observed in the response of serum 25(OH)D level,
Diet and Supplement interventions were significantly improved serum 25(OH)D level in males while only the Sun exposure benefited in females' serum 25(OH)D level compared to the Waitlist group. With the interventions and seasonal changes, females' serum 25(OH)D level in all treatment groups was increased at the 3rd month continued to improve at the 9th month, however apart from Sun exposure no significance between interventions and time length was observed. The improvement trend of serum 25(OH)D level in women may be due to the sexual difference in the responses to vitamin D supplementation (208) and their lower baseline serum vitamin D levels (209) however need more future RCTs to clarify the underlying mechanisms. As women are the main group who use anti-sunlight protection, prolonged sun exposure from Sun exposure intervention in the D4D trial and seasonal changes may suggest this significant benefit in females' serum 25(OH)D levels.

Interventions of Diet, Sun exposure and Supplement were more effective on males' HbA1c levels, especially after the long-term intervention, as males in the Diet, Sun exposure and Supplement groups showed a significant decrease in HbA1c at the 9th month compared to males in the Waitlist group. This was possibly due to the long lifespan (120 days) of red blood cells hence HbA1c results is the estimation of patients' glycaemic control over the last 2-3 months (210).

6.1.2 Racial Impact

Chinese and Caucasians are the main two racial groups in the D4D trial. The Chinese group in the D4D trial had a higher prevalence of VDD compared to the Caucasian group at the baseline and remained higher than Caucasians at the 9th month. Skin pigmentation is the major reason for a lower baseline serum vitamin D level in the Asian or Chinese group. A study found that skin pigments and covered clothes would make the risky population needing a 300-600% longer sunlight exposure time to get the equivalent effects compared to the light-skinned individuals (211, 212); hence the Asian or Chinese group may generate less serum vitamin D than Caucasians when spending the same time of sun exposure.

Other than that, Chinese background participants have the tradition of using clothing protection and/or sunscreen to reduce Ultraviolet (UV) exposure, although the purpose is for skin beauty rather than skin cancer (213). It was noted that in the D4D trial, over 85% of Chinese participants were using protections against sun exposure. Most of the participants (77-85%) have chosen a physical sun blocker (i.e. cloth protection) as the main method and the sunscreen lotion was not commonly used. It is consistent with a study from China that compared to chemical sunblock to other methods, which found that more than 90% of Chinese-origin participants emphasised the physical sunblock by using the umbrella, hat and long sleeve clothing (214). Therefore, the darker skin colour as well as the limited skin exposure (5-7%) may explain the higher VDD prevalence in the Chinese group in the current D4D trial.

Caucasians in the Diet group at 3rd month showed a significant higher serum 25(OH)D compared to Chinese. Chinese group in the Diet and Sun exposure group after nine-month

interventions observed a significantly increased serum 25(OH)D level compared to the Waitlist group. It indicated a long-term non-medical intervention is important to present the significant impacts on serum 25(OH)D level for Chinese. Nevertheless, a quicker but short-lasting effect of Diet was observed in Caucasians at 3rd month, which may be due to their higher baseline serum 25(OH)D level and possible 25(OH)D threshold limits after three-month interventions.

HbA1c level of Chinese in Diet, Sun exposure, and Supplement were significantly reduced after nine months intervention, and Chinese in the Supplement group at 9th month presented a significantly lower HbA1c level compared to Caucasians; no other significant differences were observed among other interventions. Diet also presented a superior effect compared to supplement in reducing the HbA1c level of Caucasians, the effects on HbA1c which may be explained by the nature of food rather than vitamin D function.

6.2 **Effects on Serum 25(OH)D Level**

In our study, the treatment duration and intervention type showed a significant effect on serum 25(OH)D concentration. A longer intervention period is important for sun exposure and supplementation interventions to show their effects on serum 25(OH)D levels. The Sun exposure group at the 3rd month, Diet and Supplement group at the 9th month all showed a significant increase compared to their baseline. The Dietary intervention significantly affected serum 25(OH)D level and could be the most effective way of improving serum 25(OH)D level. The serum vitamin D level in the Diet group at the 9th month was significantly increased compared to the Waitlist Group and was significantly higher than the Supplement group.

6.2.1 Dietary Vitamin D and Serum 25(OH)D Level

The Diet group in the D4D trial had presented a significant increment in serum 25(OH)D level over nine months compared to the Waitlist and Supplement group, the increment was sharper from the 3rd month to the 9th month compared to the baseline to 3rd month phase. The Dietary intervention's effects on serum 25(OH)D may be explained by the following factors: 1) Previous underestimation and improved knowledge of the dietary vitamin D; 2) The food culture on meat choices; and 3) High bioavailability of vitamin D_3 and retention of dietary 25(OH)D compared to supplementation.

Improved Understanding of Dietary Vitamin D

In Australia, very few foods contain a significant amount of vitamin D, and it is believed that dietary sources could only provide 5-10% of the vitamin D requirement (215). The food sources with the highest content of vitamin D (vitamin D_2 and or D_3) are fatty fish (include salmon, sardine, herring and mackerel), meat, milk, eggs and fortified margarine (215). According to the national survey in 1998-1999, fortified margarine is the top consumed dietary resource of vitamin D (48%) followed by canned fish (16%) and eggs (10%) (216). The 2006 Nutrients Reference Value Report confirmed that very few foods contain a significant amount of vitamin D, and that the fortified margarine is still the major dietary source (217).

However, we might underestimate the vitamin D content in food by only counting vitamin D_2 and D_3. The research identified a large discrepancy (17 to 91%) between dietary and serum vitamin D status and highlighted the possible potency of the metabolite vitamin D form, i.e., the 25(OH)D from animal-based food (218). The 25(OH)D from food sources previously was not incorporated into the vitamin D amount; however, the current study

has demonstrated it is about five times more potent than vitamin D_3 in the food (91) and the inclusion of dietary 25(OH)D could result in a 2-18 times higher dietary vitamin D content than traditional thoughts, which may well explain the discrepancy (219).

The United States Department of Agriculture and the Office of Dietary Supplements, National Institutes of Health, worked collaboratively to demonstrate that there was an underestimation of vitamin D intake in the United States and agreed that the embrace of dietary 25(OH)D could increase approximately 1.7-2.9 μg/day (68-116 IU/day) vitamin D intake that accounts 15-30% of the Estimated Average Requirement (91). A study suggested that if dietary 25(OH)D is included in vitamin D count, simply ingesting 120g beef or two eggs could easily deliver 100% of adequate vitamin D needs (220).

It is noted that the dietary vitamin D reference value was not available in the previous 2006 database: National Food Composition Database - NUTTUB (NUTrient TABles for use in Australia, now called The Australian Food Composition Database, AFCD), and the survey-specific database AUSNUT (AUStralian Food and NUTrient Database) due to limited available information. With the improved understanding as mentioned above and accuracy level in vitamin D measurement that improved from 5 μg/100g of vitamin D_3 to 0.03 μg/100g of vitamin D_3 and 0.05 μg/100g of 25(OH)D, more foods have been found with an accountable level of vitamin D. Based on these changes, the dietary vitamin D in the Australian database was updated in 2019 (Australian Food Composition Database – Release 1).

The Australian recommendation of vitamin D intake may need to be updated with improved knowledge as well. Currently, the National Health and Medical Research

Council (NHMRC) (217) have suggested the following Adequate Intake (AI) of Vitamin D: 5 μg/day (200 IU/day) for people under 50 years old; 10 μg/day (400 IU/day) for 51-70 years old and 10-15 μg/day (400-600 IU/day) for those above 70 years. The average intake of vitamin D from food is 2.6-3.0 μg/day (104-120 IU/day) for men and 2.0-2.2 μg/day (80-88 IU/day) for women (215). However, studies suggested that these levels are outdated and need to be revised (87). The most recent Recommended Daily Allowances (RDAs) of vitamin D is from the United States which is 15 μg/day (600 IU/day) for people aged between 1-70 years and 20 μg/day (800 IU/day) for those aged older than 70 years, with the upper limit 2,000 IU/day (50 μg/day) for children and 4,000 IU/day (100 μg/day) for adults (221).

Food Culture on Meat Choices

The Asian group has commonly been reported as having a high prevalence of VDD. This study did observe the higher prevalence of VDD in the Asian group compared to the Caucasian group in the D4D trial; however interestingly, the Asian group had presented as having an average sufficient serum 25(OH)D level before and after the interventions. In the D4D trial, the Chinese group reported a baseline serum 25(OH)D level of 55.91±14.22 nmol/L and was later improved to 61.63±15.48 nmol/L. Although the limited skin exposure in the Chinese group increases VDD risk; culture impacts on the food and meat choice, particularly poultry, pork and fish intake, may benefit the vitamin D status and this may be shown from the baseline adequate vitamin D status.

A higher concentration of cholecalciferol (vitamin D_3) and its metabolite 25(OH)D_3 was found in poultry (mainly chicken) and pork compared to beef and lamb (222). Such differences may be explained by the livestock cultivation pattern in Australia that, as

poultry and pigs are fed mostly by fortified feeds while beef and lamb are mainly free-ranging and feed predominantly on grass (223).

In the vitamin D dietary plan that was offered to the Diet group, chicken and pork were the main protein sources and accounted for approximately 60-65% of total protein intake (excluding dairy). Chicken and pork (with fat) are also the most common meats cooked in traditional Chinese cuisine (224). Besides, approximately 60% of Chinese participants were originally from China Southern areas (FuJian and GuangDong Province), where mussels and salmon are the other two common protein sources frequently consumed as two to four times per week. Studies from Japan confirmed that frequent fish intake could benefit serum 25(OH)D status especially in winter (225), and that the elderly group who consumed fish four days per week during winter could have a 10 nmol/L increment in serum 25(OH)D level (226). Therefore, food choices of poultry, pork and seafood may largely assisted the Chinese-origin group maintain and improve their serum 25(OH)D level adequately during the trial.

The Caucasian group obtained dietary vitamin D mainly from beef and fortified margarine (fortification with a minimum of 220 IU/100g under law). Around 85% of the Caucasian participants consumed margarine daily, and their frequency in beef intake (i.e., 3-4 times/week) is about two times higher than observed for the Asian group. However, the consumption of fish is comparably lower with only 0-1 serve/week. Therefore, although the dietary vitamin D sources for Caucasians are different from the Chinese group, a frequent intake of fortified margarine and beef may also help Caucasians maintain an adequate vitamin D level even with a lower intake of fish.

Bioavailability of Dietary Vitamin D

Differences in bioavailability and potency from various forms of vitamin D have been previously documented. Firstly, a systematic review suggested a superior effect of vitamin D_3 in raising serum 25(OH)D level compared to vitamin D_2 (227). The high potency of D_3 compared to D_2 could result from a higher metabolism and clearance of vitamin D_2 in the liver and kidney (228). Secondly, foods in different countries may have richer different vitamin D forms and hence cause a difference in serum 25(OH)D. Dietary sources of vitamin D_2 were found more commonly in US food, including the UV radiated mushrooms and poultry fed with vitamin D_2 fortified feed, but is not usually seen in Australia (222). Australian food is high in vitamin D_3 and may be more effective in raising serum 25(OH)D levels than foods in other countries. These also suggest that studies from different countries may need to be interpreted differently and carefully. Thirdly, as mentioned above, dietary 25(OH)D has been reported with 3-7 times higher bioavailability than vitamin D_3 (229, 230), which could result from 25(OH)D's polarity and greater retention than its non-hydroxylated parental vitamin D_3 (231, 232). Therefore counting 25(OH)D as part of the vitamin D intake could help to understand the discrepancy of dietary intake vitamin D and serum 25(OH)D level and suggest 25(OH)D could be more effective than vitamin D_3 and D_2 in improving serum 25(OH)D level.

However, many other dietary characteristics could affect vitamin D's bioavailability, including food matrix, food amount, type and amount of dietary lipids, dietary fibre and cooking method. Such factors will affect vitamin D absorption from food and supplementation (232). The impact of the cooking method seems minimal. In general, the retention of vitamin D after cooking can range from 35% to 175% and averagely around 80-90% (222). Hence considering all the influencing factors, the study concluded that the

25(OH)D enriched or fortified food sources could be more efficient in raising serum 25(OH)D level (229).

6.2.2 Sunlight Exposure and Serum 25(OH)D Level

The participants in the Sun exposure group of the D4D trial reported an average sun exposure time of 10-15 minutes/day with 8-10% skin exposure at the baseline (June 2019), and had a just-sufficient serum 25(OH)D level (51.20±23.18 nmol/L). With maintained or increased sun exposure time, we observed a consistent increment in serum 25(OH)D level in the Sun exposure group over nine months, and it was statistically higher at 3rd month compared to baseline. In the D4D trial, sun exposure worked effectively on serum 25(OH)D, but the participants faced many challenges of obtaining enough UVB due to: 1) Reduced Sun Exposure Time; 2) Seasonal and Geographic factor; and 3) Sun Blockers such as modern lifestyle, skin pigmentation, cloth coverage, and sunscreen usage.

Reduced Sun Exposure Time

Other than the dietary intake mentioned above, it was previously believed that cutaneous synthesis from sun exposure is the main source of vitamin D for people living in Australia and New Zealand (87). Adequate time under sunlight (UVB 290-320 nanometre) with 15% of skin exposure can provide 80-100% of vitamin D needs (233, 234).

To be more specific, in Australia, exposure of about 15% of body surface (hands, face and arms) under the sun anytime from 10 am to 4 pm for 3-4 minutes per day from December to January (summer), or 5-6 minutes per day from February to June (autumn) and September to November (spring) or 8 minutes per day from July to August (winter), can provide approximately 500 IU/day (12.5 μg/day) of vitamin D, and this amount is

equivalent to 1-2.5 times of Adequate Intake (AI) of vitamin D for all Australians (87). Research showed that 1 Minimal Erythemal Dose (MED), or the amount of sunshine that just enough produces a faint redness of the skin, equals the effect by taking 375 μg (15,000 IU) vitamin D orally (235).

However, many factors can affect the UV radiation exposure and the cutaneous synthesis of vitamin D, including geographical position (202), season and weather, time of the day, air pollution, ageing (236), skin pigmentation, clothing coverage, sunscreen and protection usage and modern lifestyle with longer indoor time. Meanwhile, the study also pointed out that the sunlight between 10 am to 2 pm in summer (11 am to 3 pm daylight saving time) should be avoided when considering the skin cancer risk (237).

Season and Geography

Geographically, south-eastern Australia, especially New South Wales (NSW) in summer reported the highest VDD prevalence (19%) than the other states (5). The current trial was conducted in Sydney, Australia at latitude 33.8688° S, 151.2093° E, from June 2019 to September 2020. The geographic and seasonal factors at the trial location are in accordance with the borderline serum vitamin D level among our participants at the baseline.

Sun Blockers: Modern lifestyles, Skin pigmentation, Cloth cover and Sunscreen

It is understood that the modern lifestyle brings a high pace of living and limited sun exposure for most Australians. People work indoors; driving instead of walking or cycling, and hence spend less time outdoors under sunshine.

Skin pigmentation and clothing coverage can affect cutaneous synthesis under the sunlight. Jordanian researchers reported that women wearing the hijab tend to have a lower vitamin D concentration and the exposure of only hand and face is insufficient for vitamin D synthesis (238). Over-used of sunscreen will also inhibit vitamin D synthesis in the skin especially in dark-skinned and/or veiled people who already have a VDD risk (239). However, a study reported that normal usage of sunscreen would not cause VDD and should be encouraged when considering skin cancer risks (234).

Approximately 78.3% of participants in the D4D trial were Chinese, African and Indian. Chinese-background participants have the tradition of using clothing protection and/or sunscreen to reduce the UV radiation (213) for skin beauty purposes; therefore we have observed a less optimum and only borderline serum 25(OH)D level at the baseline measurement time. Due to skin pigmentation and possible clothing coverage, their serum 25(OH)D levels were not significantly increased at 9th month period compared to baseline and the other groups.

Basal Serum 25(OH)D Level and Sensitivity

In addition to the improved sun exposure time in summer weather, the lower basal serum 25(OH)D level in the Sun exposure group may also contribute to its significant increase observed at the 3rd month. A study found that participants with a lower baseline serum 25(OH)D level usually had a larger increase after interventions, and the lower basal serum 25(OH)D level may be more important than lighter skin pigmentation in obtaining vitamin D following ultraviolet light exposure (240).

6.2.3 Vitamin D Supplementation and Serum 25(OH)D Level

Vitamin D supplementation seems to work consistently in the current D4D trial as it was observed a continuous increment in serum 25(OH)D level over the nine-month trial period. The vitamin D supplement significantly increased the serum 25(OH)D level at the 9th month and showed a significantly smaller effect than the Diet group. Characteristics of both supplements themselves and supplement receivers can affect the improvement of serum 25(OH)D level. These characteristics include the following: 1) Supplementation Type and Carrier Vehicle; 2) Supplement Absorption and Interaction with Food; and 3) Supplement Dosage and Intervention Time.

Supplementation Type and Carrier Vehicle

Vitamin D supplements in Australia mainly use cholecalciferol (vitamin D_3) as the major source while manufacturers in the US tend to use ergocalciferol (vitamin D_2) because vitamin D_2 at 50,000 IU was the only prescribed vitamin D treatment for rickets since 1941 (241). The chemical difference between vitamin D_3 and D_2 is only in the side-chain structures; however, supplements using vitamin D_3 as a major ingredient are more effective than vitamin D_2 in raising serum 25(OH)D level (242). This may relate to the differences in hydroxylation in the liver and kidney and the binding efficacy to Vitamin D Receptor (VDR) (243).

Cholecalciferol (vitamin D_3) in the oil vehicle was the supplement form used in the current D4D trial. The research found that the oil-based microencapsulated form of vitamin D was the most bioavailable vehicle compared to powder and ethanol-based forms (244). Therefore, the vitamin D supplement in the D4D trial should present a high bioavailability in improving serum 25(OH)D level, but the absorption can be influenced

by the receivers' absorption status and interaction with foods.

Supplementation Absorption and Interaction with Food

Vitamin D supplementations are well absorbed in the small intestine through passive diffusion. Vitamin D absorption involves emulsification, dissolution in micelles, diffusion through the stagnant water layer and penetration across enterocytes membranes (245). Before vitamin D reaches the small intestine, the acidic gastric juice may affect the bioavailability of vitamin D; however, no evidence is available to confirm this. Later, the chylomicron and vitamin D Binding Protein (DBP) help transfer vitamin D to the liver for hydroxylation. The absorption efficacy of vitamin D via the above oral-gut route is about 50%, whereas the 25(OH)D generated in the liver, the chylomicron and DBP will account for 40% and 60%, respectively (246).

Vitamin D absorption is associated with lipids and assisted by the bile salts. Normal dietary fat intake (30% of total calories) in the gut can enhance vitamin D_3 absorption by 32% compared to a fat-free diet (247). Biliary salts are also important for vitamin D_2 and D_3 absorption (248); however, a study on rats reported that the absorption of polar vitamin D metabolites (i.e. 1,25(OH)D and 25(OH)D) might not involve bile salts and micelle formation (249) hence have a higher bioavailability. Other than that, food amount (250), food matrix (232), lipid type and amount (251), dietary fibre (252) may also affect the bioavailability of vitamin D.

Baseline serum 25(OH)D level could be another important factor regulating the supplement vitamin D absorption and presentation (209). A study reported that the baseline vitamin D level was inversely correlated with serum 25(OH)D level increases

after the intervention (253) and this might be because the nature of hepatic hydrolyzation of vitamin D is a saturated process (254). In the current D4D trial, the Asian group with a lower baseline vitamin D presented a larger improvement in serum 25(OH)D level than the Caucasian group, apart from the saturation processed mentioned above, a study suggested that Asians may convert vitamin D_3 into 25(OH)D more rapidly than Caucasians because of the genotype differences and DBP affinity (255, 256).

Dosage and Intervention Time

Serum 25(OH)D level in the Supplement group were significantly increased compared to its baseline; however, the serum 25(OH)D level in the Supplement group did not present a significant difference compared to the Waitlist group but was significantly lower than the Diet group at the 9^{th} month period. As mentioned before, dietary vitamin D presented a superior effect on serum 25(OH)D level that may result from improved knowledge and its higher bioavailability; the insignificance of supplementation may result from the small dosage (500 IU/day), supplement frequency and intervention duration in the D4D trial.

Although a study reported 500 IU/day vitamin D supplementation could significantly increase the serum 25(OH)D level among healthy people in eight weeks (257), this level may not be sensitive enough for the T2DM risk group. Research showed that to boost 97.5% of serum 25(OH)D level to more than 50 nmol/L in African Americans, a dosage of 800 IU/day (20 μg/day) vitamin D was required (258). Hence the RACGP currently recommended that: for groups who cannot access enough sun exposure, or with moderate or severe VDD, it is best to use supplements at 3,000-5,000 IU/day (70-125 μg/day) for 6-12 weeks. A recheck is recommended after twelve weeks, and most people will need an ongoing maintenance dose of 1,000-2,000 IU/day (25-50 μg/day) (259).

Apart from supplement dosage, supplement frequency and intervention duration can influence serum 25(OH)D level as well. A previous study suggested a 2000 IU dosage with at least three days per week frequency over a five-month intervention would generate a better result (260). Hence despite the moderate dosage we used in the current study, the frequency and duration of the supplementation in the D4D trial were efficient in increasing serum 25(OH)D, which lead to a significant increase in 9th month compared to the baseline.

Over Usage of Supplements and Unnecessary Testing

The vitamin D supplement has been recommended as the safest and most effective way of boosting the serum 25(OH)D level, especially for groups with VDD risks (87). However, the over-use of supplements and unnecessary testing have raised justifiable concerns, not only in Australia but worldwide (261).

In Australia, the current AI of vitamin D is 500 IU/d (12.5 μg/day) for the general population with Upper Level (UL) at 3,200 IU/day (80 μg/day), while for infants the value goes down to 1,000 IU/day (25 μg/day) (217). Vitamin D supplementation might be easily overused compared to the above levels, and the specific AI and UL levels for risk groups such as dark-skinned or/and veiled population might need a re-examination, as the supplements' dosage needs to be balanced with the actual sun exposure time, risk of hypocalcaemia and related risk of kidney stone from over intake (Favus & Coe 1997).

With a more comprehensive understanding and increased public interest in vitamin D's health benefits, more people are taking vitamin D supplements, and the sales of vitamin D supplements have almost tripled from A$34.1 million in 2000 to A$94.0 in 2010 with

a large increment in winter when there is a shorter sun exposure time (262).

Serum vitamin D testing seems less encouraging to people with vitamin D supplement usage, or people will have vitamin D supplements with or without testing, hence the necessity of testing has been brought into the discussion (263). More testing has been done since 2000. The serum vitamin D test was increased from 0.4 per 1000 population in 2000 to 36.5 tests per 1000 population in 2011 and the cost to Medicare has increased from AUD$1.1 million in 2000 (263) to AUD$ 151 million in 2012-2013 (264). In 2014, the Australian government had regulated the vitamin D test with updated criteria (265), it brought a short-term decrease in test number but was later increased again from 119 to 159 tests per 1000 population due to either the ordering doctors being unaware or they did not support the testing criteria (266).

Eighty-six per cent of the participants in the D4D trial had their serum vitamin D tested previously and are aware of their suboptimal vitamin D status; however, they were not on supplements either because they ignored the importance of vitamin D and/or doctor's advice, or they believed their minor deficiency would be corrected in summer anyhow. Hence the increased testings did not benefit the vitamin D level and could be a huge waste. While we continue to emphasise the importance of vitamin D supplements for risk groups or people with severe VDD, a more appropriate and sustainable solution such as lifestyle changes in food and sun exposure should be promoted.

6.3 Effects on HbA1c Level

6.3.1 Dietary Vitamin D and HbA1c Level

Dietary vitamin D intake linked with a decreased HbA1c level; however, the association

between dietary vitamin D and T2DM is controversial in the literature, and should be interpreted carefully and comprehensively with confounding factors and study limitations. Vitamin D can directly affect β-cell function, insulin secretion and sensitivity, and is related to inflammation. The mechanisms have been reviewed in **Section 2.1.3 Possible Mechanism**. Apart from the direct effect of dietary vitamin D on HbA1c, other superior effects from dietary intervention, including increased protein intake and low Glycaemic Index food choices, may also benefit the HbA1c level.

Dietary Vitamin D

The dietary intervention in the D4D trial provided a higher level of dietary vitamin D compared to other studies. The Diet group in the trial was required to consume 0.7-1 egg and two serves of meat or fish per day to achieve 500 IU/day (12.5 μg/day) of vitamin D, matching with the vitamin D intake obtained from the sun exposure and supplementation interventions.

Lower intake of dietary vitamin D often failed to observe the association of vitamin D and HbA1c level. A large EU prospective cohort study found that the dietary vitamin D intake was not significantly associated with T2DM risks; however, the dietary vitamin D intake in the study was low for both men (144 IU/day or 3.6 μg/day) and women (96 IU/day or 2.4 μg/day) (267). The nation of Sweden in this study, among all EU countries, reported the highest dietary vitamin D intake (284 IU/day or 7.1 μg/day in men and 188 IU/day or 4.7 μg/day in women); however, this level is still less than the amount that was used in the current D4D trial (500 IU/day).

Another large-scale Nurse' Health Study suggested that oral vitamin D intake could

reduce T2DM risks (268), and the average intake of total vitamin D (both dietary and supplementary) in their long-term follow-up cohort study was about 309 IU/day (7.7 μg/day). To be more specific, women with a large dosage of total vitamin D intake (>800 IU/day or >20 μg/day) had a lower risk for T2DM incidence compared to women with a small total vitamin D intake (<200 IU/day or <5 μg/day). However, no association was observed in this study between a pure dietary vitamin D intake and T2DM, and it is worthy to note that dietary vitamin D intake in this study ranges from 0-10 μg/day (0-400 IU/day), with only 5% of the subjects having an intake over 5 μg/day (200 IU/day).

Protein Intake

The increased and adequate protein intake following the vitamin D enriched dietary plan in the D4D trial may help to reduce the HbA1c level. In the D4D trial, the Diet group was required to consume a total of two serves of fatty fish, two serves of red meat, four to five serves of chicken, four to five serves of pork plus five eggs in a week, to reach a total 56.5-60 g/day of protein. This amount has not yet reached the level of a high protein diet in that protein accounts for more than 20% of total calories, but certainly improved the general protein intake and balanced the protein food choices.

Many studies reported that a minor increase in protein intake would benefit the HbA1c level. For example, the Palaeolithic diet (Old Stone Age Diet) that is high in fruit, vegetable, fish, meat and eggs, but low in grains and dairy products, can benefit glycaemic control (269). The large European Prospective Investigation of Cancer (EPIC)-Norfolk cohort study also suggested that an increased protein intake particularly from fish, could decrease the T2DM risks; except the shellfish which may be linked with increased T2DM risks (270).

Nevertheless, it should be noted that current evidence suggests that too much red meat intake may increase T2DM risks (271). A systematic review of twelve cohort studies summarised that consuming red meat and processed meat more than three times a week may significantly increase the T2DM risks (272). Apart from red meat, other protein types such as soy and dairy showed protective effects on T2DM, while egg and fish are inconclusive (273). Current dietary guidelines (217) recommend that T2DM patients should consume 0.8-1g protein per body weight (kg) per day. Considering the above recommendations, the iron requirement, the potential harms and the cardiovascular risks from over-intake of red meat (274), we chose to use the dietary plan with a maximum of two serves/week red meat plus other balanced protein choices from poultry, pork and fish to reach the total protein amount of 56.5-60 g/day.

Glycaemic Index and Glycaemic Load

We observed a reduction of high Glycaemic Index (GI) carbohydrate intake in the Dietary group after introducing the vitamin D rich food meal plan. This change may affect the HbA1c level in these participants. The average intake of the carbohydrate in the Diet group at baseline is approximately 6-7 serves/day and mainly were the high GI options such as white bread, jasmine or Thai rice and white flour-based food including noodles and buns. With the increases in vitamin D rich food that is mainly protein sources, the Diet group had reduced high GI carbohydrate food by 15-20% at the 3rd month and 20-30% at the 9th month. Apart from reducing the high GI food to 3-4 serves/day from the initial 6-7 serves/day, we also observed a reduction in fruit from 2-3 servers/day at the baseline down to 1-2 serves/day at the 9th month.

High GI and GL foods are significantly associated with increased serum HbA1c level and T2DM risks (275, 276). A meta-analysis showed that participants who consumed a diet high in GI and GL and low in fibre could have 50% higher risk of T2DM (276) and in particular, white rice intake was associated with a 17% increased risk of T2DM (38). Hence the reduction of high GI food intake may benefit glycaemic control to some extent. Furthermore, the Diet group showed a quick decrease in serum HbA1c level during the first three months but a slower reduction from the 3rd month to the 9th month, which may be affected by the holiday eating pattern and suggest a threshold of HbA1c change (277).

6.3.2 Sunlight Exposure and HbA1c Level

The HbA1c level presents with seasonal variation (278) in accordance with the T2DM incidence (279); hence there is a hypothesis that sunlight exposure may play an important role in glycaemic control and T2DM development, and is possible through vitamin D production. Indeed, many studies suggest that sun exposure may benefit T2DM; however, the overall evidence is not strong.

There are significant gaps in the correlation between sun exposure and T2DM risks due to the limited evidence on this topic (195). A large prospective cohort study from Sweden reported that women with active sun exposure had a reduced risk of T2DM (196). Intervention studies of UVB light on humans are limited and inconsistent. One study reported that UVB light exposure had a positive effect on insulin secretions (280); while another study failed to show the association between UVB light exposure and its regulation of blood glucose and insulin levels (281).

The pathway through which sunlight exposure may benefit HbA1c levels and glucose

control is also unclear. A review (195) suggested that sun exposure is likely to benefit physiological processes such as melatonin secretion (282), circadian rhythm (283), and inflammatory response (284, 285) that indirectly prevent the T2DM development, and may not be relevant to the current hypothesis of cutaneous vitamin D. It is also worth noting that long-term sun exposure may be harmful to T2DM patients. For example, a national-wide cross-sectional study in Korea found that sunlight exposure ≥5 hours/day is significantly associated with an increased risk of diabetic retinopathy among T2DM patients (286).

The current D4D trial found that the Sun exposure group had no significant change in HbA1c level, while the HbA1c levels in the Diet and Supplements groups were both reduced. Therefore, our study suggests that sun exposure may have a limited or nil effect on HbA1c levels in T2DM patients, albeit a significant increase in serum vitamin D level. Higher doses of sun exposure at a safe level should be trialled in future studies.

6.3.3 Vitamin D Supplements and HbA1c Level

Several studies have reported an inverse association between vitamin D supplementation and the serum HbA1c level. A study from Saudi Arabic observed a decreased HbA1c level among diabetes patients after taking vitamin D supplementation (287). A three-year study conducted in the African American population reported a significant reduction in serum HbA1c level after taking vitamin D supplementation and suggested the importance of using a high dosage vitamin D supplementation for T2DM control among the obese and overweight group (288). This finding was echoed by the nurse's health study mentioned earlier (268). Similarly, our study observed that the serum HbA1c level in the Supplement group was significantly decreased at the 3rd month; however, it was not

significant at the 9th month.

The smaller and insignificant decline in serum HbA1c level at the 9th month in Supplement Group may be due to the improved serum 25(OH)D level hence the insensitivity to the vitamin D supplementation, it also could due to the small supplement dosage (500 IU/day) that was used in the D4D trial.

The supplement dose of 500 IU/day may not be large enough to raise serum 25(OH)D level, therefore failing to improve glycaemic control in this group at the 9th month. A systematic review on prospective RCTs concluded that vitamin D supplement intake at 4,000 IU/day (100 μg/day) among T2DM patients increased serum 25(OH)D levels and significantly reduced HbA1c level, FPG, and HOMA-IR index (289). To be more specific, vitamin D supplementation at a higher daily dosage of 4,200 IU/day (105 μg/day) for a minimum of seven months can help T2DM patients achieve the optimum serum 25(OH)D level of 100-130 nmol/L and benefit glucose control and insulin resistance. Therefore, the low dosage of vitamin D supplements used in the current D4D trial is considered safe but may be too low to improve glycaemic control.

It needs to be noted that serum HbA1c levels in the control group were increased over the 9-month trial period, although not statistically significant. This deterioration is commonly observed in patients with T2DM, albeit aggressive medications, which was presented in the Supplement group. This suggests that the dose of vitamin D supplement in this study is still effective in reducing the risk of deterioration in patients with T2DM. Higher doses should be trialled in future studies for the potential therapeutic effects against T2DM.

6.4 Changes in Lipid profile and relation to Serum 25(OH)D Level and HbA1c Level

In the D4D trial, we observe the generally decreased TC, TG and LDL levels in all intervention groups, HDL in intervention groups fluctuated with time length, however none of these changes are statistically significant.

Current evidence of the link between vitamin D and cholesterol are conflicting. Epidemiologic and observational studies usually observed an association between low vitamin D level and dyslipidaemia and suggested that an optimal vitamin D level may benefit lipid profile and reduce the risks of cardiovascular disease and metabolic syndrome (290, 291). However, an RCT showed that people with improved vitamin D levels are likely to have high cholesterol levels.

A study that used vitamin D supplementation (50,000 IU/week) for two months reported that vitamin D did not improve the lipids and may even worsen the lipid profile in some patients (292). They found that the increased serum 25(OH)D level could decrease the PTH levels and increased serum calcium levels, and were strongly associated with increased LDL cholesterol. Nevertheless, a recent systematic review of 41 RCTs showed that vitamin D supplementation lowered the TC, TG and LDL levels, but has no effects on HDL (212). It needs to be noted that most of the RCTs included in this review were short-term RCTs, with only 24% of them having a longer follow-up duration of more than six months. Hence research with a shorter follow-up duration may present an opposite result compared to long-term studies.

Vitamin D may affect lipids profile through the transcriptional activity of Vitamin D

Receptor (VDR) and Insulin-induced gene-2 (Insig-2) (181, 293). VDR and Insig-2 inhibit Sterol Regulatory Binding Protein-2 (SREBP-2) activation and 3-Hydroxy-3-Methyl Glutaryl-coenzyme A reductase (HMG-CoA) expression, as SREBP-2 is an important transcription factor regulating the cholesterol synthesis and HMG-CoA is a critical enzyme for cholesterol synthesis, hence reducing the cholesterol synthesis (293).

Furthermore, changes in food choices may affect the lipid profile to some extent. Foods rich in vitamin D such as fatty fish, egg, poultry, and pork were recommended to the Diet group to reach 2-3 serves of protein hence 500 IU/day (12.5µg/day) vitamin D per day. Increased intake of animal sources without fat trimming could increase the intake of Saturated Fatty Acids (SFAs), and it is well established that SFAs can increase LDL and TC and hence is a strong risk factor of CVD (294). Salmon is a rich source of Unsaturated Fatty Acids (USFAs) including long-chain n-3 polyunsaturated fatty acids, eicosapentaenoic acid, and docosahexaenoic acid (295) that are beneficial to the CVDs; hence salmon, both cooked and uncooked, can lower the TC, TG, and LDL cholesterol levels (296). Besides, fish protein was proven to benefit lipid metabolism and reduce CVD risks (297). Therefore, increased intake of salmon and n-3 USFAs in the D4D trial may benefit the reduction in TC and LDL in the Diet group at 9^{th} months.

Bodyweight reduction can also benefit the lipid profile. A study reported that weight loss, especially in the obese group achieved either through diet or exercise, can improve the HDL cholesterol level and reduce the TG level (298). Patients who lost more than 5% weight experienced a significant reduction in TC, TG and LDL cholesterol levels (299). In the current D4D trial, participants in the Diet group lost 2.81% of the initial body weight, which is the largest weight reduction compared to the other groups. Although this

reduction did not reach the 5% level, we still observed a reduction in TC and LDL cholesterol in the Diet group, although it was not statistically significant; this may still suggest that weight loss plays an important role in reducing TC and LDL.

The current evidence of the relationship between vitamin D supplementation and TG is weak. A cell study showed that vitamin D metabolites can upregulate lipoprotein lipase (300) and may benefit insulin resistance through its anti-inflammatory effects on lowering the insulin resistance marker - TG, especially in the diabetic group (301). However, a review of 41 RCTs on lipid profile (302) suggested that vitamin D supplements may not benefit TG level, but the conclusion could be over-augmented due to lack of publication bias in certain included trials. Similarly, the current study failed to observe a significant change in TG in the Supplement group. However, the correlation between serum 25(OH)D level and TG was significant in the Supplement group, suggesting serum 25(OH)D level may need to be increased to a threshold to achieve the statistical significance in TG reduction.

Besides, TG level was shown correlated with serum HbA1c level in patients with T2DM (303). A significant correlation between TG and HbA1c in the Diet and Waitlist groups suggested a strong link between TG and HbA1c level. However, a prospective study reported that the correlation between TG and HbA1c was not clinically significant; hence hypertriglyceridemia may not be an important factor when interpreting HbA1c results (304) but is still useful to determine CVD risks.

6.5 **Confounding Factors**

The major confounding factors in the D4D trials were the unexpected external impacts factors; these include the increased dietary intake during the holiday season, 2019-20 Australian Bushfire and COVID-19 pandemic from February 2020.

Participants generally reported an increase in food intake at approximately 10% from late November 2019 and 13-15% around 2019 Christmas time, and many of the increments were from seasonal confectionery foods. The increase in food and calorie intake may directly affect the HbA1c level, in all groups, which is more evident from the increase in the Waitlist group.

Secondly, the smoke hazard caused by the 2019-2020 Australia bushfire largely affected the outdoor activity time and possibly the UVB exposure quality. Participants in the Diet, Supplement, and Waitlist groups who were having to sunbath while doing outdoor activity may have had to change to indoor mode, which may have reduced their normal physical activity level and time. Reduced sun exposure time thereafter affected serum 25(OH)D and HbA1c levels in all groups. For participants in the Sun exposure group, we changed the sunbath frequency from daily to weekly to adjust to the bushfire crisis. This change allowed participants to reimburse the missed sun exposure if the air quality was not appropriate; however, the effects on serum 25(OH)D and HbA1c levels are uncertain.

Thirdly, the Australian government announced the total restriction from March 2020 due to the COVID-19 pandemic. This affected all participants in food consumption, exercise as well as sun exposure. We observed increased food and calorie intake (13-24%) from February 2020 and approximately 88% less in physical activities in participants during

March to June 2020 during the COVID-19 total lockdown in Sydney, which largely affected participants' serum 25(OH)D and HbA1c levels. The continuous increase in HbA1c in the Waitlist Group could be partially explained by the impact from the above reasons.

6.6 **Strengths and Limitations of Our Study**

To the best of knowledge, the current D4D trial is the first study that assessed the impact of vitamin D from all the possible sources on glycaemic control in T2DM patients, which compared interventions of diet, sun exposure, and supplements. Other strengths include the dietary assessment method, supplement dosage and the intervention time. Firstly, dietary intervention was followed by a food list, and the dietary assessment was comprehensive by using a three-day food recall with both weekdays and a weekend day and an FFQ-VDQ. Thus the possible effects of a variance vitamin D intake were excluded. Secondly, the dosage of supplementation used in the trial may be small but was feasible and meaningful for a real-life pattern. Other than that, the study length of a nine-month period is generally an appropriately long time for RCT to detect the association according to the review on current RCTs.

There are several limitations of the D4D trial. Firstly, the unavoidable single-blind study design may introduce certain biases. Although data collectors and data analysers were blinded to the interventions, the participants were aware of the treatments, which are unavoidable due to the nature of the interventions. This may cause certain knowledge bias regarding the treatment allocations on outcomes reporting. Secondly, the small sample size in our study may not be powerful enough to draw the conclusion, and the result and conclusion may not well represent the Caucasian group and population in Australia. In

this study, we used a convenience sampling method to recruit participants in two medical centres. Future trials need to increase the sample size and include racial diversity and allow analysis on gender differences. Thirldy, the dosage and frequency of vitamin D interventions may not be large enough to reflect the change in HbA1c over the nine-month period. Besides, vitamin D obtained through diet and sun exposure may fluctuate compared with vitamin D supplements. Fourthly, self-report data could be another bias to our study. Data of dietary intake and sun exposure time were obtained through self-reporting questionnaires which may not be accurate. Finally, skin pigmentation was generally reported according to ethnicity or checked by the naked eye in the D4D trial; hence this factor has not been thoroughly assessed and analysed.

6.7 **Recommendation for Future Studies**

There are considerable opportunities for future research on vitamin D in T2DM control. Future RCTs should consider embracing a larger sample size to allow the analysis of cultural diversity and gender differences. As the dietary sources showed a powerful impact on serum vitamin D and HbA1c levels with the recent improvement in knowledge and measurement methods, future studies could evaluate the effectiveness of dietary 25(OH)D in decreasing HbA1c levels compared to individual macronutrients and the whole food matrix. The dietary culture and a nutrition balance should also be considered as it may present a benefit in reducing HbA1c level and total cholesterol hence reducing the CVD risks.

Additionally, future studies are suggested to include sunlight exposure as an intervention when assessing the association between vitamin D and T2DM as current RCTs on UVB sunlight are limited.

Although supplementation could benefit serum 25(OH)D levels in risk groups, it usually requires a large dosage for a medium to long intervention time to show the effects on T2DM biomarkers. Considering the feasibility, potential hazard and affordability of vitamin D supplementation in some regions, future studies are encouraged to assess vitamin D's effects from natural resources for both risk groups and the general population.

To conclude, the D4D trial results suggested that diet is the most effective way to increase serum 25(OH)D level and improve glycaemic control in patients with T2DM. For future studies assessing the association of vitamin D and glycaemic control, a full picture of adjustment and balance from diet, sun, and supplementation with feasible and patient-oriented suggestions are recommended.

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Appendix

Appendix A. NHMR Participant Information and Consent Form (PICF, version IV)

University of Technology Sydney

Participant Information Sheet and Consent Form Interventional Research

Title	**The Role of Vitamin D in Controlling and Reducing Diabetes Mellitus Risks**
Short Title	**D4D Research**
Project Sponsor	**UTS**
Principal Investigator	**Associate Professor Christopher Zaslawski**
Site	**University of Technology, Sydney**
Protocol	**Protocol Version IV**

Part I – What does my participation in the study involve?

1 Introduction

You are invited to take part in the research: The Role of Vitamin D in Controlling and Reducing Diabetes Mellitus Risks (D4D Research). Before you make your decision, we would like to help you to understand more about this study such as the purpose of the study, what it will involve and how your information will be used. Please take time to carefully read the following information. One of our research staffs will help you to go through this information sheet and answer any questions you may have.

2 What is the purpose of this research?

The D4D research is designed as a multi-centre, single-blinded, randomised study aims to evaluate the possible association between vitamin D and Type II Diabetes, and to see if the corrected vitamin D status could benefit patients with Type II Diabetes.

3 Why have I been chosen?

You have been chosen as a potential participant because you fulfil the following selection criteria:
1) You are older than 18 years; 2) you have been diagnosed with Type II Diabetes in the past 12 months, and not on diabetic medication or insulin treatment; 3) Your vitamin D level from your recent blood test is between 28 nmol/l and 85 nmol/l and not on vitamin D supplements or any relevant medications *(please note serum vitamin D 28-49 nmol/l is classified as vitamin D mild deficiency; 49 to 85 nmol/l is classified as vitamin D sufficient but will still be chosen for the reason of study sensitivity)*; 4) You do not have thyroid disease, liver disease, kidney disease, cancer, osteoporosis, dementia, mental disease or taking relevant medications; 5) You are not pregnant or breastfeeding.

You would not be eligible for the current trial if you are:
1) Unable to give written informed consent form or follow the research instructions; 2) Currently using a vitamin D supplement; 3) Have thyroid disease, liver disease, kidney

disease, cancer, osteoporosis, dementia, mental health disorder or taking relevant medications; 4) Women who are pregnant or breastfeeding; 5) Vegan and cannot ingest any animal product.

4 Do I have to take part in the research?

The participation in the D4D research is completely voluntary. If you do decide to participate, you will be given this **Participant Information Sheet** & **Consent Form (PICF)** to sign, and given a copy to keep. You can change your mind later and withdraw from the study at any stage, for any reason.

5 Other relevant information

There will be a total of 20 to 140 participants involved in this study. Recruitment and assessment will be conducted in Earlwood Medical Centre and Bangor Medical Centre. A NATA (National Association of Testing Authorities, Australia) accredited pathology laboratory will conduct the blood test of vitamin D level and Diabetes biomarkers every three to six months. Data will be analysed in the University of Technology, Sydney.

We strongly recommend that you inform your family doctor of your participation in this study. You can take your regular medicine as long as it was checked and approved by the research team. If you are having any diabetes-related medication or vitamin D supplements (including calcium and Vitamin D supplements) during the study period, please let out research team know and you may be excluded from the current study. If you would like to know more information, please visit our website www.d4dresearch.com or talk to our research team.

6 What will happen to me if I take part?

Once you make the decision, you will need to sign the **Patient Information and Consent Form (PICF)** and it has to be completed prior to any assessments being performed.

Secondly, you will be randomly assigned to one of four treatment groups as: vitamin D supplement group, dietary intervention group, sun exposure group or wait-list control group. You would be known for which treatment you are receiving during the trial but the research staffs including the statisticians are blind to the treatment allocation i.e. which treatment group you are in and hence which treatment you are receiving.

To be specific, if you are in the vitamin D supplement group, you will be given oral vitamin D supplementation with a dosage of 500 IU per day. If you are in the Diet group, you will be required to follow a dietary plan with help of an Accredited Practicing Dietitian to obtain 10-15 µg/d of vitamin D. If you are in the sun exposure group, you will be asked to expose about 15% of the body surface to obtain approximately 12.5 µg/d of vitamin D per day. No intervention will be given to the waitlist control group.

The total time of your attendance in this study is 9 months. You are required to attend the initial baseline assessment, and the follow-up assessment on a monthly basis. Information collection would be conducted in each monthly assessment but the blood test will be only conducted at the baseline, 3rd month, and 9th month assessment.

Please feel free to contact the research team if you experience any discomfort or an upsetting symptom, we would assess the problem and if necessary, cease your participation immediately and provide you with medical treatment if required.

There are no additional costs associated with participating in this study, nor will you be paid. *All tests and medical care required as part of the study will be provided to you free*

of charge. However, you may be reimbursed $25 voucher for any reasonable travel, parking, meals and other expenses associated with the study visit.

7 What do I have to do?

As we mentioned before:

- If you are in the vitamin D supplement group, you will need to take one tablet of vitamin D (500IU) per morning and you should keep a written record of supplement taken for the monthly assessment.

- If you are in the dietary intervention group, please follow the dietary plan designed by the Accredited Practising Dietitian to obtain 10-15 µg/d of vitamin D from food and keep a dietary diary for the monthly assessment. Dietary diary could be recorded in paper form or through the smart phone application.

- If you are in the sun exposure group, you will be required to expose approximately 15% of your body surface (i.e. hands, face and arms) in sunlight for 3-11 minutes every day and to keep a sun exposure dairy for the monthly assessment.

You are required to attend the initial baseline assessment, and the follow-up assessment on a monthly basis for 9 months. Information collection would be conducted in each monthly assessment but the blood test will be only conducted at the baseline, 3rd month, and 9th month assessment.

Please note it is your responsibility and commitment for taking the research supplements, or following the dietary plan or sun exposure time; and attending the monthly assessment including a necessary blood test, in accord with the instructions provided.

8 What will happen to my test samples?

Your test sample including your data and blood sample will be collected in monthly assessment in Earlwood Medical Centre or Bangor Medical Centre. All information will be de-identified with a code to maintain the privacy or confidentiality and will later be analysed by research staff in University of Technology, Sydney.

9 What are the possible benefits of taking part?

Some possible benefits may include a corrected vitamin D level and a healthier lifestyle of diet and exercise, however we cannot guarantee or promise that you will receive any benefits from the D4D research.

10 What are the side effects of the study medications?

You may worry about the dosage of vitamin D used in the current D4D study. However the daily vitamin D dosage (500 IU/d) used in the study is set following the Adequate Intake (AI) of vitamin D in Australia and is considered as a safe level. Hence it is unlike to cause any side effects or symptoms by having this dosage through sun exposure, dietary change or supplementary intake.

Participants in dietary intervention group may experience food allergy or intolerance by adding seafood or eggs into the daily meals, even if you are cleared from the allergy history at the initial screening. Please inform our research team if you experience any severe allergic reaction or food intolerance, we will assess the condition and provide allergy test or medical treatment if necessary, and will exclude you from the current study.

11 What are the possible disadvantages and risks in taking part?

If you have thyroid diseases, liver impairment, renal failure (eGFR <50 ml/min), cancer, osteoporosis, dementia or mental diseases and are taking any relevant medications,

study staff will exclude you from the study as they may interfere with diabetes and blood glucose control.

If you become upset or distressed as a result of your participation in the research, the study staff is able to arrange the supportive counselling service or other appropriate support if necessary.

FOR FEMALE PARTICIPANTS: Although vitamin D changes in the current study would not harmful to an embryo or foetus, you must not participate in this study if you are pregnant, or trying to become pregnant, or breastfeeding. If you do become pregnant whilst participating in this study you should advise your study doctor immediately. The research staff may withdraw you from the study and advise on further medical attention should this be necessary.

12 What if new information becomes available?

The study team will inform you if any new information becomes available and discuss with you whether you want to continue in the study. If you decide to withdraw, our study team will assist you on that and if you decide to continue with the study, you will be asked to sign an updated consent form.

13 Can I have other treatments during this study project?

Whilst you are participating in this study project, you may not be able to take some or all of the medications or treatments you have been taking for your condition or for other reasons. It is important to tell the research staff about any treatments or medications you are undertaking and going to take, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments.

You should also tell the research staff about any changes of these conditions or treatment during your participation in the research. The study staff will also advise you if the treatments or medications need to be stopped or advise your family doctor during the period you are in the study.

14 What do I do if I wish to withdraw from the research?

If you wish to withdraw from this study please advise the research team. You will be asked to sign a **Withdrawal of Consent Form** and your will be provided a copy of this form including the information sheet .

The research staff will stop to collect additional information from you. **A consent or arrangement of using your data** will be sought from you. With the agreement from you; the data collected up to the time of withdrawing will be included in the study results. However you have your right to have your existed data removed, please contact the research team if you want to do so.

15 Could this study be stopped unexpectedly?

This study may be stopped unexpectedly for a variety of reasons including unacceptable side effects or decisions made in the interests by local regulatory/health authorities.

16 What happens when the study ends?

You don't need to follow the treatments i.e. vitamin D supplements, sun exposure time and meal plan when the study is finished. A study result summary can be provided upon the request once the study is completed, please contact the research team if you would like to have a copy of result summary.

It is suggested to consult with your family doctor about the appropriate future treatment plan for your Diabetes condition after the study.

Part II – How is the study being conducted?

17 What will happen to information about me?

By signing the consent form, you consent to the relevant research staff of collecting and using personal information about you for the D4D study.

All information and documentations will be identified by a code ID number only, to maintain your privacy and confidentiality. All local databases will be securely encrypted and password protected in an independent hard disk, which is only accessed by principal investigators. The hard disk will be backed up twice a month for data security. Study staff will be required to sign agreements to preserve the confidentiality of all participants. Your information will only be used for the purpose of this study project and it will only be disclosed with your permission, except as required by law.

Participant files will be maintained in storage for a period of 7 years after completion of the study. At the end of the period, research data and primary materials will be disposal of either permanently by achieving or destroying. For any type of studies including sub-studies, ancillary studies or any research outside of the D4D, which is using data or samples collected by the D4D, it will require a review and agreement from current principal investigator committee.

For the purpose of this research, information about you may be obtained from your health records held at this and other health services. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in D4D study. Your health records and any information collected and stored by the study staff during the study may be reviewed (for the purpose of verifying the procedures and the data) by the UTS human research ethics committee, regulatory authorities, or as required by law. By signing the consent form, you authorise the release of, or access to, this confidential information as noted above. Information about your participation in this study may be recorded in your health records.

The study results will be released to the participating physicians, referring physicians, and participants. It is anticipated that the results of this study will be published and or presented in a variety of public or professional forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your express permission.

In accord with relevant Australian and/or NSW privacy and other relevant laws, you have the right to request access to your personal information collected and stored by the study team. You also have the right to request any information with which you disagree be corrected. Please contact the research team named at the end of this document if you would like to access your information.

18 What if something goes wrong?

Please contact our research team immediately if you suffer any side effects or complications including distress or psychological injury as a result of this study. Our research team will assist you for assessment and in arranging appropriate medical treatment if necessary.

19 Who is organising and funding the research?

This study is being mainly conducted by Ms Fay YU as part of her PhD study at the University of Technology, Sydney under the supervision of Associate Professor Christopher Zaslawsi and Adjunct Professor Danforn Lim. This study is being funded by the Australian Traditional Medicine Society (ATMS), which is a not-to-profit company.

20 Who has reviewed the study?

This study has been reviewed and given approval by the UTS Human Research Ethics Committee. The conduct of this study at UTS and data collected from Earlwood Medical Centre and Bangor Medical Centre has been authorised by the UTS Human Research Ethics Committee.

21 Further information and who to contact

Please contact our research team if you would like any further information on this study .

If you would like to talk to someone not directly involved with the study for any further information regarding your rights or should you wish to make a complaint to people independent of the study team, you may contact the UTS Ethics Secretariat on 02/9514 9772 and quote the HREC reference number: ET16-0640.

Question	Who to contact	Phone / Facsimile
General questions or concerns during the study	Study Coordinator	Ms. Fay YU 02 9554 7788 contact@D4Dresearch.com
	Principal Investigators	A/Prof Chris Zaslawski 02 9514 7856 or chris.zaslawski@uts.edu.au Adj Prof Danforn Lim 02 9554 7788
Questions about the way the research is being conducted	Principal Investigators	A/Prof Chris Zaslawski 02 9514 7856 or chris.zaslawski@uts.edu.au
	Institutional Research Governance Officer	Adj Prof Danforn Lim 02 9554 7788
Questions regarding side effects	Ms. Fay YU	Ms. Fay YU 02 9554 7788 or contact@D4Dresearch.com

University of Technology Sydney

PARTICIPANT CONSENT FORM

Title	**The Role of Vitamin D in Controlling and Reducing Diabetes Mellitus Risks**
Short Title	**D4D study**
Project Sponsor	**UTS**
Principal Investigator	**Associate Professor Christopher Zaslawski**
Site	**University of Technology, Sydney**
Protocol	**Protocol Version IV**

1. I have read the attached Participant Information Sheet outlining the nature and purpose of the research study and I understand what I am being asked to do.
2. I have discussed my participation in this study with a member of the study team named below. I have had the opportunity to ask questions and I am satisfied with the answers I have received.
3. I have been informed about the possible risks of taking part in this study.
4. I consent to medical practitioners, other health professionals, hospitals or laboratories outside this institution releasing information concerning my condition and treatment which is needed for this study and understand that such information will remain confidential.
5. I freely consent to participate in the research project as described in the attached Participant Information Sheet.
6. I understand that my participation is voluntary and that I am free to withdraw at any time during the study without affecting my future health care.
7. I understand that if I decide to discontinue the study treatment, I may be asked to attend follow-up visits to allow collection of information regarding my health status. Alternatively, the investigator/sponsor will request my permission to access my medical records for collection of follow-up information for research and analysis.

Name of Participant	Signature of Participant	Date

Name of Witness to Participant's Signature	Signature of Witness	Date

*Witness is not to be the Investigator or member of the study team nor their delegate
* Please note that in the event that an Interpreter is used, the Interpreter is not a witness to the consent process

ALL WITNESSES MUST BE OVER 18 YEARS OF AGE

Name of Witness to consent process (GCP Guidelines 4.8.9)	Signature of Witness	Date

*Witness is not to be the Investigator or member of the study team nor their delegate
* Please note that in the event that an Interpreter is used, the Interpreter is not a witness to the consent process

Name of Investigator	Signature of Investigator	Date

Participant will be provided with a copy of the Participant Information Sheet and this Consent Form
All parties signing the Consent Form must date their own signature

University of Technology Sydney

WITHDRAWAL OF PARTICIPATION

Title	**The Role of Vitamin D in Controlling and Reducing Diabetes Mellitus Risks**
Short Title	**D4D study**
Project Sponsor	**UTS:**
Principal Investigator	**Associate Professor Christopher Zaslawski**
Site	**University of Technology, Sydney**
Protocol	**Protocol Version IV**

I hereby wish to WITHDRAW my intent to participate further in the above research project and understand that such withdrawal will not jeopardise my future health care.

Participant's Name (printed) ----------

Signature ----------

Date ----------

In the event the participant decided to withdraw verbally, please give a description of the circumstances. Coordinating Investigator to provide further information here:

Coordinating Investigator to sign the withdrawal of consent form on behalf of a participant if verbal withdrawal has been given:

Participant's Name (printed) ----------

Signature of Investigator ----------

Date ----------

Appendix B. NHMR Participant Information and Consent Form (PICF, version V for COVID 19)

University of Technology Sydney

Participant Information Sheet and Consent Form Interventional Research

Title	**The Role of Vitamin D in Controlling and Reducing Diabetes Mellitus Risks**
Short Title	**D4D Research**
Project Sponsor	**UTS**
Principal Investigator	**Associate Professor Christopher Zaslawski**
Site	**University of Technology, Sydney**
Protocol	**Protocol Version V**

Part I – What does my participation in the study involve?

22 Introduction

You are invited to take part in the research: The Role of Vitamin D in Controlling and Reducing Diabetes Mellitus Risks (D4D Research). Before you make your decision, we would like to help you to understand more about this study such as the purpose of the study, what it will involve and how your information will be used. Please take time to carefully read the following information. One of our research staffs will help you to go through this information sheet and answer any questions you may have.

23 What is the purpose of this research?

The D4D research is designed as a multi-centre, single-blinded, randomised study aims to evaluate the possible association between vitamin D and Type II Diabetes, and to see if the corrected vitamin D status could benefit patients with Type II Diabetes.

24 Why have I been chosen?

You have been chosen as a potential participant because you fulfil the following selection criteria:
1) You are older than 18 years; 2) you have been diagnosed with Type II Diabetes in the past 12 months, and not on diabetic medication or insulin treatment; 3) Your vitamin D level from your recent blood test is between 28 nmol/l and 85 nmol/l and not on vitamin D supplements or any relevant medications *(please note serum vitamin D 28-49 nmol/l is classified as vitamin D mild deficiency; 49 to 85 nmol/l is classified as vitamin D sufficient but will still be chosen for the reason of study sensitivity)*; 4) You do not have thyroid disease, liver disease, kidney disease, cancer, osteoporosis, dementia, mental disease or taking relevant medications; 5) You are not pregnant or breastfeeding.

You would not be eligible for the current trial if you are:
1) Unable to give written informed consent form or follow the research instructions; 2) Currently using a vitamin D supplement; 3) Have thyroid disease, liver disease, kidney disease, cancer, osteoporosis, dementia, mental health disorder or taking relevant

medications; 4) Women who are pregnant or breastfeeding; 5) Vegan and cannot ingest any animal product.

25 Do I have to take part in the research?

The participation in the D4D research is completely voluntary. If you do decide to participate, you will be given this **Participant Information Sheet** & **Consent Form (PICF)** to sign, and given a copy to keep. You can change your mind later and withdraw from the study at any stage, for any reason.

26 Other relevant information

There will be a total of 20 to 140 participants involved in this study. Recruitment and assessment will be conducted in Earlwood Medical Centre and Bangor Medical Centre. A NATA (National Association of Testing Authorities, Australia) accredited pathology laboratory will conduct the blood test of vitamin D level and Diabetes biomarkers every three to six months. Data will be analysed in the University of Technology, Sydney.

We strongly recommend that you inform your family doctor of your participation in this study. You can take your regular medicine as long as it was checked and approved by the research team. If you are having any diabetes-related medication or vitamin D supplements (including calcium and Vitamin D supplements) during the study period, please let out research team know and you may be excluded from the current study. If you would like to know more information, please visit our website www.d4dresearch.com or talk to our research team.

27 What will happen to me if I take part?

Once you make the decision, you will need to sign the **Patient Information and Consent Form (PICF)** and it has to be completed prior to any assessments being performed.

Secondly, you will be randomly assigned to one of four treatment groups as: vitamin D supplement group, dietary intervention group, sun exposure group or wait-list control group. You would be known for which treatment you are receiving during the trial but the research staffs including the statisticians are blind to the treatment allocation i.e. which treatment group you are in and hence which treatment you are receiving.

To be specific, if you are in the vitamin D supplement group, you will be given oral vitamin D supplementation with a dosage of 500 IU per day. If you are in the Diet group, you will be required to follow a dietary plan with help of an Accredited Practicing Dietitian to obtain 10-15 μg/d of vitamin D. If you are in the sun exposure group, you will be asked to expose about 15% of the body surface to obtain approximately 12.5 μg/d of vitamin D per day. No intervention will be given to the waitlist control group.

The total time of your attendance in this study is 9 months. You are required to attend the initial baseline assessment, and the follow-up assessment on a monthly basis. Information collection would be conducted in each monthly assessment, but the blood test will be only conducted at the baseline, 3rd month, and 9th month assessment.

Please feel free to contact the research team if you experience any discomfort or an upsetting symptom, we would assess the problem and if necessary, cease your participation immediately and provide you with medical treatment if required.

There are no additional costs associated with participating in this study, nor will you be paid. *All tests and medical care required as part of the study will be provided to you free of charge. However, you may be reimbursed $25 voucher for any reasonable travel,*

parking, meals and other expenses associated with the study visit.

28 What do I have to do?

As we mentioned before:

- If you are in the vitamin D supplement group, you will need to take one tablet of vitamin D (500IU) per morning and you should keep a written record of supplement taken for the monthly assessment.

- If you are in the dietary intervention group, please follow the dietary plan designed by the Accredited Practising Dietitian to obtain 10-15 μg/d of vitamin D from food and keep a dietary diary for the monthly assessment. Dietary diary could be recorded in paper form or through the smart phone application.

- If you are in the sun exposure group, you will be required to expose approximately 15% of your body surface (i.e. hands, face and arms) in sunlight for 3-11 minutes every day and to keep a sun exposure dairy for the monthly assessment.

You are required to attend the initial baseline assessment, and the follow-up assessment on a monthly basis for 9 months. Information collection would be conducted in each monthly assessment but the blood test will be only conducted at the baseline, 3rd month, and 9th month assessment.

Please note it is your responsibility and commitment for taking the research supplements, or following the dietary plan or sun exposure time; and attending the monthly assessment including a necessary blood test, in accord with the instructions provided.

***PLEASE NOTE WE HAVE MAKE FOLLOWING CHANGES AMID COVID-19**

Our monthly data collection of sun exposure, dietary intake and supplements intake will be completed with you remotely from home. Our research staff will contact you via phone calls or email to obtain the data monthly.

However, for the blood test at the initial baseline, 3rd month and 9th month, you are required to attend the clinic's pathology lab to complete the blood test. Our research staff will contact you before the blood test help to make appointments and explain the safety request in the medical centre and pathology laboratory and to understand and answer your concerns if you have any about COVID-19. Please feel free to contact our research staff if you decide not to proceed or delay with their blood test anytime.

29 What will happen to my test samples?

Your test sample including your data and blood sample will be collected in monthly assessment in Earlwood Medical Centre or Bangor Medical Centre. All information will be de-identified with a code to maintain the privacy or confidentiality and will later be analysed by research staff in University of Technology, Sydney.

30 What are the possible benefits of taking part?

Some possible benefits may include a corrected vitamin D level and a healthier lifestyle of diet and exercise, however we cannot guarantee or promise that you will receive any benefits from the D4D research.

31 What are the side effects of the study medications?

You may worry about the dosage of vitamin D used in the current D4D study. However the daily vitamin D dosage (500 IU/d) used in the study is set following the Adequate Intake (AI) of vitamin D in Australia and is considered as a safe level. Hence it is unlike to cause any side effects or symptoms by having this dosage through sun exposure, dietary change or supplementary intake.

Participants in dietary intervention group may experience food allergy or intolerance by adding seafood or eggs into the daily meals, even if you are cleared from the allergy history at the initial screening. Please inform our research team if you experience any severe allergic reaction or food intolerance, we will assess the condition and provide allergy test or medical treatment if necessary, and will exclude you from the current study.

32 What are the possible disadvantages and risks in taking part?

If you have thyroid diseases, liver impairment, renal failure (eGFR <50 ml/min), cancer, osteoporosis, dementia or mental diseases and are taking any relevant medications, study staff will exclude you from the study as they may interfere with diabetes and blood glucose control.

If you become upset or distressed as a result of your participation in the research, the study staff is able to arrange the supportive counselling service or other appropriate support if necessary.

FOR FEMALE PARTICIPANTS: Although vitamin D changes in the current study would not harmful to an embryo or foetus, you must not participate in this study if you are pregnant, or trying to become pregnant, or breastfeeding. If you do become pregnant whilst participating in this study you should advise your study doctor immediately. The research staff may withdraw you from the study and advise on further medical attention should this be necessary.

33 What if new information becomes available?

The study team will inform you if any new information becomes available and discuss with you whether you want to continue in the study. If you decide to withdraw, our study team will assist you on that and if you decide to continue with the study, you will be asked to sign an updated consent form.

34 Can I have other treatments during this study project?

Whilst you are participating in this study project, you may not be able to take some or all of the medications or treatments you have been taking for your condition or for other reasons. It is important to tell the research staff about any treatments or medications you are undertaking and going to take, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments.

You should also tell the research staff about any changes of these conditions or treatment during your participation in the research. The study staff will also advise you if the treatments or medications need to be stopped or advise your family doctor during the period you are in the study.

35 What do I do if I wish to withdraw from the research?

If you wish to withdraw from this study please advise the research team. You will be asked to sign a **Withdrawal of Consent Form** and your will be provided a copy of this form including the information sheet .

The research staff will stop to collect additional information from you. **A consent or arrangement of using your data** will be sought from you. With the agreement from you; the data collected up to the time of withdrawing will be included in the study results. However you have your right to have your existed data removed, please contact the research team if you want to do so.

36 Could this study be stopped unexpectedly?

This study may be stopped unexpectedly for a variety of reasons including unacceptable

side effects or decisions made in the interests by local regulatory/health authorities.

37 What happens when the study ends?

You don't need to follow the treatments i.e. vitamin D supplements, sun exposure time and meal plan when the study is finished. A study result summary can be provided upon the request once the study is completed, please contact the research team if you would like to have a copy of result summary.

It is suggested to consult with your family doctor about the appropriate future treatment plan for your Diabetes condition after the study.

Part II – How is the study being conducted?

38 What will happen to information about me?

By signing the consent form, you consent to the relevant research staff of collecting and using personal information about you for the D4D study.

All information and documentations will be identified by a code ID number only, to maintain your privacy and confidentiality. All local databases will be securely encrypted and password protected in an independent hard disk, which is only accessed by principal investigators. The hard disk will be backed up twice a month for data security. Study staff will be required to sign agreements to preserve the confidentiality of all participants. Your information will only be used for the purpose of this study project and it will only be disclosed with your permission, except as required by law.

Participant files will be maintained in storage for a period of 7 years after completion of the study. At the end of the period, research data and primary materials will be disposal of either permanently by achieving or destroying. For any type of studies including sub-studies, ancillary studies or any research outside of the D4D, which is using data or samples collected by the D4D, it will require a review and agreement from current principal investigator committee.

For the purpose of this research, information about you may be obtained from your health records held at this and other health services. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in D4D study. Your health records and any information collected and stored by the study staff during the study may be reviewed (for the purpose of verifying the procedures and the data) by the UTS human research ethics committee, regulatory authorities, or as required by law. By signing the consent form, you authorise the release of, or access to, this confidential information as noted above. Information about your participation in this study may be recorded in your health records.

The study results will be released to the participating physicians, referring physicians, and participants. It is anticipated that the results of this study will be published and or presented in a variety of public or professional forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your express permission.

In accord with relevant Australian and/or NSW privacy and other relevant laws, you have the right to request access to your personal information collected and stored by the study team. You also have the right to request any information with which you disagree be corrected. Please contact the research team named at the end of this document if you would like to access your information.

39 What if something goes wrong?

Please contact our research team immediately if you suffer any side effects or complications including distress or psychological injury as a result of this study. Our research team will assist you for assessment and in arranging appropriate medical treatment if necessary.

40 Who is organising and funding the research?

This study is being mainly conducted by Ms Fay YU as part of her PhD study at the University of Technology, Sydney under the supervision of Associate Professor Christopher Zaslawsi and Adjunct Professor Danforn Lim. This study is being funded by the Australian Traditional Medicine Society (ATMS), which is a not-to-profit company.

41 Who has reviewed the study?

This study has been reviewed and given approval by the UTS Human Research Ethics Committee. The conduct of this study at UTS and data collected from Earlwood Medical Centre and Bangor Medical Centre has been authorised by the UTS Human Research Ethics Committee.

42 Further information and who to contact

Please contact our research team if you would like any further information on this study .

If you would like to talk to someone not directly involved with the study for any further information regarding your rights or should you wish to make a complaint to people independent of the study team, you may contact the UTS Ethics Secretariat on 02/9514 9772 and quote the HREC reference number: ET16-0640.

Question	Who to contact	Phone / Facsimile
General questions or concerns during the study	Study Coordinator	Ms. Fay YU 02 9554 7788 contact@D4Dresearch.com
	Principal Investigators	A/Prof Chris Zaslawski 02 9514 7856 or chris.zaslawski@uts.edu.au Adj Prof Danforn Lim 02 9554 7788
Questions about the way the research is being conducted	Principal Investigators	A/Prof Chris Zaslawski 02 9514 7856 or chris.zaslawski@uts.edu.au
	Institutional Research Governance Officer	Adj Prof Danforn Lim 02 9554 7788
Questions regarding side effects	Ms. Fay YU	Ms. Fay YU 02 9554 7788 or contact@D4Dresearch.com

University of Technology Sydney

PARTICIPANT CONSENT FORM

Title	**The Role of Vitamin D in Controlling and Reducing Diabetes Mellitus Risks**
Short Title	**D4D study**
Project Sponsor	**UTS**
Principal Investigator	**Associate Professor Christopher Zaslawski**
Site	**University of Technology, Sydney**
Protocol	**Protocol Version V**

8. I have read the attached Participant Information Sheet outlining the nature and purpose of the research study and I understand what I am being asked to do.
9. I have discussed my participation in this study with a member of the study team named below. I have had the opportunity to ask questions and I am satisfied with the answers I have received.
10. I have been informed about the possible risks of taking part in this study.
11. I consent to medical practitioners, other health professionals, hospitals or laboratories outside this institution releasing information concerning my condition and treatment which is needed for this study and understand that such information will remain confidential.
12. I freely consent to participate in the research project as described in the attached Participant Information Sheet.
13. I understand that my participation is voluntary and that I am free to withdraw at any time during the study without affecting my future health care.
14. I understand that if I decide to discontinue the study treatment, I may be asked to attend follow-up visits to allow collection of information regarding my health status. Alternatively, the investigator/sponsor will request my permission to access my medical records for collection of follow-up information for research and analysis.

Name of Participant	Signature of Participant	Date

Name of Witness to Participant's Signature	Signature of Witness	Date

*Witness is not to be the Investigator or member of the study team nor their delegate
* Please note that in the event that an Interpreter is used, the Interpreter is not a witness to the consent process

ALL WITNESSES MUST BE OVER 18 YEARS OF AGE

Name of Witness to consent process (GCP Guidelines 4.8.9)	Signature of Witness	Date

*Witness is not to be the Investigator or member of the study team nor their delegate
* Please note that in the event that an Interpreter is used, the Interpreter is not a witness to the consent process

Name of Investigator	Signature of Investigator	Date

Participant will be provided with a copy of the Participant Information Sheet and this Consent Form
All parties signing the Consent Form must date their own signature

University of Technology Sydney

WITHDRAWAL OF PARTICIPATION

Title	**The Role of Vitamin D in Controlling and Reducing Diabetes Mellitus Risks**
Short Title	**D4D study**
Project Sponsor	**UTS:**
Principal Investigator	**Associate Professor Christopher Zaslawski**
Site	**University of Technology, Sydney**
Protocol	**Protocol Version IV**

I hereby wish to WITHDRAW my intent to participate further in the above research project and understand that such withdrawal will not jeopardise my future health care.

Participant's Name (printed) ----------

Signature ----------

Date ----------

In the event the participant decided to withdraw verbally, please give a description of the circumstances. Coordinating Investigator to provide further information here:

Coordinating Investigator to sign the withdrawal of consent form on behalf of a participant if verbal withdrawal has been given:

Participant's Name (printed) ----------

Signature of Investigator ----------

Date ----------

Participant will be provided with a copy of this Withdrawal of Consent Form

Appendix C. Ethics Committee Approval

HREC Approval Granted - ETH16-0640

Research.Ethics@uts.edu.au <Research.Ethics@uts.edu.au> Thu, Jan 24, 2019 at 9:36 AM
To: Chris.Zaslawski@uts.edu.au, Xuefei.Yu@student.uts.edu.au, Research.Ethics@uts.edu.au

Dear Applicant

Thank you for your response to the Committee's comments for your project titled, "The Role of Vitamin D in Controlling and Reducing Diabetes Mellitus Risks (D4D)". Your response satisfactorily addresses the concerns and questions raised by the Committee who agreed that the application now meets the requirements of the NHMRC National Statement on Ethical Conduct in Human Research (2007). I am pleased to inform you that ethics approval is now granted.

Your approval number is UTS HREC REF NO. ETH16-0640.

Approval will be for a period of five (5) years from the date of this correspondence subject to the provision of annual reports.

Your approval number must be included in all participant material and advertisements. Any advertisements on the UTS Staff Connect without an approval number will be removed.

Please note that the ethical conduct of research is an on-going process. The National Statement on Ethical Conduct in Research Involving Humans requires us to obtain a report about the progress of the research, and in particular about any changes to the research which may have ethical implications. This report form must be completed at least annually from the date of approval, and at the end of the project (if it takes more than a year). The Ethics Secretariat will contact you when it is time to complete your first report.

I also refer you to the AVCC guidelines relating to the storage of data, which require that data be kept for a minimum of 5 years after publication of research. However, in NSW, longer retention requirements are required for research on human subjects with potential long-term effects, research with long-term environmental effects, or research considered of national or international significance, importance, or controversy. If the data from this research project falls into one of these categories, contact University Records for advice on long-term retention.

You should consider this your official letter of approval. If you require a hardcopy please contact Research.Ethics@uts.edu.au.

To access this application, please follow the URLs below:
* if accessing within the UTS network: https://rm.uts.edu.au
* if accessing outside of UTS network: https://vpn.uts.edu.au , and click on " RM6 – Production " after logging in.

We value your feedback on the online ethics process. If you would like to provide feedback please go to: http://surveys.uts.edu.au/surveys/onlineethics/index.cfm

If you have any queries about your ethics approval, or require any amendments to your research in the future, please do not hesitate to contact Research.Ethics@uts.edu.au.

Yours sincerely,

Associate Professor Beata Bajorek
Chairperson
UTS Human Research Ethics Committee
C/- Research & Innovation Office
University of Technology, Sydney
E: Research.Ethics@uts.edu.au
I: https://staff.uts.edu.au/topichub/Pages/Researching/Research%20Ethics%20and%20Integrity/Human%20research%20ethics/human-research-ethics.aspx

Appendix D. Major Questionnaire (Version IV)

QUESTIONNAIRE
'The Role of Vitamin D in Controlling and Reducing Diabetes Mellitus Risks (D4D)'
Please note: All questions contained in this questionnaire are strictly confidential

Part I – PERSONAL INFORMATION			
Name *(Last, First, M.I.):* M ☐ F ☐		Date of Birth:	
Marital status: ☐ Single ☐ Partnered ☐ Married ☐ Separated ☐ Divorced ☐ Widowed			
Current weight (kg):		Height (cm):	
Are you of Aboriginal or Torres Strait Islander origin? ☐ Y ☐ N		Do you speak a language other than English at home? And if yes, what is it? ☐ Y ☐ N	
In which country where you born?		What is your ancestry?	
Have your mother, father, brother(s), sister(s) ever had diabetes? ☐ Y ☐ N		Have you taken any medications, supplements or vitamin for most of the last 4 weeks? And if yes, what is it? ☐ Y ☐ N	
Has a doctor ever told you that you have (please circle):	Skin cancer / melanoma / prostate cancer / heart diseases / high blood pressure/ stroke / diabetes / blood clot / asthma / depression / Parkinson's diseases / **none of these**		

Part II. EXERCISE & SUN EXPOSURE	
All questions contained in this questionnaire are optional and will be kept strictly confidential.	
Exercise	☐ Sedentary (No exercise)
	☐ Mild exercise (i.e., climb stairs, walk 3 blocks, golf)
	☐ Occasional vigorous exercise (i.e., work or recreation, less than 4x/week for 30 min.)
	☐ Regular vigorous exercise (i.e., work or recreation 4x/week for 30 minutes)
Sun exposure	*H.E.C. Hanwell et al. / Journal of Steroid Biochemistry & Molecular Biology 121 (2010) 334–337*

	Time Outdoors			Amount of Skin Exposed			
	<5 min	5-30 min	>30min	Hands and face	Hands, face, arms	Hands, face, legs	Bathing suit
Monday	0	1	2	1	2	3	4
Tuesday	0	1	2	1	2	3	4
Wednesday	0	1	2	1	2	3	4
Thursday	0	1	2	1	2	3	4
Friday	0	1	2	1	2	3	4
Saturday	0	1	2	1	2	3	4
Sunday	0	1	2	1	2	3	4

PART III – DIETARY INFORMATION			
Diet	Are you dieting?	☐ Yes	☐ No
	If yes, are you on a physician prescribed medical diet?	☐ Yes	☐ No

	# of meals you eat in an average day?				
	Rank salt intake	☐ Hi	☐ Med	☐ Low	
	Rank fat intake	☐ Hi	☐ Med	☐ Low	
Caffeine	☐ None	☐ Coffee	☐ Tea	☐ Cola	
	# of cups/cans per day?				
Alcohol	Do you drink alcohol?			☐ Yes	☐ No
	If yes, what kind?				
	How many drinks per week?				
	Are you concerned about the amount you drink?			☐ Yes	☐ No
	Have you considered stopping?			☐ Yes	☐ No
	Have you ever experienced blackouts?			☐ Yes	☐ No
	Are you prone to "binge" drinking?			☐ Yes	☐ No
	Do you drive after drinking?			☐ Yes	☐ No

24 HR FOOD RECALL			
Food or Beverage Items **(List all foods and beverages for every meal and snack during the 24-hr, including water, coffee, tea and any vitamins and supplements taken)**	**Portion size** (How many pieces, slices, spoon, tablespoon, cup or gram)	**How was it prepared?** (was is it grilled, fried, boiled, steamed, light fired or raw?)	**Was anything added to it?**
Breakfast			
Morning tea			
Lunch			
Afternoon snack			
Dinner:			
Evening snack:			
Fluid intake including water / soup etc			

Appendix E. Questionnaire - 3 Days Food Recall (Version IV)

3-Day Food Recall[1] No.				
Meals	**Time**	**Date: Day 1**	**Date: Day 2**	**Date: Day 3**
Breakfast				
Morning tea				
Lunch				
Afternoon tea				
Dinner				
Supper				
Water	-			
Exercise	-			
Comments	-			

Appendix F. Questionnaire – Vitamin D Specific Food Frequency Questionnaire (FFQ-VDQ)

Do you eat **fatty** fish (salmon, mackerel, herring)? ☐NO ☐YES

If YES: How often?

- ☐At least twice weekly
- ☐3-4 times per month
- ☐1-2 times per month
- ☐More seldom

Do you drink milk or is milk part of your diet? ☐NO ☐YES

If YES: How much milk do you consume?

- ☐More than 3 dl (10 oz) per day
- ☐1-3 dl (3.5-10 oz) per day
- ☐Less than 1 dl (3.5 oz) per day

If YES: Which type of milk do you consume most often?

- ☐Full-fat milk (standardmjölk)
- ☐Medium-fat milk (mellanmjölk)
- ☐Low-fat milk (lättmjölk)
- ☐Skim milk (minimjölk)
- ☐Other (e.g. oat milk), please specify:.....

Is yoghurt and/or sour milk part of your diet? ☐NO ☐ YES

If YES How much yoghurt/sour milk do you consume?

- ☐More than 3 dl (10 oz) per day
- ☐1-3 dl (3.5-10 oz) per day
- ☐Less than 1 dl (3.5 oz) per day

If YES What kind do you consume most often?

- ☐fruit-/vanilla yoghurt/sour milk
- ☐plain yoghurt/sour milk
- ☐turkish/greek yoghurt

Do you use margarine as a spread (t ex. Bregott, Lätta, Flora, Milda, Becel)? ☐ NO ☐YES

If YES: How many sandwiches with margarine do you eat?

- ☐at least 4/day
- ☐2-3/day
- ☐4-7/week
- ☐1-3/week
- ☐fewer

Appendix G. External Organization Support Letter – Earlwood Medical Centre

Earlwood Medical Centre
Kids' Dr | Earlwood Imaging | Sydney CosMedic |
Integrative Medicine & Wellness Clinic
356 Homer Street, Earlwood, NSW 2206
"Your One Stop Health Station in Earlwood"
Our Clinical services include:
Specialist GP, Onsite Pathology Collection, Onsite X-Ray, Doppler, DEXA and Ultrasound Scans, Registered Nurses, Podiatrists, Chiropractors, Dietitian, Psychologists, Physiotherapist, Speech Pathologist, Acupuncturist, Chinese Herbal Medicine Practitioner, and Pharmacist
Tel: 02 9554 7788 Fax: 9554 7733
www.specialistmedicalservices.com.au

12/09/2015

To the UTS HREC manager

This is to confirm that the Earlwood Medical Centre is happy to assist and provide support for the screening and recruitment of potential subjects for the research project being conducted by Ms Fay Yu titled "The prevalence of vitamin D deficiency among newly diagnosed type 2 diabetes patients".

Production Note:
Signature removed
prior to publication.

Dr Lisa Cheng
Director

EARLWOOD
MEDICAL CENTRE
356 HOMER STREET
EARLWOOD NSW 2206

Appendix H. External Organization Support Letter – Bangor Medical Centre

Bangor Medical Centre

(A Member of Specialist Medical Services Group)
Shop 6, Bangor Shopping Centre, 121 Yala Road, Bangor NSW 2234
Tel: 02 8582 1318 Fax: 02 8582 1313
www.specialistmedicalservices.com.au

13/11/2018

To the UTS HREC manager,

This is to confirm that the Bangor Medical Centre is happy to assist and provide support for the screening and recruitment of potential subjects for the research project being conducted by Ms Fay Yu titled "The Role of Vitamin D in Controlling and Reducing DM Risks (The D4D study)".

Production Note:
Signature removed prior to publication.

Crystal Fung

Vice President

Specialist Medical Services Group

Appendix I. Recruiting Form for Study Therapist Use

Recruitment Week no.		**Selection Criteria of D4D research**						**Legibility**	**Notes**
Date	**Name Medicare no Contact no**	**1) Aged ≥ 18 years old;**	**2) Diagnosed with DM in the preceding 12 months + NOT on diabetic medication or insulin treatment;**	**3) Serum 25OHD is 28 - 85 nmol/l and NOT on vitamin D supplements or any relevant medications;**	**4) Do NOT have thyroid diseases, liver impairment, renal failure, cancer, osteoporosis, dementia, mental diseases or taking relevant medications;**	**5) NOT pregnant or breastfeeding women.**	**6) NOT vegen and can ingest any animal product.**	**YES / NO**	

Appendix J. Recruitment Flyer

Researchers from D4D Research are looking for adult participants to investigate the prevalence of Vitamin D deficiency and the potential link between vitamin D and Type 2 Diabetes.

We Would Like To Hear From You If You Are

- At least 18 years old;
- And, have Type 2 Diabetes that has been diagnosed in the past 12 months, and **NOT** on diabetic related medication or insulin treatment;
- And, diagnosed with vitamin D deficiency and **NOT** on vitamin D supplements or any relevant medication

Contact For More Info

Email: contact@D4Dresearch.com
Website: www.d4dresearch.com

Earlwood Medical Centre
(02) 9554 7788
356 Homer St,
Earlwood NSW 2206

Bangor Medical Centre
(02) 8582 1318
Shop 6, Bangor Shopping Centre,
121 Yala Rd,
Bangor NSW 2234

All Enquires Will Be Treated Privately And Confidentially

University of Technology, Sydney | Visit us on: www.D4Dresearch.com

Appendix K. Confidentiality Agreement (study therapists)

CONFIDENTIALITY AGREEMENT

Title of Research Project:
THE ROLE OF VITAMIN D IN CONTROLLING AND REDUCING DM RISKS (D4D)

Local Principal Investigator:
Ms Fay YU
Organization: School of Life Sciences, University of Technology, Sydney
Address: School of Life Sciences, University of Technology, Sydney
Telephone no.:
Email: contact@d4dresearch.com or Xuefei.Yu@student.uts.edu.au

As a member (study therapist) of this research team I understand that I may have access to confidential information about study sites, participants and research data. By signing this statement, I am indicating my understanding of my responsibilities to maintain confidentiality and agree to the following:

- I understand that names and any other identifying information about study sites, participants and research data are completely confidential.
- I agree not to divulge, publish, or otherwise make known to unauthorized persons or to the public any information obtained in the course of this research project that could identify the persons who participated in the study.
- I understand that all information about study sites, participants and research data obtained or accessed by me in the course of my work is confidential. I agree not to divulge or otherwise make known to unauthorized persons any of this information, unless specifically authorized to do so by approved protocol or by the local principal investigator acting in response to applicable law or court order, or public health or clinical need.
- I understand that I am not to read information about study sites, participants or research data, or any other confidential documents, nor ask questions of study participants for my own personal information but only to the extent and for the purpose of performing my assigned duties on this research project.
- I agree to notify the local principal investigator immediately should I become aware of an actual breach of confidentiality or a situation, which could potentially result in a breach, whether this be on my part or on the part of another person.

Signature	Date	Printed name
Signature of local principal investigator	Date	Printed name